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Diversity and molecular phylogenetics of Psychodidae (Insecta, Diptera)

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Diversity and molecular phylogenetics of Psychodidae (Insecta, Diptera)

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Summary

Moth flies (Diptera: Psychodidae) exhibit remarkable diversity with over 3,500 worldwide described species. Despite their small size, typically ranging from 1 to 5 millimeters, these flies are easily recognizable by their setose appearance and distinctive wing shape. These flies often holding their wings horizontally over the abdomen when resting, resembling small moths, hence the common name. Within the family Psychodidae, six extant subfamilies are broadly recognized: Bruchomyiinae, Horaiellinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyiinae, alongside one extinct subfamily, Datziinae.

This Ph.D. thesis represents a comprehensive effort to advance our understanding of the morphology, ecology, and phylogeny within the subfamily Psychodinae. Employing an integrative taxonomy approach, the research combines traditional morphological analyses with Next Generation Sequencing techniques. By leveraging these methodologies, the study aims to address ongoing taxonomic discussions within this subfamily, shedding light on previously unresolved issues.

Structured as a series of publications in scientific journals or manuscripts, this thesis covers a broad spectrum of geographical regions, including the Neotropical, Palearctic, and Oriental Regions. Through the examination of various genera, the research not only describes new species but also documents numerous new geographic records for both species and genera across the studied taxa. This extensive geographic scope enriches our understanding of the distribution patterns and ecological preferences of Psychodinae species.

A central objective of this Ph.D. thesis is to elucidate the intricate phylogenetic relationships within the Psychodinae. By inferring the phylogenetic relationships within this subfamily, the study aims to revise the tribal classification within Psychodinae, providing a more

robust framework for understanding their evolutionary relationships. To achieve this goal, our research team designed 18,651 hybrid-capture baits targeting 1,445 coding regions across 1,161 ortholog groups. Remarkably, this approach successfully captured targeted loci for 82 moth fly species spanning 46 genera, representing a significant proportion of the total diversity within Psychodinae. This comprehensive dataset serves as a valuable resource for phylogenetic inference and taxonomic revision within the subfamily.

Overall, this Ph.D. thesis represents a significant contribution to our knowledge of moth flies, offering insights into their morphology, ecology, and evolutionary history. By employing a multidisciplinary approach and leveraging cutting-edge techniques, the research not only expands our understanding of Psychodinae but also lays the groundwork for future studies aimed at unraveling the complexities of this diverse group of flies.

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Disclaimer

In accordance with the International Code of Zoological Nomenclature (ICZN) articles 8.2 and 8.3, this thesis is NOT to be considered a published work for nomenclatural purposes. Species names, and nomenclatural changes proposed herein, specifically in chapters 8, 9, 14, 15, and 19, with appendixes (6, 7, 12, 13, and 17) have no official standing in zoological nomenclature, and will only be validated in subsequent publications satisfying the criteria of ICZN §8.

Chapter 1 – Introduction

1.1 Introduction to the German Barcode of Life

The German Barcode of Life (GBOL) is a comprehensive biodiversity research project focused on the identification and documentation of Germany's vast array of species through DNA barcodes. It is an example of a comprehensive initiative that utilizes DNA barcoding to build a reference library of species in a specific region or country. GBOL phases I (2011–2015) and II (2016–2019) were a collaborative effort between multiple institutions and organizations in Germany with a network of professional and non-professional taxonomists. The primary goal of GBOL is to generate DNA barcode data for the estimated 45,000 species found in Germany, including animals, plants, and fungi (Geiger et al., 2016).

A DNA barcode refers to a short, standardized segment of DNA that serves as a unique genetic marker for identifying and classifying species. By building a comprehensive DNA barcode reference library, GBOL enables scientists and researchers to quickly and accurately identify species, even when traditional methods based on morphology are challenging or inconclusive. It is derived from a specific region of an organism's genome, typically a region that exhibits variability between species but remains relatively conserved within the individuals of the same species. Moreover, DNA barcoding has proven to be a powerful tool in various fields, including biodiversity research, conservation biology, forensics, and ecological studies. It allows, among others, for the rapid identification of species, the discovery of new species, the detection of invasive species, and the assessment of genetic diversity within and among populations.

GBOL's third phase, entitled “GBOL III: Dark Taxa” was launched in 2020 aiming to complete the DNA Barcode reference library dealing with hyper-diverse and understudied groups of insects, known as “Dark Taxa”, mainly the orders Diptera and Hymenoptera. These two targeted

groups correspond to about half of the animal species present in Germany (around 10,000 species each) (Hausmann et al., 2020).

Overall, the German Barcode of Life project has significant implications for various fields of research and conservation. It facilitates the identification of species involved in ecological interactions, such as pollinators, predators, or prey, and decomposers, aiding in the understanding of ecosystem dynamics. It also assists in the detection of invasive species, monitoring biodiversity changes, and assessing the impact of environmental factors on different taxa.

Furthermore, GBOL provides valuable data for the conservation and management of Germany's biodiversity. By identifying species accurately and efficiently, policymakers and conservationists can make informed decisions regarding the protection and preservation of vulnerable or endangered species and their habitats.

Altogether, the German Barcode of Life is a pioneering project that utilizes DNA barcoding to generate a comprehensive reference library of Germany's diverse species. By combining advanced molecular techniques with traditional taxonomic knowledge, GBOL contributes to the understanding, conservation, and management of biodiversity, while also supporting scientific research and promoting collaboration both nationally and internationally.

1.2 Dark Taxa, taxonomic impediment, and integrative taxonomy

The term “Dark Taxa” was coined in 2011 (Page, 2011, 2016) to refer to species with sequences in GenBank that lacked formal scientific names. Nowadays, however, the term dark taxa usually refer to hyper-diverse and understudied groups of life forms (e.g. bacteria, fungi, and, invertebrates), which are usually taxonomically neglected groups (Hartop et al., 2022; Meier et al.,

2022). When specifically talking about insects, dark taxa are usually small-bodied specimens, belonging to very species-rich groups, and usually the undescribed taxa exceed the described fauna (Hartop et al., 2022). Due to the described circumptancies, dealing with these kinds of samples using conventional morphological techniques is an extremely slow process. As a result, unidentified species are frequently either completely ignored or only a few select specimens are chosen for examination (Hartop et al., 2022).

The term "Taxonomic impediment" dates back to the 70s (Taylor, 1976) and it refers to the limited knowledge of the known species and the full magnitude of the true diversity (Green, 1998). Several other factors affecting the taxonomic impediment have been pointed out since the late 90s such as the resources needed for taxonomical research, the access to specimen collections and scientific literature, and lack of specialists (Green, 1998). In more recent years, several other factors, like biodiversity loss, the bureaucracy to conduct research, the lack of financial resources, and the shortage of qualified professionals or amateur taxonomists have been related to the taxonomic impediment, and despite that new techniques (e.g. DNA barcoding) tried to tackle this issue, it seems to be a persistent problem that remains unsolved (Evenhuis, 2008; Pante et al., 2015; Audisio, 2017). Dark taxa are one of the major causes of the taxonomic impediment. There have been active discussions about what the taxonomic impediment represents and how it can affect the current biodiversity crisis (Engel et al., 2021). In general, three key points have been attributed to the taxonomic impediment: 1) the delay in species discovery; 2) the slow pace of formal taxonomical revision; and 3) the lack of funding or taxonomic jobs and/or specialists (Vinarski, 2020).

One of the concepts that is being used today to tackle some points of the taxonomic impediment is the integrative taxonomy approach. The core concept underlying integrative taxonomy is that, due to the challenging nature of defining species, it is advisable to utilize a wide array of available methods to recognize species. In other words, the diversity of data types

(behaviour, biology, DNA sequences, images, morphology, sounds etc.) should be examined and combined to achieve a comprehensive understanding of species delimitation (Goulding & Dayrat, 2016). Integrative taxonomy is often seen as solution great tool to tacklethe taxonomic impediment (Gomes et al., 2015; Cao et al., 2016; Goulding & Dayrat, 2016; Vinarski, 2020) as it allows taxonomists to have a more robust species concept.

The dark taxa groups, the taxonomic impediment, and the tools provided for an integrative taxonomy approach are some of the reasons why projects like "GBOL III: Dark Taxa" are crucial, not only in Germany, but globally. These projects provide the tools (e.g. DNA barcode reference libraries), the human power (e.g. forming new taxonomists), and the knowledge (e.g. new species discovery, new distributional records) for a more comprehensive biodiversity understanding. The outcomes of such projects ultimately can affect decision-making when it comes to biodiversity protection, management, and other branches of knowledge.

These concepts fit into a larger discussion about the nature of systematics and of taxonomic information and the role they have in conservation (e.g. Cotterill, 1995; Blaxter, 2004; Carvalho et al., 2005, 2007; Wheeler, 2005; DeSalle et al., 2005; Ebach & Holdrege, 2005; Godfray, 2002, 2007; Godfray & Knapp, 2004; Janzen, 2004; Knapp et al., 2002; McNeely, 2002; Thiele & Yeates, 2002; Evenhuis, 2007; Miller, 2007; Santos & Amorim, 2007).

In recent years, three pivotal factors have emerged as significant contributors to the current challenges faced in taxonomic research, primarily, a global decline in the number of taxonomists, coupled with a pervasive lack of funding and insufficient appreciation for this specialized area of research, creating a confluence of obstacles (Dubois, 2003; Ebach et al., 2011; Britz et al., 2020). To date, species description relies heavily on the expertise of taxonomists. Despite the emergence of new technologies and recent approaches aimed at addressing the hyperdiverse taxa (eg.

Integrative taxonomy), these approaches continue to hinge upon taxonomic expertise, cycling back to the pressing usue of the decreasing number of taxonomists.

The challenge further intensifies with the impediment to training new taxonomists, a consequence of reduced funding in universities or research institutions specializing in organismal studies or taxonomy (Britz et al., 2020). This decline in training opportunities not only exacerbates the shortage of taxonomic expertise but also diminishes the prospects of replenishing the field with new talent. Consequently, the repercussions of this multifaceted dilemma are grim. The urgent need to fund taxonomic initiatives and support taxonomy becomes evident. Adequate resources must be channeled into educational programs, research centers, and initiatives aimed at nurturing taxonomic expertise, fostering a new generation of specialists equipped to tackle the challenges of biodiversity assessment and conservation.

The destruction of habitats due to urbanization, deforestation, pollution, and climate change has reached unprecedented levels, jeopardizing the delicate balance of ecosystems worldwide. Taxonomic information plays a fundamental role in understanding these habitats' biodiversity, species interactions, and ecological dynamics. Improved taxonomic knowledge directly contributes to effective conservation strategies. Accurate identification and classification of species within ecosystems are fundamental for informed decision-making in conservation efforts. Without precise taxonomic information, conservation initiatives may fall short, misidentifying species at risk, underestimating biodiversity values, or implementing inadequate protection measures (Amorim, 2009).

Furthermore, enhancing the quality of taxonomic information is vital for advancing ecological and conservation sciences. Poor taxonomy hampers our ability to comprehend species' roles within ecosystems, hindering the study of ecological interactions, population dynamics, and community structures. This deficiency in understanding can impede the formulation of sustainable

management practices and conservation policies tailored to safeguarding these vital habitats. Therefore, the imperative to invest in improving taxonomic accuracy and depth of information becomes clear. Robust taxonomic research is essential for preserving habitats, protecting biodiversity, and ensuring the sustainable management of ecosystems in the face of ongoing environmental challenges. Amorim (2009) underscores the urgency of this matter, emphasizing that enhancing taxonomic information is not merely an academic pursuit but a critical step toward effective ecological understanding and conservation action.

1.3 Target group – Family Psychodidae

The order Diptera, commonly known as mosquitoes and flies, is one of the most diverse insect orders on Earth, with over 160,000 described species and many more awaiting discovery (Borkent et al., 2018). The sheer magnitude of dipteran diversity is so remarkable that it represents a significant portion of the total animal diversity on our planet. Flies account for approximately 12% of all known animal species; in other words, 1 of every 10 known species is a fly (Grimaldi, 2005). This fact highlights their remarkable ecological success and evolutionary resilience. From tiny midges to robust horse flies, and from pollinators to decomposers, Diptera encompasses a vast range of ecological roles and habitats (Yeates & Wiegmann, 2005; Pape et al., 2009; Marshall, 2012). Exploring the immense diversity within this group not only unravels the intricacies of insect evolution, but also offers insights into the broader functioning of ecosystems worldwide (Weigmann et al., 2011; Wiegmann & Yeates, 2017).

The monophyly of the order Diptera has never been disputed (Hennig, 1973, 1981; Meier, 2005; Bertone et al., 2008; Weigmann et al., 2011). The group was traditionally divided into two suborders, namely Nematocera (adults with long multi-segmented antennae, with similar shaped flagellomeres; and larvae with cephalic capsule well-developed and mouth with mandibles) and Brachycera (adults with a few heterogeneous flagellomeres and with a stylus/arista; and larvae

with modifications in the mandibles and without complete cephalic capsule). While the monophyly of Brachycera is not questioned, the nematoceran families are not recovered as a clade (Wood & Borkent, 1989; Oosterbroek & Courtney, 1995; Amorim & Yeates, 2006; Bertone et al., 2008; Woodley et al., 2009; Wiegmann et al., 2011). Generally, there are four recognized infraorders within the nematoceran families, i.e., Bibionimorpha, Culicomorpha, Psychodomorpha, and Tipulomorpha (Wiegmann & Yeates, 2017). The target group of this Ph.D. dissertation, the family Psychodidae Newman, 1834, belongs to the infraorder Psychodomorpha.

Commonly referred to as moth flies, sand flies, or owl flies, the family Psychodidae (Diptera: Psychodomorpha) (Figure 1) has nearly 3,500 described species distributed worldwide, including the Antarctic Region (Frenot et al., 2005; Cordeiro & Wagner, 2018; Galati, 2018; Curler et al., 2019; Wagner & Ibáñez-Bernal, 2009; Andrade et al., 2022; Galati & Rodrigues, 2023). Adults usually measure 1–6 millimiters and can be easily recognized due to their setose appearance and distinctive wing shape and venation. When at rest, many of these flies hold their wings horizontally over the abdomen, resembling small moths, which is the reason for their common name of moth flies (Figure 1) (Curler & Courtney, 2009; Wager & Ibáñez-Bernal, 2009).

Several different intrafamilial classifications have been proposed thorough history and the family subdivision is still under debate (Curler & Moulton, 2012; Galati & Rodrigues, 2023). One extinct subfamily, Datziinae (5 spp), and six extant subfamilies are generally recognized, namely Bruchomyiinae (74 spp), Horaiellinae (6 spp), Phlebotominae (1,060 spp), Psychodinae (2,050 spp), Sycoracinae (46 spp), and Trichomyiinae (215 spp).

A number of studies have targeted the classification of the Psychodidae, particularly the subfamily Psychodinae, to better understand its evolutionary relationships (e.g. Vaillant, 1971; Ježek, 1983; Duckhouse, 1987; Rispail & Leger, 1998, Kvifte, 2018). Recent studies that analysed DNA sequences from both nuclear and mitochondrial sources (Curler & Moulton, 2012; Espíndola

et al., 2012; Kvifte, 2018) have revealed that the tribal classification proposed by Vaillant (1971) and Ježek (1984a) does not accurately represent the evolutionary relationships. Currently, there is no consensus for Psychodinae among different classification systems based on various character datasets, especially at the tribal level (Kvifte, 2018).

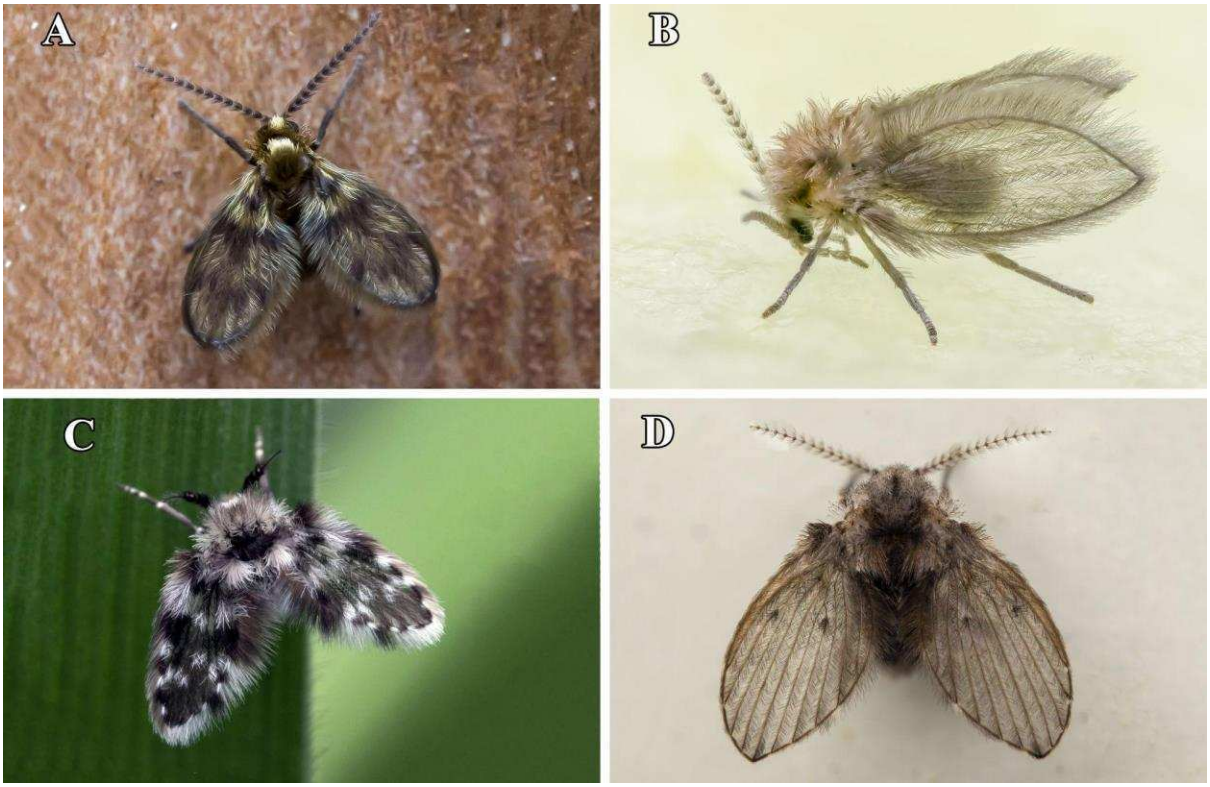

Figure 1. Habitus pictures of Psychodidae species. **A.** *Trichomyia urbica* Haliday, 1839. **B.** *Psychoda* sp. **C.** *Clytocerus ocellaris* (Meigen, 1804). **D.** *Clogmia albipunctata* (Williston, 1893). All photographies by Santiago Jaume Schinkel.

1.3.1 General morphology of Psychodidae

Generally speaking, the body of insects is typically divided into three distinct regions: head, thorax, and abdomen. The head is the foremost part of the insect body and contains vital sensory organs, such as the antennae, compound eyes, and mouthparts. The thorax is the middle region of the insect body and is divided into three segments: the prothorax, mesothorax, and metathorax. Each segment typically bears a pair of legs, resulting in three pairs of legs in adult insects. Additionally, the mesothorax and metathorax usually each have a pair of wings in winged insects, making a total of two pairs of wings. The wings and legs are attached to the thoracic segments, allowing insects to move, fly, and perform various locomotor activities. The abdomen is the posterior part of the insect body and is composed of multiple segments, although the number of visible segments can vary across different insect species. The abdomen contains vital organs such as the digestive, reproductive, and respiratory systems (Gullan & Cranston, 2014).

The distinguishing feature of flies (Order Diptera) within insects is the possession of a single pair of functional wings (the first pair of wings), with the second pair modified into halters (small, knobbed structures aiding in balance during flight). This unique wing arrangement distinguishes them from other winged insects and contributes to their remarkable agility in the air. Additionally, Diptera often exhibit diverse mouthparts adapted for various feeding habits, ranging from piercing-sucking mouthparts in blood-feeding species like mosquitoes to sponging or sponging-lapping mouthparts seen in fruit flies and houseflies (McAlpine, 1981).

Adult Psychodidae are small to medium sized dipterans, with a wing length of about 1–6 mm (Figure 1). The body shape is slightly humpbacked, covered with a setose vestiture. The head presents oval compound eyes, with or without an eye bridge; ocelli absent; antenna usually with 13–14 flagellomeres, antennal flagellomeres often carrying hyaline sensory rods (referred to as ascoids), palpus with three to five segments. The wings are held erect, roof-like or horizontally

over the abdomen at rest; the wing shape is variable and has asubcostal (Sc), four to five radial veins (R), four medial veins (M), and one to two cubital veins (Cu); and the wing crossveins are reduced, or confined to Sc (Kvifte & Wagner, 2017). Adult Psychodidae can be morphologically variable between the different subfamilies and can superficially resemble adults of other families, like Corethrelidae, Simuliidae, Thaumaleidae, and Cecidomyiidae; although they can be separated by the higher number of developed wing veins (Kvifte & Wagner, 2017) (Figure 2).

Regarding species identification, the final segments of the abdomen, known as the terminalia or genitalia, hold significant importance. Specifically, within the Psychodidae family, the male genitalia are particularly crucial for species distinction. The established and commonly used morphological terms for Diptera are summarized in Cumming & Wood's works (2009, 2017). In male Psychodidae, the main elements of the terminalia encompass the copulatory structures found in the primary genital segment (abdominal segment 9) and the proctiger. These components include the epandrium (tergite 9) with two posterolteral appendages (surstyli or epandrial appendages), the hypandrium (sternite 9), paired two-segmented gonopods (Gonocoxites and gonostyli), a central tubular structure called the aedeagus, parameres, and the proctiger (Cumming & Wood, 2017) (Figure 3).

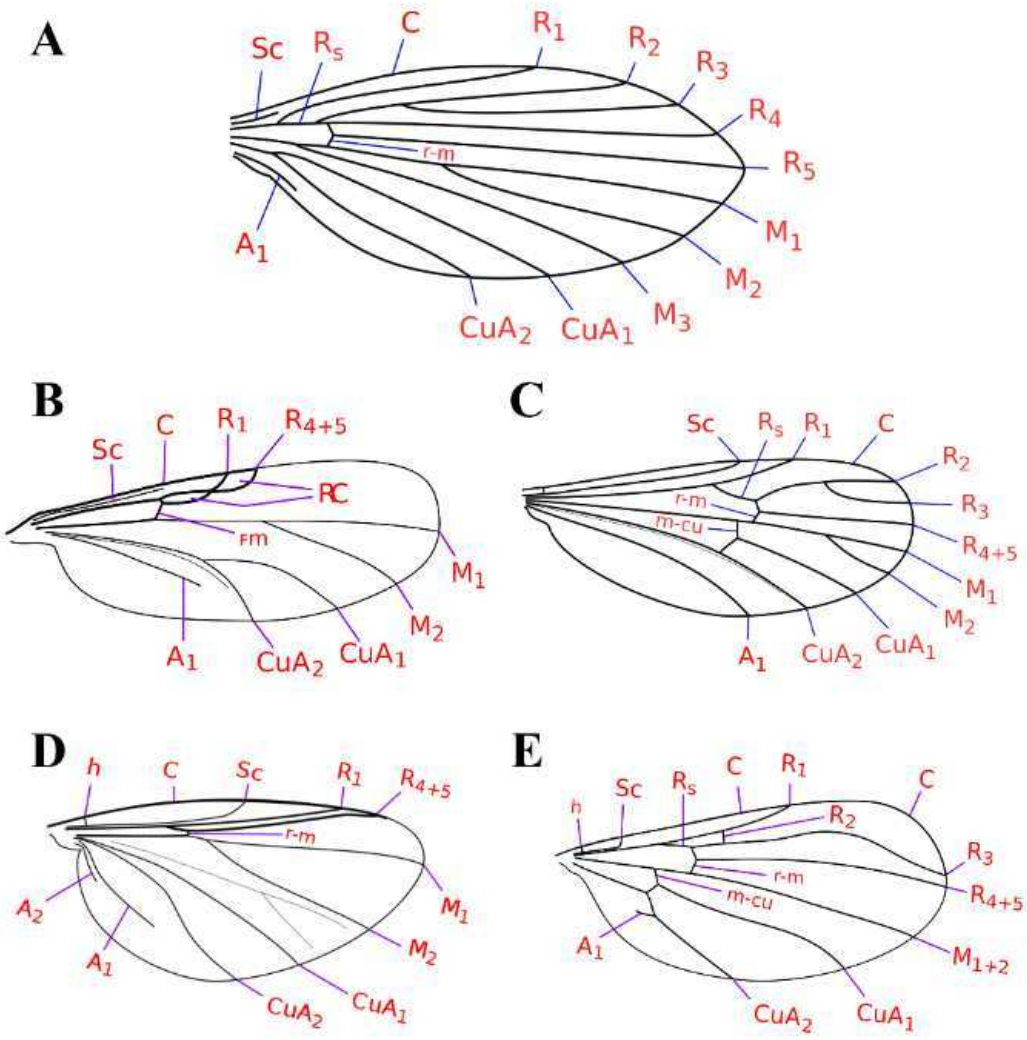

Figure 2. Wings of different Diptera families. **A.** Psychodidae. **B.** Ceratopogonidae. **C.** Corethrelidae. **D.** Simuliidae. **E.** Thaumaleidae. Abbreviations: Cells: **RC** = first and second radial cells. Longitudinal veins: **C** = costa; **Sc** = subcosta; **R** = radius; **M** = media; **Cu** = cubitus; **A** = anal. Crossveins: **h** = humeral; **r-m** = radial-medial; **m-cu** = medial-cubital. All wing images obtained from wikimedia commons.

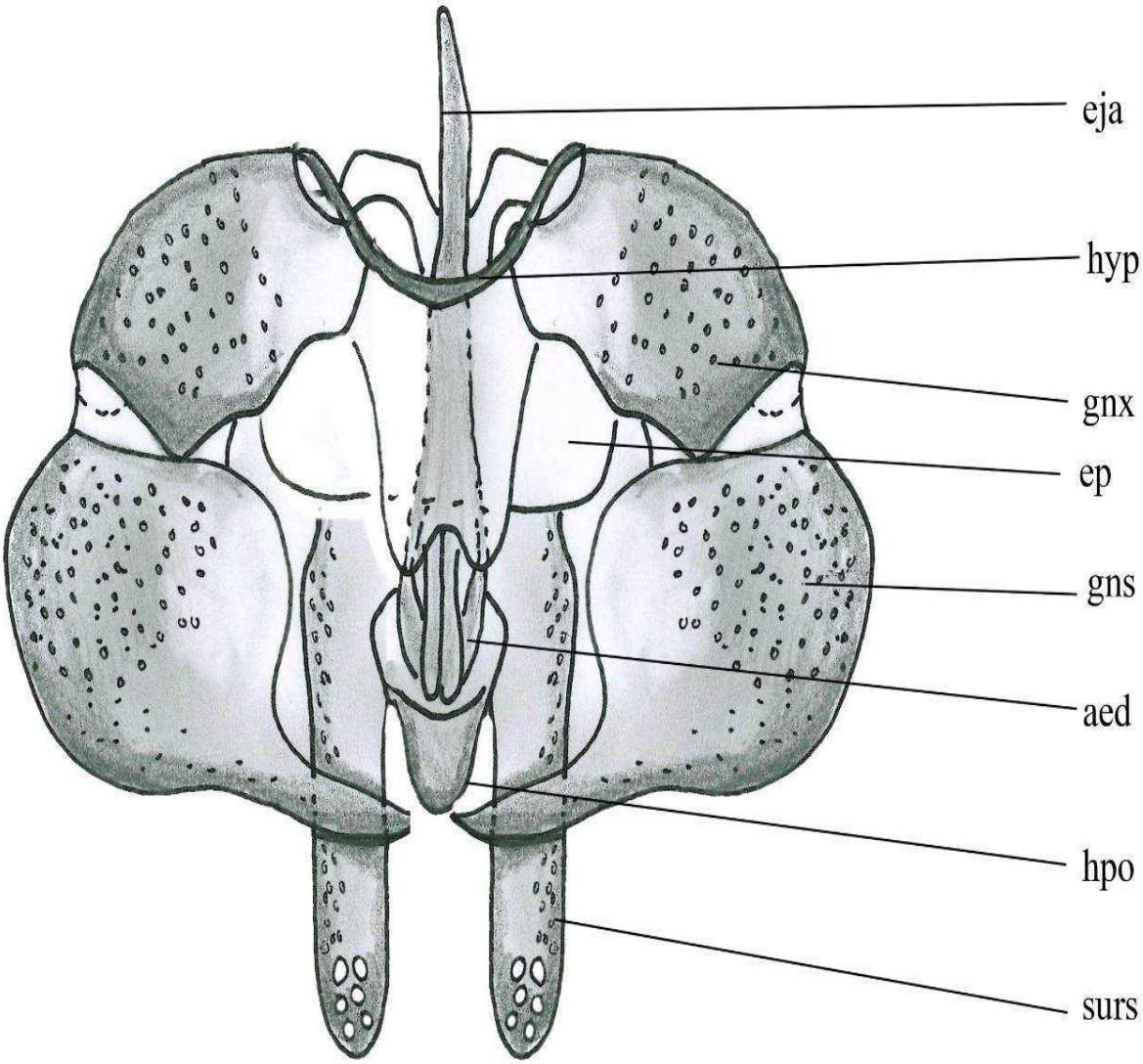

Figure 3. General morphology of male genitalia of Psychodidae. Abbreviations: **aed** = aedeagus; **eja** = ejaculatory apodeme; **epa** = epandrium; **gns** = gonostylus; **gnx** = gonocoxite; **hyp** = hypandrium; **hpo** = hypoproct; **surs** = surstylus.

In male Psychodidae, the gonocoxites are often cylindrical or elongated structures, while the gonostyli may vary in shape and may or may not have spines or additional projections. The hypandrium range from heavy to weakly sclerotized varying in shape across different species. The epandrium is usually cuadrate or rectangular and sclerotized, with two epandrial appendages that vary in shape and size across the family. The aedagus and parameres shape are species-specific and vary in shape and size, as well as the ejaculatory apodeme. The proctiger (segment 10, anal region) is usually conformed by the epiproct and hypoproct (See Figure 3).

The larvae of Psychodidae are shaped like cylinders, sometimes appearing flattened, and have a heavily sclerotized surface (Figure 4). Some genera have suction discs on the ventral surface of the abdomen, especially if the larvae develop in running water bodies (streams and rivers), as the suction discs allow them to hold into the surface without being washed away by the flow. The larval stages seem to have around 26 to 27 rings (annuli) that give them a segmented appearance. On their heads, they have an antenna with several setulae coming out of a round base. The thoracic segments are split into two parts, while the first abdominal segment is divided into two or three, and segments 2 to 7 are divided into three rings each. Usually, these rings have plates on the top side, and the larvae have the same number and positions of setae on each segment: prothorax with 22 pairs, mesothorax to abdominal segment 7 with 18 pairs. There are other additional setae (accesory setae) whose number and shape can vary. Toward the end of the larvae's bodies (segments 8 to 11), there are some structures that seem different from the rest, probably adapted to the larvae's living environment. These structures help keep the openings of the breathing holes dry. Overall, their breathing system seems to be located in these structures and involves keeping these holes dry while allowing them to breathe both underwater and in the air.

The descriptions of pupae within Psychodidae remain notably sparse (Omad et al., 2013). Typically, these pupae exhibit a cylindrical body with dorso-ventrally flattened bodies. The thorax presents a pair of prototHoracic respiratory organs, commonly referred to as respiratory horns

(Quate & Vockeroth, 1981) (see Figure 4). The wing covers extend towards the midsection and slightly past the terminal segments of the legs. Additionally, the abdominal segments exhibit one or more transverse rows of spiniform setae, forming distinctive rings. The posterior abdominal segments display a modified, somewhat rectangular shape, accompanied by pairs of spines, one dorsal and one ventral (Quate & Vockeroth, 1981; Ibañez-Bernal, 2000).

Psychodidae pupae generally share similar characteristics, posing challenges in differentiation between species. Consequently, further detailed descriptions are imperative to effectively distinguish among various species (Omad et al., 2013).

Figure 4. *Clogmia albipunctata* (Williston, 1893). **A.** Larva. **B.** Pupa. **C.** Adult. Photos reproduced with permission of the author Matt Bertone, North Carolina State University.

Adult Brychomyiinae typically have a slim appearance, with their legs being longer than their bodies. They also have a lot of fine setae covering their bodies. Their heads have oval-shaped compound eyes but lack a dorsal extension between the eyes (eye bridge). Their antennae usually have 14 long and tube-like segments (flagellomeres) but some species can have up to 111. Antennal flagellomeres present bulbous ascoids that are shorter than the segments carrying them. Their palpi, have five segments. Females within the subfamily have functional mandibles, whilst males do not have functional mandibles.

The wings of Bruchomyiinae are usually narrow and rounded at the ends, with a particular vein, R_5, branching out. In male Brychomyiinae, the terminal segments of the abdomen (the Terminalia) are twisted due to the arrangement of segments seven and eight. The gonocoxites are cylinder-shaped, and the gonostyli typically do not have spines. Males have two testes and ducts used for ejaculation, while a part called the epandrium lacks additional parts. On the other hand, females have one spermatheca (a structure for storing sperm) and a single duct connected to it.

Phlebotominae adults have a slender appearance, with their legs being longer than the body. The body is usually covered in fine setae. Their heads present oval compound eyes lacking the dorsal extension (eye bridge). The antennae are usually conformed by 14 elongate and cylindrical flagellomeres. The flagellomeres presnt digitiform ascoids that are shorter than the flagellomeres carrying them. The palpi are five-segmented. Females of Phlebotominae have functional mandibles, while males do not.

Their wings are long and lanceolate, and usually distally pointed; with vein R with five branches. In the males, the terminalia is inverted by the torsion of segments seven and eight; the gonocoxites are cylindrical, gonostyli with one to several lobes, usually with several spiniform setae. Males have paired testes with ejaculatory ducts and aedeagal filaments; the epandrium presents fixed appendages, with or without apical tenacula. On the other hand, females have paired

spermatheca and paired spermathecal ducts connected to the spermatheca (Kvifte & Wagner, 2017).

Adult Psychodinae have a sturdy appearance, with their legs being shorter or almost the same size as their bodies. They are covered by a setose vestiture. The head presents reniform eyes, usually with an eye bridge present. The antennae have eight to 14 flagellomeres. The flagellomeres haveascoids which are highly variable in shape and size. The palpi are four-segmented. In some species, the males present a pair of club- or sac-shaped structures posteriorly projecting on the head, called cornicula. Both males and females do not have functional mandibles.

The wings of Psychodinae are usually lanceolate to oval, and can be distally rounded or pointed. Wing vein R is 5-branched. Male terminalia is inverted by the full rotation of segment nine; gonocoxites are usually curved and cylindrical, with a parameral complex partially sclerotized; gonostyli usually taper towards the end, but are variable in shape. Males with paired testes and three pairs of accessorial glands, ejaculatory duct unpaired and unsclerotized, with one phallotrema; epandrium with jointed muscular appendages present, with variable tenacula. In females with a single genital opening; spermatheca and spermathecal ducts are reduced, and structures superficially similar to spermatheca are often present (Kvifte & Wagner, 2017).

Sycoracinae adults have a stout appearance, with their legs being shorter than their body. The stose vestiture of the body is reduced. Their heads have oval eyes, and the dorsal extension (eye bridge) is missing. Their antennae usually have 13–14 flagellomeres. The antennal flagellomeres presnet ascoids that can be shorter or longer than the flagellomere carrying them. Their palpi have four segments. The females have functional mandibles, while the males do not.

The wings of Sycoracinae are oval and distally rounded. The wing vein R has four branches. In the males, the terminalia may or may not be inverted. The gonocoxites are cylindrical and present complex parameres, the gonostyli with one or multiple sclerotized spines. Males present paired and unsclerotized testes, with paired ejaculatory ducts, and a single phallotrema. On the other hand, females fave paired spermathecae (Kvifte & Wagner, 2017).

Adult Trichomyiinae have a sturdy build, with legs that are as short or as long as their bodies. The setose vestiture may or may not be developed. Their heads feature oval-shaped eyes without an eye bridge between them. The antennae usually consist of 13 to 14 tube-like segments, with multiple small, finger-like ascoids. Their palpi, have three or four segments. Females within the subfamily have functional mandibles, whilst males do not.

The wings are oval-shaped. Wing vein R is 4-branched. The male Terminalia is inverted by the torsion of segments seven and eight. The gonocoxites are cylindrical, often with complex parameres, the shape of gonostyli is variable. The males have paired unscletorized testes, with paired ejaculatory ducts; and one or two phallotremata. On the other hand, females have paired spermathecae and paired spermathecal ducts, with a single genital opening (Kvifte & Wagner, 2017).

Horaiellinae adults have a slender appearance, with their legs being longer than their body. The setose vestiture is well developed. Their heads have oval-shaped compound eyes and lack the eye bridge. Their antennae have 14 flagellomeres. Antennal flagellomeres present digitiform ascoids that vary in shape. The palpi have three to four segments. Females within this subfamily have functional mandibles, while the males do not.

The wings of Horaiellinae are oval-shaped and rounded at the ends. The wing venation is variable within species. In male Horaiellinae, the terminalia presents different degrees of inversion, ranging from uninverted to 90 or 180 degrees. The gonocoxites are cylindrical and the gonostyli are usually elongated, with multiple spiniform setae that vary between the species (Curler et al., 2019).

1.3.2 Biology and behavior

Despite their somewhat erratic and weak flights, psychodids have successfully adapted to various habitats and niches, ranging from high montane streams to heavily polluted wastewater and drains. Their larvae can be found in diverse environments, including aquatic, semi-aquatic, and even relatively dry habitats such as decaying wood, carrion, fungi, dung, and soil (Wager & Ibáñez-Bernal, 2009). Although most known moth fly larvae are considered detritivores (relying on decaying organic matter as their substrate or food source), the larval biology of numerous species remains unknown (Wager & Ibáñez-Bernal, 2009; Kvifte & Wagner, 2017).

Adults of Bruchomyiinae, Psychodinae, and Trychomyiinae have unknown feeding habits (Kvifte & Wagner, 2017). Adult females of the Phlebotominae have functional mouthparts and feed on vertebrate blood. Both females and males have been reported feeding on natural sugar-rich sources such as sap of plants and honeydew from aphids (Killick-Kendrick, 1999). This subfamily has medical importance as they are vectors of *Leishmania* spp. and microfilarial worms (Kvifte & Wagner, 2017). Similarly, adult Sycoracinae females have functional mouthparts and are known to feed on vertebrate blood, with some European species capable of transmitting microfilarial worms between frogs (Desportes, 1941). Males of Sycoracinae have been found resting on frogs (a common blood-resource for females) (Jezek et al., 2015) but it remains unknown if males feed. Likewise, adult females of Horaiellinae have functional mandibles, and it has been suggested that

they might be blood-feeders (Tonnoir, 1933; Duckhouse & Duckhouse, 2004), male feeding habits, on the other hand, remain unkown.

Bruchomyiinae and Phlebotominae larvae have been observed feeding on decaying organic matter (Kvifte & Wagner, 2017). The Phlebotominae larval stages typically develop in the soil, with some species being associated with small mammal burrows (Quate & Vockeroth, 1987; Wagner, 1997a). Psychodinae larvae inhabit a diverse range of environments, relying on water, moist substrates, and decomposing organic matter for their development (Kvifte & Wagner, 2017; Wagner & Ibañez-Bernal, 2009). They have been found in decaying wood, carrion, vertebrate feces, compost, and fungal bodies, with only a few doubtful (improbable) cases of opportunistic myiasis (Taylan-Ozkan et al., 2004; Tu et al., 2007; Mathison et al., 2024). Horaiellinae larvae have been found in aquatic environments, collected in moistened rocks on the side of waterfalls (Duckhouse & Duckhouse, 2004) and it is suggested that the larvae can withstand prolonged submergence (Duckhouse & Duckhouse, 2004), no feeding habits have been recorded. Sycoracinae larvae are typically found on aquatic mosses, leaf litter, or lime-rich habitats (Duckhouse, 1972; Kvifte & Wagner, 2017; Wagner, 1997b) probably feeding on decaying organic matter. Lastly, all known larvae of Trichomyiinae develop inside decaying wood (Kvifte & Wagner, 2017).

1.3.3 Previous moth fly classifications

The family Psychodidae was erected by Newman in 1834 to comprise the genus *Psychoda* Latreille, 1797 with *Tipula phalaenoides* Linneaus, 1748 as type species [nowadays known as *Psychoda phalaenoides* (Linnaeus)]. Since then, several names and spellings have been used by different authors, such as Psychodoidae Agassiz, 1846; Psychodidi Bigot, 1854; Psychodides Zetterstedt, 1837; Psicodinae Rondani, 1840; Psychodida Marshall, 1873; Psychopidae Hardy,

1960 (Sabrosky, 1999). To date, the family Psychodidae has nearly 3,500 described species distributed worldwide (Galati & Rodrigues, 2023)

During its history, Psychodidae has been divided into different groups. Originally Bruchomyiinae was described and considered as a subfamily of Tanyderidae (Alexander, 1921), while Trichomyiinae has been considered a separate family inside the superfamily Psychodidea (Rhodendorf, 1962). Some authors have suggested treating Phlebotominae as a distinct family (e.g. Azar et al., 1999; Walker, 1851; Lewis, 1973; Rohdendorf, 1974; Williams, 1993), although the system of six extant subfamilies (Bruchomyiinae Alexander, 1921; Horaiellinae Enderlein, 1937; Phlebotominae Rondani, 1840; Psychodinae Newman, 1834; Sycoracinae Jung, 1954; Trichomyiinae Tonnoir, 1922) and one extinct subfamily (Datziinae Stebner, Solórzano-Kraemer, Ibañez-Bernal & Wagner, 2015) is currently widely accepted.

Some phylogenetic studies have placed the subfamily Sycoracinae as a sister group of the family Tanyderidae and discussed the possibility of a paraphyletic Psychodidae (Curler & Moulton, 2012). However, the evolutionary relationships between the moth fly subfamilies remain unresolved, and therefore this placement for Sycoracinae is not universally accepted (Henning, 1972; Quate & Vockeroth, 1981; Curler & Moulton, 2012; Bertone et al., 2008; Kvifte & Wagner, 2017; Wiegemann et al., 2011).

The target group of this Ph.D. thesis, the subfamily Psychodinae, has suffered several changes in its classification, especially at the tribal level. Enderlein (1935, 1937) proposed the first tribal division. This classification was presented as a taxonomical key based mainly on wing venation. Enderlein's classification ignored some important diagnostic characters (especially characters in the male genitalia) and most subsequent workers dismissed his work (Kivfte, 2018). Later, Vaillant (1971, 1982, 1986, 1990) developed a tribal classification using a wider range of characters but his classification was impaired by several violations of the International Code of

Zoological Nomenclature (ICZN); see Duckhouse (1978). Nevertheless, European authors continued using Vaillant's classification (i.e. Andersen & Håland, 1995; Salamanna & Raggio, 1985; Wagner, 1990, 1997, 2004; Krek, 1999; Bernotienè, 2002; Svensson, 2009).

In the following years, Ježek (1984b, 1985; Ježek & Goutner, 1993; Ježek & van Harten, 2005) followed some of Duckhouse's (1978) critiques of Vaillant's classification and presented an alternative tribal classification. Later, Duckhouse (1985, 1987) proposed another classification followed by Quate (1996) and Kvifte (2012). Later, Espíndola et al. (2012) evaluated the monophyly of the subfamily Psychodinae, finding that the tribal classification had partial contradiction with previous classifications and that some tribes were not monophyletic. Finally, Kvifte (2018) revised the tribal classification based on a molecular phylogeny, proposing the most recent tribal classification for Psychodinae. Following Kviftes's (2018) classification, the subfamily Psychodinae currently has four recognized tribes, namely Psychodini, Brunettiini, Maruinini, and Pericomaini.

1.4 Introduction to Palearctic Psychodidae

Since the 16th and 17th centuries, natural history museums in Europe experienced significant growth alongside the expansion of European academia. During this period, an influential development emerged in the realm of scientific nomenclature, stemming from the pioneering work of Carolus Linnaeus in mid 1700s (Pape, 2009). This era laid the foundation for much of the present family-level classification for Diptera, heavily influenced by its European origins (Pape, 2009). Understanding the diversity of dipteran species in the Palearctic region has seen fluctuations over time. In the 1800s, scholars like Robineau-Desvoidy, Meigen, and Wiedemann produced extensive monographs. However, it is worth noting that less than a third of the names they proposed are considered valid in today's classification systems (Pape, 2009). Recent efforts to catalog the Palearctic Diptera fauna have been published in comprehensive

catalogs such as those by Becker et al. (1903–1907) and Soós & Papp (1984–1993) Grootaert et al. (1991), Ježek (1987), Chvála (1997), Papp (2001), and Pakalniškis et al. (2000, 2006).

The proportion between the known species and the expected diversity of Diptera in the Palearctic Region differs between each family, but the Palearctic fauna of Diptera is certainly one of the best studied in the world (Pape, 2009). There are around 45,000 described species (Evenhuis & Pape, 2023), but it has been estimated that two or even three times that number is not unrealistic (Pape, 2009).

Europe has the fauna of Psychodidae most thoroughly studied in the world. Five of the six recognized subfamilies are present in Europe, namely Bruchomyiinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyinae (Wagner, 2001, 2004). There have been several taxonomical works through the years dealing with Psychodidae in Europe and more than 500 described species are currently recorded (Wagner, 2004; Kvifte, 2015). Despite this, the taxonomic knowledge for moth flies in Europe is uneven, though, with a few countries having high numbers of studies and records (e.g., Bulgaria, Czech Republic, France, Germany, and Slovakia), and many others lack systematic collection of specimens and have low number of records (e.g., Austria, Croatia, Denmark, Finland, Greece, Hungary, Spain, and, The Netherlands). This current situation results in many unknown species' distributions and poor taxonomic treatment in Europe, which ultimately ends in new geographical records and the description of new species (Wagner, 2000, 2004; Kvifte & Andersen, 2012; Kvifte et al., 2016; Kvifte, 2023; Jaume-Schinkel et al., 2023).

Early studies of European Psychodidae were conducted by a series of essays by Eaton from 1893-1904 (Svensson, 2009). Almost two decades later, between 1919-1922, Tonnoir continued to work on moth flies in Europe until his work was supplemented in 1940 (Tonnoir, 1940; Svensson, 2009). Feuerborn (1922a, 1922b) and Satchell (1947a, 1947b, 1948) made significant contributions to the studies of Psychodidae. During the 50s, Jung (1956) contributed significantly

to the European knowledge of the family (Svensson, 2009). In the 1980s Vaillant contributed with a very comprehensive review included in Lindner's *Die Fliegen der Palaearktischen Region* (1971–1983), summarizing all the previous knowledge and describing many new species (Svensson, 2009). In the last decades, the European work has improved thanks to the works of Krek (1999), Wagner (e.g., 1973, 1979, 1980, 1990, 1997, 2000), Withers (e.g., 1988, 1989), and Ježek (e.g., 1981, 1983, 1984b, 1990, 1998) (see Svensson, 2009). More recently, Psychodidae knowledge in Europe has continued to grow due to the work of a few specialists, e.g., Kvifte (e.g., 2019, 2023), Oboňa et al. (e.g., 2021, 2023), Salmela (e.g., 2005, 2008), Jaume-Schinkel et al. (e.g., 2022, 2023).

1.5 Introduction to Neotropical Psychodinae

Knowledge of the dipteran diversity in the Neotropical Region has gradually increased since the 18th century (Amorim, 2009). The history of Neotropical dipterology has been described by Papavero (1971, 1973) and summarized by Amorim (2009).

The proportion between the known species and the actual diversity in the Neotropical dipterans certainly differs according to each family (Amorim, 2009; Brown, 2005). Moreover, a number of dipteran families are absent in the Neotropics, and some families are endemic to the Neotropical Region (Amorim, 2009). There are more than 31,000 described species, it has been estimated that between 150,000 and 320,000 species could be present in the Neotropics (Amorim, 2009).

Five of the six recognized subfamilies are present in the American continent, namely Bruchomyiinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyinae (Wagner & Ibáñez-Bernal, 2009). Through the years, several taxonomical works have dealt with Psychodidae in the Neotropics, but moth fly taxonomic knowledge in the Neotropics remains uneven, strongly biased

towards the medically important Phlebotominae. When it comes to sand flies (Phlebotominae), 530 of the approximate 1,000 species described worldwide are known to occur in the American continent (Shimabukuro, 2016), and up to 2009, there were 274 species recorded only for Central America (Wagner & Ibáñez-Bernal, 2009). Regarding Psychodidae (not only Phlebotominae), a few countries have high numbers of studies and records, for instance Brazil with 540 species (Shimabukuro et al., 2020), Colombia with 199 species (Bejarano & Estrada, 2016), and Mexico with 99 species (Ibañez-Bernal & Duran-Luz, 2022). Many countries lack a systematic collection of specimens resulting in a low number of recorded species. This neglect towards other subfamilies (Bruchomyiinae, Psychodinae, Sycoracinae, and Trichomyiinae) derives in new geographical records and new species discovery (e.g. Jaume-Schinkel, 2022, 2023; Jaume-Schinkel & Kvifte, 2022; Jaume-Schinkel & Mengual, 2023)

Earlier publications are summarized in the *Catalog of Psychodidae for America South of the United States* (Duckhouse, 1973). Later, Young & Duncan (1994) included all Neotropical species known to that date in their catalog. The first general revision of Neotropical Psychodidae including keys, descriptions, and illustrations was done in the mid-1990s (Quate, 1996). Since then, recent works have described new species, new geographical records, or catalogues for specific countries, mostly summarized in Wagner & Ibañez-Bernal (2009). Due to their medical importance, the geographic distribution of American Phlebotomines has been thoroughly studied, especially in the last three decades (de Aguiar & Vieira, 2018). On the contrary, the Psychodinae fauna has not been studied so thoroughly, being not medically important and highly diverse, resulting in gaps in the general knowledge.

1.6 Introduction to Oriental Psychodidae

From the early years of taxonomy (1758 to 1908), very few species were described from the Oriental Region (Grootaert, 2009). There have been some peaks of taxonomic work being

conducted in the region (e.g., 1908–1940, 1965, 1988) (see Grootaert, 2009) and in recent years the knowledge has increased at a constant rate.

The proportion of the known Diptera species and the undescribed taxa is high, with 22,545 described species up to 2007, with 93 % of which (20,000) are endemic to the region (Grootaert, 2009). The currently known Diptera species is about half the number of species described from the Palearctic Region (22,000 in the Oriental; 45,000 in the Palearctic), but it has been estimated that about 81,000 species are expected to occur in this part of the world (Grootaert, 2009).

Currently, the six recognized subfamilies are recorded in the Oriental Region (Ježek 2010, Curler & Priyadarsanan 2015; Ježek et al., 2015; Kvifte & Andersen, 2016). Several taxonomical works have been published through the years dealing with Psychodidae in the region, with emphasis on the medically important Phlebotomines (e.g. Fairchild & Herting, 1952; Fairchild, 1952; Lewis, 1957; Quate & Fairchild, 1961). Duckhouse (1973) catalogued 227 species in the Oriental Region; later, Ipe et al. (1986) added 33 species, and both Duckhouse (1973) and Ipe et al. (1986) summarized the previously published works (see Ježek, 2010). According to Kvifte & Andersen (2016), 288 species (excluding Phlebotominae) have been recorded for the Oriental Region. Duckhouse & Lewis (1989) compared the numbers of the Oriental and the Australasian Regions known to date (256 and 426 species respectively) mentioning that some of the genera in the Australasian Region originated from the Oriental. Jaume-Schinkel & Kvifte (2022) recorded a genus, previously only known from the Neotropics, in the Oriental Region, pointing out that many more interesting records are awaiting discovery.

Some subfamilies, such as Bruchomyiinae were recently revised (Polseela et al., 2019) for the Oriental Region, and some like Phlebotominae are in constant work due to the medical importance of the group. Despite this, moth fly taxonomic knowledge in the region remains uneven, strongly biased towards the medically important Phlebotominae, and it is well known that

several new species remain undescribed and new records are likely to appear while working on the group (Duckhouse & Duckhouse, 2004; Curler 2009; Ježek 2010; Kvifte & Andersen, 2016).

Earlier publications are summarized in the regional catalogues of Duckhouse (1973), Duckhouse & Lewis (1989), and Bugledich (1999). Since then, recent works have described new species or new geographical records (e.g. Curler, 2009; Curler & Courtney, 2009; Ježek 2010; Kvifte & Andersen, 2016; Polseela et al., 2019). Phlebotomine fauna has been well studied in the Oriental Region, while the remaining subfamilies have not been studied so thoroughly, resulting in gaps in the general knowledge.

1.7 Introduction to Molecular Studies

DNA barcoding (Hebert et al., 2003) encompasses the idea of a unique DNA sequence for each species, and it is analogue to the barcodes used in retail products (Hebert et al., 2003). This approach to taxa recognition is becoming more and more common among researchers (Savolainen et al., 2015) as the costs for sequencing are decreasing over time. The advantages and limitations of DNA barcoding have been extensively debated, with some scientists considered it the end of taxonomical work, and others envisioned a bright future (Hebert & Gregory, 2005; Bucklin, et al., 2010). Basically, DNA barcoding is a system that relies on genomic variance and helps with the identification of species. In animals, DNA barcoding usually uses the 5'-end segment of the Cytochrome *c* oxidase gene subunit 1, also known as *COI*, *CO1,* or *Cox1*, a conservative protein-coding gene inside the mitochondrial genome of animals (Folmer et al., 1994; Hebert et al., 2003).

In today's context, the creation of a dependable DNA barcode for a specific taxon requires the collection and identification of individual specimens. This identification process demands a certain level of taxonomic expertise, which highlights the taxonomic impediment. Conversely, the molecular tools employed in this process are, to a certain extent, independent of taxonomic

knowledge. Nevertheless, it remains crucial to deposit the specimen in a collection as a voucher specimen and store the molecular data in a publicly accessible database. The culmination of this effort allows for the comparison of gathered information with both historical and contemporary data, ultimately contributing to the refinement and increased reliability of available information.

High throughput sequencing technologies (HTS) have brought about a revolution in molecular biology (Andermann et al., 2020) as they enable cost-effective and genome-scale data collection, facilitating the processing of large amounts of taxa (Andermann et al., 2020; Lemmon & Lemmon, 2013). While whole genome sequencing (WGS) generates vast datasets, it presents significant bioinformatic challenges, such as data storage and management (large volumes of data), computational complexity (physical computational resources (memory and processor) and computational skills (for analysis), and costs (high cost associated with WGS). To address these challenges, various genome subsampling methods have emerged, offering distinct advantages and disadvantages over WGS (Andermann et al., 2020). These subsampling HTS methods enable the sequencing of specific portions of the genome, reducing both the data volume and associated costs. Among these techniques, we can find sequence-capture methods, also known as target enrichment or targeted sequencing (Mamanova et al., 2010). Sequence-capture selectively enriches sequence libraries for specific regions of interest within a genome (Faircloth et al., 2012; Gnirke et al., 2009; Lemmon et al., 2012; Lemmon & Lemmon, 2013).

The approach of enriching orthologous single-copy protein-coding genes has been effectively applied to multiple plant families (Li et al., 2017) and various metazoan taxa, such as stony corals (Anthozoa: Scleractinia; Quek et al., 2020), sea spiders (Pycnogonida: Pantopoda; Dietz et al., 2019), isopods (Malacostraca: Isopoda; Stringer et al., 2021), wasps (Insecta: Hymenoptera; Bank et al., 2017; Klopfstein et al., 2019; Maletti et al., 2021; Mayer et al., 2016; Pauli et al., 2021), butterflies and moths (Insecta: Lepidoptera; Call et al., 2021; Mayer et al.,

2021), cockroaches (Insecta: Blattodea; Evangelista et al., 2021), and hover flies (Insecta: Diptera; Mengual et al., 2022).

Chapter 2 – Material and methods

Within the framework of this dissertation project, specimens of Psychodidae from different geographical regions have been examined. In this section, a general view of the methodologies utilized in the chapters and publications of the thesis is given. For specific methodologies of each publication mentioned in the chapters, see the section on material and methods on each publication.

2.1 Geographic scope

For the purposes of this dissertation, I follow the general biogeographic regions according to Morrone (2015, 2020) as seen in Figure 5. With specific definitions as described below.

2.1.1 The Palearctic Region

This region corresponds to the Arctic and temperate Eurasia, the Mediterranean, and Africa north of the Sahara, including the islands from the Arctic, the Sea of Japan, the eastern half of the North Atlantic, and Macaronesia. However, most of the Palearctic work was done in Europe, and for that I follow the proposed geographic boundaries of Europe by de Jong et al. (2014), using the following geographic limits: East: Ural (E 60°), West: Atlantic Ocean (W 30°), South: Mediterranean (N 35°), and North: Atlantic Islands (N 82°).

2.1.2 The Neotropical Region

Following Morrone (2015, 2020) this region corresponds to Central and South America, southern central Mexico, and the West Indies.

2.1.3 The Oriental Region

According to Morrone (2015, 2020) the Oriental region (also known as Indo-Malayan region) comprises the tropical areas of Eurasia and Southeast Asia, including India, the Himalayas, Myanmar, Malaysia, Indonesia, the Philippines, Micronesia, Polynesia, and Hawaii. However, it is worth mentioning that Indonesia, Philippines, Micronesia, Polynesia, and Hawaii have been assigned to either the Oriental or Australian Regions, being treated as separate regions or subregions (Udvardy, 1975; Holt et al., 2013). The actual limits of the geographic regions are beyond the scope of this dissertation, and I mostly follow the limits proposed by Morrone (2015, 2020) with the exception of not including the Philippines, Micronesia, Polynesia and Hawaii in the Oriental Region (see Figure 5)

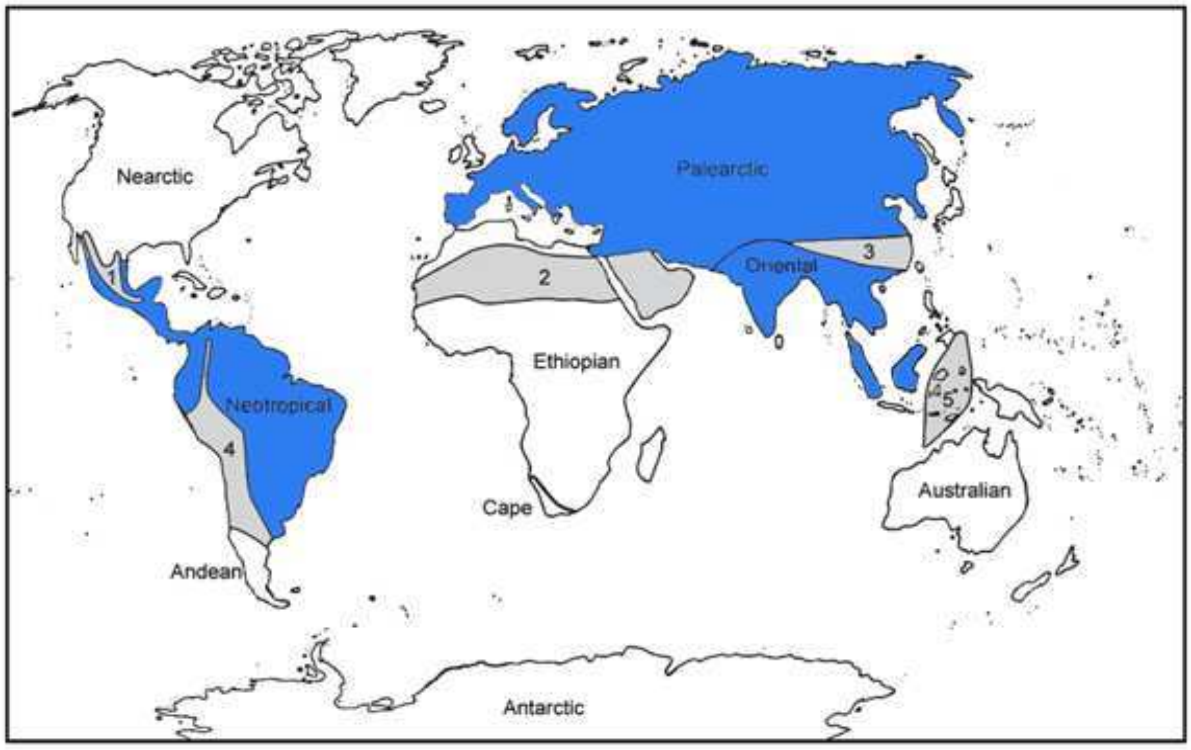

Figure 5. World Biogeographical regions with transition zones. Transition zones: 1. Mexican, 2. Saharo-Arabian, 3. Chinese, 4. South American, 5. Indo-Malayan. Transition zones are highlighted in grey. The focus regions of this dissertation are highlighted in blue. (Modified from Morrone (2015)).

2.2 DNA barcoding

In the framework of this dissertation, most DNA barcodes were obtained using a non-destructive methodology from complete specimens, performed in the facilities of Leibniz Institute for the Analysis of Biodiversity Change, Museum Koenig Bonn. We amplified the 5'-end of the cytochrome c oxidase subunit I (COI) gene using the primers HCO2198-JJ (forward) and

LCO1490-JJ (reverse) (Astrin & Stüben, 2008), with the help of a Qiagen (Hilden, Germany) BioSprint 96 magnetic bead extractor, and the corresponding kits were used following the manufacturers' specifications. PCR was carried out using a TouchDown PCR (TD-PCR) protocol as proposed by Korbie & Mattick (2008) using a QIAGEN Multiplex PCR Kit. Later the PCR products were shipped to Beijing Genomic Institute (BGI) (China, Hong Kong) for bidirectional sequencing. For a more detailed methodology refer to the Matherial and methods of Chapter 18 in Appendix 16 (also see Jafari et al., 2023).

2.3 DNA barcoding sequence analysis

DNA sequences were assembled, aligned, and edited using Geneious Prime ver. 2022.1.1 (Biomatters, Auckland, New Zealand). The total sequence length was set to 658 bp. Sequences were uploaded to BOLD (www.boldsystems.org) and to the German Barcode of Life website (https://data.bolgermany.de/ergebnisse/results). Furthermore, some of the sequences used during the publications were submitted to GenBank (see publications for GenBank accession numbers). If not stated otherwise in the publications, default parameters were used during alignments and gene trees.

2.4 Exon-capture

In contrast with the methodology for DNA barcoding, specimens were dissected, separating the genitalia, head, and wings from the thorax and abdomen. The latter were stored in ethanol for subsequent DNA extraction using Qiagen Blood & Tissue Kits, following the manufacturer's protocol. Detailed procedures can be found in Chapter 19 of Appendix 17.

Library preparation involved the use of the Agilent SureSelect XT HS2 Library Preparation Kit for ILM, followed by purification of PCR reaction products using Agencourt AMPure XP

beads. Libraries were quantified using the Quantus Fluorometer. Enrichment was performed as per the protocol by Mayer et al. (2021). Final extracted products were sequenced at Macrogen Europe using a NovaSeq 6000 System, producing 150 bp paired-end sequencing data with an estimated output of 15 Gbp per library pool. Detailed methodology is available in Appendix 17.

2.5 Exon-capture sequence analysis

The sequence analysis methodology involved a comprehensive workflow. Initially, we used fastp (Chen et al., 2018) for precise sequence trimming, followed by SPAdes (Prjibelski et al., 2020) for sequence assembly. Post-assembly, we used the tool Ortograph for pinpointing the coding sequences (CDS) of interest. Our analytical pipeline, designed in a Snakemake workflow following Mayer et al. (2021), encompassed multiple steps (eg. sequence trimming, assembly, identification of orthologous loci, alignment, and stringent data filtering). To unravel evolutionary relationships, we conducted phylogenetic analyses using IQ-TREE 1.6.3 (Nguyen et al., 2015). For a more comprehensive understanding, a detailed methodology is available in Appendix 17 (see also the approach outlined in Mayer et al. (2021).

Chapter 3 – *Alepia viatrix* sp. nov. (Diptera: Psychodidae), a new species of a Neotropical genus found on the Azores Archipelago (Portugal).

3.1 Bibliography of the published article

Jaume-Schinkel S, Kvifte GM, van der Weele R, Mengual X (2022) *Alepia viatrix* sp. nov. (Diptera: Psychodidae), a new species of a Neotropical genus found on the Azores Archipelago (Portugal). *Zootaxa,* 5128 (3), 384–396. https://doi.org/10.11646/ZOOTAXA.5128.3.4

3.2 Summary

The Azores Archipelago, located in the northern Atlantic Ocean, bears witness to a history marked by ecological transformation, primarily catalyzed by human activities (Santos et al., 2003; Arteaga et al., 2020). These anthropogenic interventions have encompassed deforestation and the introduction of exotic species, profoundly altering the native flora and fauna of this unique island ecosystem (Arteaga et al., 2020). Within the fauna found on the islands, several studies assessing the arthropod fauna have shown that there are more than 2,300 species, of which 42 % are introduced (Arteaga et al., 2020). Regarding the moth flies (Diptera: Psychodidae), prior to this publication, the archipelago yielded only six reported species, all of which exhibit broad distributions across Europe and none that can claim endemism to the Azores. However, the present publication resulted in the identification of a previously unknown species of Psychodidae of the Neotropical genus *Alepia* Enderlein, 1937. This revelation represents a significant milestone, marking not only the initial record of the genus *Alepia* within the Azores Archipelago but also the second known occurrence within the Palaearctic Region.

The study draws its foundation from the meticulous sampling efforts within two prominent botanical gardens, one situated on the island of Faial and the other one on the island of Terceira.

The examined material was collected over a span of six months, employing passive flight interception traps. After examination, the specimens were used for DNA extraction and DNA Barcoding. After DNA extraction some dissection procedures and permanent slide mounting for morphological analysis were conducted.

Within the context of this research, we described *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 sp. nov., a new species closely related to *Alepia vaga* Wagner & Svensson, 2006. *Alepia viatrix* can be easily differentiated through a suite of morphological features, most notably the configuration of the gonostyli, epandrium, and tunica. Moreover, through this publication, we made available the first DNA barcodes of the genus *Alepia*, and we matched males and females through barcodes, thus, describing both sexes. The description was further enriched by an array of detailed images, both of male and female specimens.

Additionally, we provided an identification key for the adult male Psychodidae species present within the Azores, accompanied by informative comments on each of these species inhabiting the region.

The revelation of a new *Alepia* species within the Azores Archipelago is an enigma that beckons inquiry into its origin. It is our hypothesis that human-mediated activities, potentially facilitated through the importation of ornamental plants like bromeliads from the Neotropics, have acted as the conduit for the dispersion of this Neotropical genus to the Azores. This hypothesis underscores the intricate interplay between human actions and the dynamics of species distribution within island ecosystems.

Furthermore, this study serves as a compelling testament to the vital role of integrative taxonomy, where the fusion of morphological and genetic data unfurls the intricate tapestry of

insect species diversity. The discovery of *Alepia viatrix* sp. nov. within the Azores Archipelago underscores the fluid nature of species distribution patterns and accentuates the imperative for sustained research endeavors aimed at unraveling the ecological intricacies and taxonomic dimensions of island ecosystems. As we delve deeper into the intricate mysteries of these ecosystems, we continue to unveil nature's secrets, underscoring the necessity of preserving these island paradises for generations to come.

The published article is the result of a collaboration of Santiago Jaume Schinkel with Gunnar M. Kvifte (Bodo University, Norway), Ruud van der Weele (Independent researcher, The Netherlands), and Ximo Mengual (ZFMK/LIB). All authors were involved in the interpretation of the data, the first draft and figure plates were prepared by SJS and subsequently edited by all authors.

Chapter 4 – What's inside the hole? A review of European dendrolimnetic moth flies (Diptera: Psychodidae: Psychodinae).

4.1 Bibliography of the published article

Jaume-Schinkel S, Morelli A, Kvifte GM, Mengual X (2022) What's inside the hole? A review of European dendrolimnetic moth flies (Diptera: Psychodidae: Psychodinae). *Diversity*, 14, 532. https://doi.org/10.3390/d14070532

4.2 Summary

Our research is the result of an exhaustive exploration of the existing literature, focusing on the records of Psychodinae species that develop inside water-filled tree holes (dendrotelmata). Besides the literature review, we revised freshly collected samples concentrating our efforts on the European species of Psychodinae associated with dendrotelmata. Following an in-depth analysis of more than a hundred publications, we identified 11 specific records pertaining to dendrolimnetic Psychodinae.

We have found six dendrolimnetic genera (*Clogmia* Enderlein, 1937*; Clytocerus* Eaton, 1904*; Lepiseodina* Enderlein, 1937*; Pneumia* Enderlein, 1935; *Psychoda* Latreille, 1797; and *Telmatoscopus* Eaton, 1904) and 13 species: *Clogmia albipunctata* (Williston, 1893), *Clytocerus xylophilus* Vaillant, 1983, *Lepiseodina tristis* (Meigen, 1830), *L. rothschildi* (Eaton, 1912), *L. latipennis* (Sarà, 1953), *Pneumonia canescens* (Meigen, 1804), *P. trivialis* (Eaton, 1893), *Psychoda alternata* Say, 1824, *P. minuta* Banks, 1894, *Telmatoscopus advena* (Eaton, 1893), *T. thuringicus* Beran, Doczkal, Pfister & Wagner, 2010, and *T. laurencei* (Freeman, 1953); all intricately intertwined with 13 species of trees.

A notable highlight of our research is the discovery and subsequent documentation of two species, *Lepiseodina latipennis* and *Telmatoscopus bartai* (Ježek, 2004) in Germany for the first time. This noteworthy addition to the geographical distribution of these species adds a new dimension to our understanding of their range and habitat preferences. Furthermore, we have undertaken a meticulous re-description of *L. latipennis*, leveraging freshly collected material and conducting a thorough examination of the holotype. This detailed examination contributes significantly to our knowledge of the species, enhancing our capacity for accurate identification and taxonomic classification.

The wealth of data generated through our research has spurred a comprehensive review of the genera *Lepiseodina* and *Telmatoscopus*. Derived from this revision, we provide an identification key specifically for the males of both of these genera. This invaluable resource will undoubtedly aid fellow researchers and enthusiasts in their quest to accurately identify and classify these intriguing species. Additionally, our study involves the synonymization of *Krivosheinoscopus* Ježek, 2001 under *Telmatoscopus*, leading to new combinations for the species *Telmatoscopus ussuricus* (Ježek, 2001) comb. nov. and *Telmatoscopus bartai* (Ježek, 2004) comb. nov. Furthermore, we have established a synonymy and placed *Tematoscopus wagneri* (Salmanna, 1982) comb. et syn. nov. under *Telmatoscopus advena* (Eaton, 1893), further enriching the taxonomic knowledge of this genus.

In addition to our taxonomic contributions, we have taken a deeper dive into the life cycle of these species. For the first time, we have described the female and eggs of *Telmatoscopus advena*, shedding light on the reproductive processes and morphological features of this species. Our comprehensive efforts extend to the realm of molecular biology, with the provision of the first published DNA barcodes (COI) for *Telmatoscopus bartai, Lepiseodina latipennis*, *Lepiseodina rothschildi*, and *Lepiseodina tristis*. This genetic marker enhances our capacity for species identification within this taxonomic group.

Ultimately, our research transcends the mere cataloging of species and delves into the intricacies of their taxonomy and ecology. By elucidating the interactions and adaptations of European dendrolimnetic species of the subfamily Psychodinae, we contribute to a richer understanding of the complex web of life within these unique ecosystems. Our findings open doors to further inquiries and inspire future investigations into the ecological dynamics and evolutionary history of these captivating insects, underscoring the enduring allure of entomology and the unending mysteries of the natural world.

The published article is the result of a collaboration of Santiago Jaume Schinkel with Alessio Morelli (Independant researcher, Pianella, Italy), Gunnar M. Kvifte (Bodo University, Norway), and Ximo Mengual (ZFMK/LIB). All authors were involved in the interpretation of the data, the first draft and figure plates were prepared by SJS and subsequently edited by all authors.

Chapter 5 – Revisionary notes on *Feuerborniella* Vaillant, 1971, with the first record of the genus from the Afrotropical Region (Diptera, Psychodidae).

5.1 Bibliography of the published article

Kvifte GM, Jaume-Schinkel S (2023) Revisionary notes on *Feuerborniella* Vaillant, 1971, with the first record of the genus from the Afrotropical region (Diptera, Psychodidae). *Deutsche Entomologische Zeitschrift*, 70 (1), 121–127. https://doi.org/10.3897/dez.70.97465

5.2 Summary

The genus *Feuerborniella*, initially erected by Vaillant (1971), has the European species *F. obscura* (Tonnoir, 1919) as the type species. In 1974 Vaillant (1974) expanded his earlier genus concept by recognizing four distinct species distributed across the Neotropical, Palearctic, and Oriental Regions.

Ježek (1985) transferred the Oriental species *Psychoda nigripennis* Brunetti, 1908 to the *Feuerborniella* genus. This taxonomic decision created doubts as it lacked morphological support. This taxonomic decision has not found widespread acceptance in subsequent scientific investigations.

The story of *Feuerborniella* taxonomic journey continues with Ibáñez-Bernal's work (2004). In this significant contribution, he revisited Vaillant's (1974) species list and added an additional Neotropical species; increasing, thus, the global tally to five distinct species. The expansion of the known distribution was a notable milestone in *Feuerborniella* taxonomic history.

Further developments emerged in 2014 when Cordeiro et al. (2014) recognized six species within the genus, presenting their findings alongside a detailed description of *Feuerborniella paramuna* Cordeiro, 2014. However, the taxonomic landscape became more intricate when Cordeiro and colleagues published another paper in 2015 (Cordeiro et al., 2015), proposing numerous combinations and changes. We discuss the consistency of these changes with the defining characteristics of the genus and discuss their validity on a case-by-case.

In the present research paper, we embrace the diagnostic criteria established by Ježek (1985) and Ibáñez-Bernal (2004), offering a comprehensive analysis of the genus. To provide a deeper insight into *Feuerborniella* taxonomy, we provide a supplementary re-description of the type species, *Feuerborniella obscura*, originally found in Europe. This re-description aims to offer a more nuanced understanding of this crucial species, shedding light on its morphological intricacies and the generic diagnostic characters.

Additionally, our research unveils a new species, *Feuerborniella sinefurcata* sp. nov., discovered in Tanzania. This exciting discovery adds a fresh dimension to the genus's distribution and showcases the ongoing exploration of its biodiversity. Furthermore, we expand the scope of *Feuerborniella* by transferring several related species into the genus, including *Psychoda morogorica* Wagner & Andersen, 2007, *Philosepedon ensiger* Quate, 1996, and *Philosepedon longistylus* Quate, 1996.

Notably, our paper marks a significant milestone by reporting the genus *Feuerborniella* in the Afrotropical Region for the first time. The expansion of the known range for this genus makes necessary new adjustments to existing identification keys.

The published article is the result of a collaboration of Gunnar M. Kvifte (Bodo University, Norway) with Santiago Jaume Schinkel. All authors were involved in the interpretation of the data, the first draft and figure plates were prepared by SJS and GMK, subsequently edited by all authors.

Chapter 6 – The Hitchhiker's Guide to Australia: The 18,000-km-long journey of *Alepia viatrix* (Diptera: Psychodidae) discovered through citizen science.

6.1 Bibliography of the published article

Jaume-Schinkel S, Mengual X, Howe A, Fagan-Jeffries EP (2023) The Hitchhiker's Guide to Australia: The 18,000-km-long journey of *Alepia viatrix* (Diptera: Psychodidae) discovered through citizen science, *Check List,* 19 (4), 589–597. https://doi.org/10.15560/19.4.589

6.2 Summary

Following the detailed description of *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 and the integration of its DNA barcode into BOLD (boldsystems.org), an intriguing revelation emerged through a citizen science initiative. Insect Investigators, an Australian citizen-science project engaging young researchers from various schools across South Australia, Western Australia, and Queensland, was initiated to collect different invertebrate specimens. The primary objective was to generate DNA barcodes for multiple insect species and ultimately inspire and educate Australia's emerging scientists.

One of the Malaise traps, placed at The Yeronga State School, unexpectedly collected specimens belonging to the *Alepia* Enderlein, 1937 genus. This discovery was surprising considering the predominant presence of *Alepia* species in the Neotropical Region, except for three species, i.e. *Alepia symmetrica* Wagner & Hibrar, 2004 found in Florida, USA and reared from a bromeliad plant; *Alepia vaga* Wagner & Svensson, 2006 described from specimens reared from a bromeliad in Sweden; and *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 described from specimens collected in the Azores Archipelago, Portugal.

In this comprehensive study, we unveiled, for the first time, the presence of the *Alepia* genus in Australia, specifically the introduced species *A. viatrix*. Additionally, we provided the first description of the species's eggs and those of the genus, aided by a scanning electron microscope. Our discussion elaborated on how the species-specific exochorionic structures in the eggs can contribute to the precise delineation of species within the Psychodidae family.

Furthermore, we put forth a hypothesis regarding the possible introduction of this species to Australia. Moreover, we delved into the effectiveness of citizen-science projects in the detection and monitoring of introduced species. We emphasized their crucial role in enhancing research knowledge by providing novel distributional records for both native and non-native species.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Ximo Mengual (ZFMK/LIB), Andy Howe (University of the Sunshine Coast, Australia), and Erin P. Fragan-Jeffries (University of the Sunshine Coast, Australia). All authors significantly contributed to data interpretation.The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 7 – New records of Psychodidae for the Dutch Fauna.

7.1 Bibliography of the published article

Jaume-Schinkel S, Kvifte GM, Njunjić I, Schilthuizen M (2023) New records of moth flies (Diptera, Psychodidae) for the Dutch Fauna. *Biodiversity Data Journal*, 11, e108636. https://doi.org/10.3897/BDJ.11.e108636

7.2 Summary

Moth flies are widely distributed globally and circa 3,400 documented species are known (Pape et al., 2011; Curler et al., 2019). This family exhibits remarkable diversity, especially in tropical regions. In Europe alone, there are over 500 documented species (Wagner, 2013), although gaps in knowledge persist regarding the known distribution of these species in Europe. The current taxonomic knowledge remains limited and recent regional surveys consistently reveal previously undocumented distributional records (Kvifte, 2019; Jaume-Schinkel et al., 2022; Morelli & Biscaccianti, 2022).

Regarding The Netherlands, the initial checklist of Psychodidae for the country was published in the early 1930s by Barendrecht (1934), listing 34 species. Later, Wagner & Beuk (2002) published an updated checklist featuring 48 species. In recent years, several new species have been added to the Dutch fauna bringing the total number of species to 61.

In the course of a citizen science entomological survey conducted in The Netherlands (specifically Amsterdam), a Malaise trap was set in an ecologically managed area of one of the city's oldest parks, Vondelpark (Achterberg et al., 2020; Schilthuizen et al., 2021). From this

survey, we reported, for the first time in The Netherlands, two species of moth flies, namely, *Psychoda uniformata* Haseman, 1907 and *Panimerus maynei* (Tonnoir, 1919).

In addition, employing an integrative taxonomical approach, we successfully matched males and females based on DNA barcoding. We also provided redescriptions of the females of the species *Panimerus notabilis* (Eaton, 1893) and *Panimerus goetghebueri* (Tonnoir, 1919). These redescriptions included a discussion on the morphological characters necessary to distinguish the known females of the genus *Panimerus*. Lastly, we have made the COI Barcodes (the sequence of the 5'-end of the cytochrome c oxidase subunit I (COI) gene) available for the first time for the species *Panimerus notabilis, P. goetghebueri*, and *P. maynei*.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway), Iva Njunjić (Leiden, Netherlands), and Menno Schilthuizen (Leiden, Netherlands). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 8 – Key to species of the European genus *Periulomyia* Krek, 1999 stat. nov. (Diptera: Psychodidae), with the description of *Periulomyia marijae* sp. nov.

8.1 Bibliography of published article

Jaume-Schinkel S, Kvifte GM (in prep.) Key to species of the European genus *Periulomyia* Krek, 1999 stat. nov. (Diptera: Psychodidae), with the description of *Periulomyia marijae* sp.nov.

8.2 Summary

In this study, our focus centered on re-evaluating the taxonomic status of the *Periulomyia* genus. Initially it was described as a subgenus of *Ulomyia* Walker, 1856 by Krek (1999), and it is equivalent to the *Ulomyia cognata* and *Ulomyia hirta* species groups proposed by Vaillant (1983). Our investigation involved a comprehensive analysis of morphological features in both adult and larval stages. The results of our examination provide compelling evidence supporting the elevation of Periulomyia to the status of a distinct full genus, separate from Ulomyia.

We transferred species to *Periulomyia* creating the following new combinations: *Periulomyia cognata* (Eaton, 1893) comb. nov., *Periulomyia hirta* (Szabò, 1960) comb. nov., *Periulomyia ophicornis* (Vaillant, 1983) comb. nov., *Periulomyia meridionalis* (Vaillant, 1983) comb. nov., *Periulomyia montium* (Vaillant, 1983) comb. nov., *Periulomyia rostrata* (Vaillant, 1983) comb. nov., *Periulomyia szaboi* (Vaillant, 1983) comb. nov., and *Periulomyia vaseki* (Ježek, 2002) comb. nov. The current total number of *Periulomyia* species is nine.

Additionally, for each of the species now in *Periulomyia* we provided the taxonomic history, a brief diagnosis including the most important morphological characters, and the currently known distribution.

Furthermore, we compiled a comprehensive catalog encompassing all known species of *Periulomyia* across the globe. Additionally, we provided an identification key for discerning the males of the known species within this newly elevated genus. Our contribution also included a thorough redescription of the type species of the genus, using freshly collected material from Germany. In addition to this, we introduced a new species, *Periulomyia marijae* Jaume Schinkel & Kvifte sp. nov., based on specimens collected in Croatia, further enriching the understanding of the genus and its diversity.

The unpublished manuscript is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway), and Rudiger Wagner (retired, Germany). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS and GMK, subsequently refined and edited by all authors.

Chapter 9 – A key to the moth flies (Diptera: Psychodidae) of Europe, including subfamilies and genera.

9.1 Bibliography of published article

Jaume-Schinkel S, Kvifte GM, Mengual X (in prep.) A key to the moth flies (Diptera: Psychodidae) of Europe, including subfamilies and genera.

9.2 Summary

The Psychodidae family encompasses nearly 3,400 described species distributed worldwide, with two introduced species in the Antarctic Region (Galati & Rodrigues, 2023). The family classification has seen various proposals over time, and its subdivision remains a topic of debate (Curler & Courtney, 2009). However, there is a general recognition of six subfamilies: Bruchomyiinae, Horaiellinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyiinae. The monophyly of the family is still under debate (Wiegemann et al., 2011). Some authors propose Phlebotominae as a separate family (Azar et al., 1999). However, this would need the elevation f all subfamilies to family status, and since the phylogenetic relationships between subfamilies remain unresolved, this proposal lacks broad acceptance (Curler & Moulton, 2012; Kvifte & Wagner, 2017).

Several studies aim to clarify the suprageneric classification of Psychodidae, particularly within the subfamily Psychodinae (e.g., Vaillant, 1971; Ježek, 1983; Duckhouse, 1987; Rispail & Leger, 1998; Kvifte, 2018). Recent studies using nuclear and mitochondrial DNA sequence data (e.g., Curler & Moulton, 2012; Espindolá et al., 2012; Kvifte, 2018) reveal that the tribal classification proposed by Vaillant (1971) and Ježek (1984) comprises non-monophyletic groups.

To date, systematic classification systems based on various character datasets do not align with the subdivision of the family (Kvifte, 2018).

The most comprehensive identification keys for the family in the Palearctic Region were published over 50 years ago and numerous nomenclatural changes have been published since then. Thus, the need arises for a new key with updated nomenclature. In this manuscript, we comprehensively discuss all the subfamilies present in Europe, offering an identification key with illustrations to distinguish each subfamily. Additionally, we introduce each subfamily with a concise summary of historical classification and known biology of larvae and adults, along with comments on the known number of genera and species. For subfamilies with multiple genera, we provide a key for genus identification. Each couplet in the identification key is illustrated, highlighting the characters necessary to follow the couplet.

This work remains ongoing, nearing completion. Our current focus involves providing an updated distribution for all species in Europe, necessitating a thorough literature review from the early 1800s to the present, to catalog all known species in Europe and the countries where they are documented to be present.

Chapter 10 – *Platyplastinx ibanezbernali* sp. nov., a new species of moth fly (Diptera: Psychodidae) from Ecuador.

10.1 Bibliography of the published article

Jaume-Schinkel S, Kvite GM (2022) *Platyplastinx ibanezbernali* sp. nov., a new species of moth fly (Diptera: Psychodidae) from Ecuador. *Acta Entomologica Musei Nationalis Pragae*, 62 (2), 383–389. https://doi.org/10.37520/aemnp.2022.020

10.2 Summary

This publication, described a new species of *Platyplastinx* Enderlein, 1937. The newly described species, *Platyplastinx ibanezbernali* sp. nov., was based on morphological characters and DNA barcodes obtained from male and female specimens collected in Ecuador. Our sequences represented the first DNA barcodes made available for the genus *Platyplastinx.* The study provided the first brief description of an egg of the genus and included this species in the key to world species of the genus, along with a key to adult males of *Platyplastinx* from Ecuador.

The original description of the genus *Platyplastinx* was based on a single broken female specimen, and the genus has been redefined over time, with key insights provided by subsequent researchers. The genus is primarily found in the Neotropical Region, with a few species extending into the Nearctic Region. This paper presents the description of a new *Platyplastinx* species from Ecuador, contributing to a total of 14 known species worldwide and expanding the knowledge base on the genus.

The study area was in the Pichincha Province of Ecuador. Specimens were collected using a Malaise trap and prepared for analysis. Detailed methods regarding specimen preparation,

terminology, measurements, and the taxonomic accounts of *Platyplastinx* are outlined. We provide a table displaying the known distribution of *Platyplastinx* species.

This publication provided a detailed taxonomic account of the species of *Platyplastinx*, and it discussed the genetic information and the differential diagnosis of the new species in comparison to other related species. The discussion emphasized the importance of biological inventories for discovering new species and highlights the significance of understanding eggshell structures for species identification within the Psychodidae family. The publication discussed the description of the egg structure of *Platyplastinx ibanezbernali* sp. nov., setting the stage for future studies.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 11 – Description of *Tonnoira conistylus* sp. nov. from Costa Rica and a new record of *Tonnoira distincta* Bravo et al. 2008 from Ecuador.

11.1 Bibliography of the published article

Jaume-Schinkel S (2022) Description of *Tonnoira conistylus* sp. nov. from Costa Rica and a new record of *Tonnoira distincta* Bravo et al. 2008 from Ecuador. *Studies on Neotropical Fauna and Environment*, 1–8. https://doi.org/10.1080/01650521.2022.2081466

11.2 Summary

This publication delved into the intricate taxonomy and distribution of the Neotropical genus of moth flies, *Tonnoira* Enderlein, 1937. The genus, initially described in 1937, encompasses a fascinating array of 26 extant species and one fossil species, distributed across ten countries within the Neotropical region. Brazil presents the highest number of species of *Tonnoira*, followed by countries like Suriname, French Guiana, Costa Rica, Panama, Venezuela, Bolivia, Ecuador, Nicaragua, and Peru.

This study resulted in the description of a new *Tonnoira* species, an intriguing discovery based on meticulous examination of a male specimen collected in Costa Rica way back in 1921. Additionally, the research unearthed a new geographic record for *Tonnoira distincta* Bravo, Alves & Chagas, 2008, this time from Ecuador. To facilitate a comprehensive understanding of the genus, the study furnished an exhaustive checklist and an updated distributional map encompassing all known species.

A significant highlight of this research is the description and characterization of *Tonnoira conistylus* sp. nov., a hitherto undiscovered species defined by distinct morphological traits and a

specific geographical range. Furthermore, the study casts a spotlight on the intriguing habitat preference of *Tonnoira* species, notably their occurrence in cave environments. This observation opens a window into the ecological dynamics and interactions of these flies within cave ecosystems, underscoring the need for further investigations. The findings pave the way for prospective studies focused on unraveling the behaviors and associations of the Psychodidae subfamily in their natural habitats, presenting a rich avenue for future exploration and discovery.

This publication was derived from a visit to the Natural History Museum in London, UK, while doing a revision of different type material of different genera.

Chapter 12 – Description of *Tonnoira chuki* sp. n. (Diptera: Psychodidae) from Ecuador with an updated taxonomical key for the genus *Tonnoira* Enderlein, 1937.

12.1 Bibliography of the published article

Jaume-Schinkel S (2023) Description of *Tonnoira chuki* sp. n. (Diptera: Psychodidae) from Ecuador with an updated taxonomical key for the genus *Tonnoira* Enderlein, 1937. *Integrative Systematics*, 6 (1), 51–58. https://doi.org/10.18476/2023.462484

12.2 Summary

This publication focused on the Neotropical genus *Tonnoira* Enderlein, 1937, and it is linked to the publication in Chapter 11. *Tonnoira* species are found across ten countries in the Neotropical Region, spanning from Nicaragua to Brazil. The genus was first described in 1937 based on a single female specimen, and to date, the male sex of the type species remains unknown. Since its initial description, 28 extant species have been identified including the herein new published species.

The study presented a new species of *Tonnoira* based on male specimens collected in Ecuador. The type locality for the new species is Cantón Pedro Vicente Maldonado in Ecuador, characterized by a tropical climate and diverse vegetation.

The examination involved DNA extraction and sequencing, shedding light on the genetic characteristics of the newly described species. The present work provided the first DNA barcodes for this genus. The publication also includes a taxonomic account of the genus, including important references, key diagnostic features, and a discussion on the distribution and biology of *Tonnoira*

species. I emphasized the need for further taxonomic surveys in the Neotropical Region to uncover additional species and enrich our understanding of this diverse genus.

Moreover, in this publication, I provide a new and updated identification key for the known males or *Tonnoira* species present worldwide.

Chapter 13 – A revision of the genus *Armillipora* Quate (Diptera: Psychodidae) with the description of two new species.

13.1 Bibliography of the published article

Jaume-Schinkel S, Mengual X (2024) A revision of the genus *Armillipora* Quate (Diptera: Psychodidae) with the description of two new species. *European Journal of Taxonomy,* 925(1), 161–178. https://doi.org/10.5852/ejt.2024.925.2459.

13.2 Summary

This comprehensive study marked a significant milestone by documenting the genus *Armillipora* Quate, 1996 for the first time in Ecuador, with the new geographical record of *Armillipora selvica* Quate, 1996, thus expanding its known geographic range. Notably, it offers critical insights into the distribution of this species. Additionally, the study unveiled two new species, namely *Armillipora muyu* sp. nov. and *Armillipora imitata* sp. nov., effectively doubling the existing number of species within the genus.

The taxonomic contributions of this study are substantial, providing meticulous descriptions of the newly discovered species and offering insights derived from male and female specimens. These descriptions serve to enrich our understanding of the genus, its morphological attributes, and its diversity in the Neotropical Region.

In a pioneering move toward integrative taxonomy, using molecular tools, this research introduced the first-ever DNA barcodes for the genus *Armillipora*. Specifically, the study presented the sequence of the 5'-end of the Cytochrome c Oxidase subunit I (COI) gene for *A. imitata* sp. nov., *A. muyu* sp. nov., and *A. selvica*. This molecular dataset is poised to become a

crucial resource for future taxonomic investigations, facilitating precise identification within the genus.

Furthermore, the study highlighted the presence of the second known and described female of the genus, an important contribution that augmented our knowledge of the morphological characteristics and sexual dimorphism within *Armillipora*.

To aid in species identification, the study offered a new taxonomic key for the known males of *Armillipora* worldwide. Lastly, the research delved into ecological aspects by constructing a Species Distribution Model that shed light on the potential distribution of the genus within the Neotropical Region. This predictive modeling serves as a fundamental resource for conservation efforts and understanding the ecological preferences and potential habitats of *Armillipora* species.

In conclusion, this study significantly advanced our understanding of the genus *Armillipora* by expanding its known distribution and species diversity. The integration of molecular and morphological data, accompanied by ecological insights, broadens the scope of knowledge within Psychodidae systematics and sets a foundation for future research and conservation endeavors within this taxonomic group.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, and Ximo Mengual (ZFMK/LIB). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 14 – Revision of the genus *Bryopharsos* Quate, 1996 (Diptera: Psychodidae) with the description of nine new species.

14.1 Bibliography of the published article

Jaume-Schinkel S, Kilian I, Pazmiño-Palomino A, Mengual X (in review) Revision of the genus *Bryopharsos* Quate, 1996 (Diptera: Psychodidae) with the description of nine new species. *European Journal of Taxonomy*.

14.2 Summary

In this study we present a groundbreaking discovery of the genus *Bryopharsos* in Colombia, marking the first documented occurrence in this country. We provide novel geographical records for *B. amazonensis* Bravo & Araújo, 2019, shedding light on its distribution. Additionally, we describe three new species from this country: *B. curvum* sp. nov., *B. tetracanthus* sp. nov., and *B. gorgona* sp. nov., further enriching our understanding of the genus. Venturing into Ecuador, we unveil new geographical records for *B. amazonensis, B. clavigum* Quate, 1996, *B. claviformosum* Quate, 1996, and *B. palpiculum* Quate, 1996. Moreover, we described two new species from Ecuador, namely *B. asymmetricum* sp. nov. and *B. septenacula* sp. nov., hailing from this country.

Further expanding our studies to Peru, we describe two new species, namely, *B. bitenacula* sp. nov. and *B. chuspi* sp. nov., contributing to the comprehensive taxonomic characterization of *Bryopharsos*. Our exploration extends to Venezuela, from where we describe *B. bifidum* sp. nov., another new addition to the genus. Continuing into Costa Rica, we uncover and describe *B. insperatus* sp. nov., significantly expanding the species diversity of *Bryopharsos*. Furthermore, we

offer crucial insights into the reproductive biology of this genus by presenting the first-ever description of an egg and a thorough redescription of the female of *B. palpiculum*.

Regarding molecular taxonomy, we make a pioneering contribution by providing the inaugural DNA barcodes for the genus *Bryopharsos*. Specifically, we present a sequence of the 5'-end of the Cytochrome c Oxidase subunit I (COI) gene for *Bryopharsos asymmetricum* sp. nov., *B. amazonensis, B. clavigum, B. claviformosum, B. palpiculum,* and *B. septenacula* sp. nov. This molecular data is a critical resource for future research, enabling accurate identification and within the genus helping to overcome the taxonomic impediment.

Lastly, we enhance the practical utility of our findings by updating the identification key for the global species of the genus *Bryopharsos*. This refined key will facilitate accurate identification and classification of Bryopharsos species worldwide. Additionally, we engage in a detailed discussion regarding the potential distribution of the genus in the Neotropical Region, leveraging distribution models to illuminate the ecological landscape and habitat preferences of *Bryopharsos*. This study not only expands our taxonomic knowledge but also lays the foundation for further exploration and conservation efforts concerning this intriguing genus.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Isabel Kilian (ZFMK/LIB), Alejandro Pazmiño-Palomino (INABIO, Ecuador) and Ximo Mengual (ZFMK/LIB). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 15 – Revision of the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae) with the description of three new species from the Neotropical Region.

15.1 Bibliography of the published article

Jaume-Schinkel S, Kvifte GM (2024) Revision of the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae) with the description of three new species from the Neotropical Region. *Journal of European Taxonomy,* 935(1), 81–100. https://doi.org/10.5852/ejt.2024.935.2547

15.2 Summary

In this comprehensive study, we conduct an in-depth review of the diagnostic characters defining the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae). This genus is prevalent in the Neotropical Region, it was initially documented in Costa Rica, Nicaragua, and Panama, this genus has now expanded its range, with the inclusion of one species from Costa Rica and two from Ecuador, elevating the total known species count to six. The study meticulously outlines these new species, offering intricate descriptions derived from male and female specimens, thereby enriching our taxonomic understanding of the *Eugenys* species.

A significant milestone is achieved through the provision of the first-ever DNA barcodes for both the genus and several of the recently uncovered species. The inclusion of genetic data, particularly the sequences of the 5'-end of the Cytochrome c Oxidase subunit I (COI) gene, offers a molecular perspective critical for future research, facilitating precise species identification. To enhance the practicality of our research, we present an identification key for the male specimens of the genus across the globe.

Furthermore, our study delves into the morphological characters of *Eugenys*, providing a comparative analysis with related taxa. This comparative evaluation tentatively positions *Eugenys* within the tribe Pericomaini s.l., shedding light on its evolutionary and taxonomic relationships within the broader taxonomic landscape.

In conclusion, this extensive study not only amplifies our understanding of the Neotropical genus *Eugenys* but also underscores the importance of molecular and morphological data integration for advancing taxonomy and overcoming the taxonomic impediment. The elucidation of additional species and their genetic signatures broadens the horizons of knowledge, promising to catalyze future research in Psychodidae systematics and contribute to conservation efforts within this taxonomic group.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 16 – First record of *Lepidiella* Enderlein, 1937 from the Oriental Region (Diptera, Psychodidae).

16.1 Bibliography of the published article

Jaume-Schinkel S, Kvifte GM (2022) First record of *Lepidiella* Enderlein, 1937 from the Oriental Region (Diptera, Psychodidae). *Zookeys,* 1115, 73–79. https://doi.org/10.3897/zookeys.1115.81668

16.2 Summary

This publication described a new species of the moth fly genus *Lepidiella* found in Thailand, thus providing the first record of this genus from the Oriental Region. In the publication we also update the generic diagnosis of *Lepidiella* and we discuss its taxonomic placement.

This study highlights the high diversity and the overall lack of studies of the Psychodidae fauna in the Oriental Region. Psychodidae includes over 420 described species just from the Oriental Region. Despite recent attention to this family, specifically to the subfamily Phlebotominae due to itsmedical importance, numerous species remain undescribed and the knowledge is limited in the region.

The genus *Lepidiella* was previously thought to be restricted to the Neotropical Region, but this publication presents the discovery of a new species of *Lepidiella* in Thailand, marking its first record outside the Neotropical Region. The publication includes a detailed description of this new species, *Lepidiella limicornis* sp. nov., and discusses its differentiation from other *Lepidiella* species based on various morphological features.

The results provide an overview of the genus *Lepidiella*, updating its diagnosis, and providing a list of species included in the genus. We highlight the uniqueness of certain morphological characters of this new species and suggests a potential evolutionary relationship between *Lepidiella* and another genus, *Clytocerus*, based on distinct morphological traits. However, further research, including molecular analysis, is recommended to better understand the evolutionary relationships within this group.

Overall, the publication contributes valuable information to the taxonomy and distribution of moth flies in the oriental region, specifically within the genus *Lepidiella*, expanding our understanding of their diversity and geographic range.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 17 – Four new species of *Gondwanoscurus* Ježek, 2001 (Diptera, Psychodidae).

17.1 Bibliography of the published article

Jaume-Schinkel S, Kvifte MG (in prep.) Four new species of *Gondwanoscurus* Ježek (Diptera, Psychodidae).

17.2 Summary

In this comprehensive taxonomic study, we presented a significant update on the genus *Gondwanoscurus* Ježek, 2001, with a particular focus on male specimens collected during an expedition in 1991 from Thailand and Laos. This research unveils four novel species: *G. kjaerandseni* sp. nov., *G. ostentatus* sp. nov., *G. sagittarius* sp. nov., and *G. quadrifurcata* sp. nov., each meticulously characterized, thereby expanding the known species within the genus to a total of 15. The taxonomic contributions include a comprehensive worldwide list of *Gondwanoscurus* species, an informative distribution map, and a taxonomical key specifically crafted to facilitate the precise identification of male individuals.

Spanning the Afrotropical and Oriental Regions, the genus *Gondwanoscurus* demonstrates its presence in countries like India, Malaysia, Tanzania, Thailand, and Yemen. Despite being classified into various tribal categories within the Psychodinae, the intricate web of phylogenetic relationships among these taxa is yet to be fully unraveled. Morphological evidence tentatively suggests *Neotelmatoscopus* as a potential relative, although molecular data presents a contrasting perspective. This study underscores the necessity for fresh samples and extensive molecular analyses to resolve the evolutionary lineage and delineate distinct species groupings within *Gondwanoscurus*.

Notably, gaps in understanding persist regarding the biology of *Gondwanoscurus*, particularly concerning the lifecycle stages. Field observations suggest that the larval stages of these species likely inhabit aquatic environments and may feed on decaying organic matter. Thus, there is a vital need for future research initiatives to prioritize systematic collections and thorough examination of specimens from both the Afrotropical and Oriental regions. This approach will unveil previously undiscovered species, enriching our understanding of this intriguing genus and contributing to the broader comprehension of Dipteran systematics and ecological dynamics.

The published article is a result of collaborative efforts between Santiago Jaume Schinkel, Gunnar M. Kvifte (Bodo University, Norway). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 18 – Preserving Morphology while extracting DNA: A non-destructive Field-to-Museum Protocol for slide-mounted specimens.

18.1 Bibliography of the published article

Jaume-Schinkel S, Muller B, Avila-Calero S, Kukowka S, Mengual X (2024) Preserving morphology while extracting DNA: a non-destructive field-to-museum protocol for slide-mounted specimens. *Biodiversity Data Journal* 12: e119448. https://doi.org/10.3897/BDJ.12.e119448

18.2 Summary

The primary objective of our research was to devise an optimized laboratory protocol that ensures the preservation of morphological structures and the extraction of high-quality DNA sequences from specimens belonging to the Psychodidae family. This study involved the analysis of 310 specimens, from different collection years (102 from 2013, 104 from 2018, 104 from 2020). Focusing on assessing the effects of various laboratory treatments on both morphological preservation and DNA yield, our approach encompassed the utilization of two shaking categories coupled with five different incubation periods, resulting in ten distinct experimental treatments during the DNA extraction process. These treatments were divided into two groups: five with constant shaking (C) and five with interrupted shaking (I), each featuring incubation periods of 16, 12, 8, 4, and 2 hours.

After our laboratory procedures, out of the 310 specimens we obtained 278 COI sequences, with a success rate of 89.6%. However, out of this 278 specimens successfully sequenced, eight sequences were excluded from the analysis for being shorter than the desired sequence length of 658 bp. Resulting in a total of 270 specimens of moth flies represented by 13 genera and 24 species.

A significant revelation from our investigation was that a substantial 82.6% of the specimens displayed noticeable morphological changes during the DNA extraction process, a phenomenon greatly influenced by the specific experimental treatments applied. Notably, we observed that prolonged incubation periods were strongly associated with increased structural losses within the specimens. On the contrary, shorter incubation periods led to comparatively minor alterations in certain structures, such as antennal flagellomeres, antennal ascoids, and legs.

Throughout our experimentation, we observed variations in DNA concentration among different treatments and genera. However, we found no significant impact stemming from factors such as collection year or species on the observed DNA concentration disparities. Despite these variations, successful extraction of the COI sequence was accomplished in an impressive 89.6% of the specimens. Furthermore, the differences in DNA fragment lengths across most treatments were negligible.

Our results effectively strike a balance between maintaining the integrity of morphological structures and maximizing the efficiency of DNA extraction from Psychodidae specimens. As a result, our findings hold substantial promise for advancing research endeavors involving Psychodidae specimens, offering a robust methodology that ensures both the preservation of morphological features and the extraction of high-quality DNA sequences. This optimized protocol serves as a critical asset in facilitating future studies within this field, allowing researchers to delve deeper into the genetic and morphological characteristics of these specimens while minimizing potential structural alterations during the extraction process.

The submitted publication is a result of collaborative efforts between Santiago Jaume Schinkel, Björn Müller (ZFMK/LIB), Sergio Avila-Calero (ZFMK/LIB), Sandra Kukowka (ZFMK/LIB) and Ximo Mengual (ZFMK/LIB). All authors contributed to data interpretation. The

initial draft and figure plates were prepared by SJS and BM, subsequently refined and edited by all authors.

Chapter 19 – Phylogeny of Psychodidae using exon-capture sequencing.

19.1 Bibliography of the published article

Jaume-Schinkel S., Avila-Calero S, Martin S, Kvifte GM, Kukowka S, Mengual X (in prep.) Phylogeny of Psychodidae using exon-capture sequencing.

19.2 Summary

The primary objective of our research was to infer a phylogeny of mothflies (Diptera, Psychodidae) based on exon-capture. In this comprehensive study, we developed 18651 hybrid-capture baits specifically designed to target 1445 coding regions spanning 1161 ortholog groups within Psychodidae. Our approach successfully captured targeted loci for 82 mothfly species across 46 genera, representing a significant portion of the genera within the family. Phylogenetic analyses based on the captured data revealed a non-monophyletic arrangement within Psychodidae, with our inferred phylograms supporting previous morphological classifications, particularly within the subfamily Psychodinae. Our results led to a revision of tribal classifications within Psychodinae, delineating seven tribes: Brunettiini, Maruinini, Mormiini, Paramormiini, Pericomaini, Psychodini, and Setomimini.

Further analyses are warranted to elucidate the relationships and tribal classification of certain genera such as *Clogmia, Clytocerus, Lepidiella, Mystropsychoda, Peripsychoda*, and *Tonnoiriella*. Additionally, morphological evidence is needed to refine the classification of the Setomimini tribe. The application of the exon-capture method, coupled with BAITFISHER assistance, proved highly successful in capturing all targeted loci and generating robust phylogenetic reconstructions within Psychodidae.

Our bait kit represents a valuable resource for advancing phylogenomic research and facilitating further investigations into moth fly evolution. The use of this sequence-capture methodology holds promise for catalyzing future in-depth phylogenetic analyses across diverse Psychodidae lineages, thereby contributing to a better understanding of the evolutionary dynamics within this intriguing insect group.

The article in preparation is a result of collaborative efforts between Santiago Jaume Schinkel, Sergio Avila-Calero (ZFMK/LIB), Sandra Kukowka (ZFMK/LIB), Sebastian Martin (ZFMK/LIB), Gunnar M. Kvifte (Bodo University, Norway), and Ximo Mengual (ZFMK/LIB). All authors contributed to data interpretation. The initial draft and figure plates were prepared by SJS, subsequently refined and edited by all authors.

Chapter 20 – General discussion.

20.1 Charting the knowledge of Psychodidae research through time and space

The foundations of modern systematics and taxonomy took root in Europe, fostering ongoing research in Diptera for over two centuries. Likewise, the study of moth flies, persisting since the 1800s, has resulted in the Palearctic Region presenting one of the world's most extensively studied Psychodidae faunas, with approximately 500 species recorded in Europe alone.

Despite Europe's comprehensive documentation of Psychodidae, knowledge about the family remains skewed, with research efforts concentrated in specific regions and countries, such as Bulgaria, France, Germany, the Czech Republic, and Slovakia. Consequently, many other countries have limited records and studies, including Austria, Croatia, Denmark, Estonia, Finland, Greece, Hungary, Spain, and the Netherlands. This imbalance in knowledge has enabled recent studies to publish new distributional records, and in some cases, the discovery and description of previously unknown species (see chapters 3 to 5, 7, and 8, and Appendixes 1 to 3, 5 and 6 respectively). The disparity in understanding the diversity of Psychodidae across various European countries comes from the uneven distribution of scientific research efforts over the past few decades. Moreover, the level of knowledge about these insects is largely correlated with the intensity of research conducted within specific countries; often centered around the country where specialists have been located.

A similar scenario, albeit slightly worse, exists in the Neotropical Region. The first extensive published work on Psychodidae in this region was published in the mid-1990s (Quate, 1996), approximately a century later than its Palearctic counterparts. Similar to Europe, specific countries within the Neotropics, such as Brazil, Colombia, and Mexico, exhibit a high number of

recorded species. And many other countries have limited or no records at all. In the same way, the Oriental Region also struggles with a knowledge gap. Some studies in this region have been published since the 1950s, mainly focusing only on certain countries (i.e., Brunei, China, Malaysia, and India).

Historically, research efforts in the Neotropical and Oriental regions have predominantly centered around medically important sand flies (Phlebotominae), leading to a substantial lack of knowledge concerning other subfamilies (i.e. Psychodinae, Horaiellinae, Trichomyiinae, Sycoracinae, and Bruchomyiinae). While the focus on sand flies is crucial due to their impact on public health and the economic concerns (particularly in regions affected by zoonotic diseases like Leishmaniasis), this emphasis has unintentionally limited our understanding of the broader Psychodidae diversity. Derived from this, we currently lack robust estimations of species numbers and have virtually no knowledge of their biology or their interactions within the environment. Consequently, this knowledge gap limits our ability to implement effective conservation strategies for these regions, the known and unknown species, and their habitats, especially if they are under threat.

Specialists in the past (and in the present) have played fundamental roles in expanding our knowledge of Psychodidae in specific geographical areas, highlighting the disparity in attention and research efforts among different regions across the globe. Additionally, Psychodidae research faces a pressing challenge: only a handful of researchers are currently active and some are close to retirement or are already retired. Consequently, the ongoing research activity in Psychodidae is constrained to a small number of specialists around the world. This limitation hampers progress as the amount of research that a single person can undertake is inherently limited compared to what is needed and thus, impeding the comprehensive exploration and understanding of the fauna around the globe.

Addressing these disparities requires global recognition of the significance of understanding Psychodidae beyond individual regions. Collaboration among researchers, increased funding, and a combined effort to explore and document these flies in less-studied areas are key elements that need to be addressed soon. Bridging these knowledge gaps not only enriches our understanding of biodiversity but also contributes to broader ecological studies crucial for conservation efforts worldwide.

Advancements in our understanding of Psychodidae arise from diverse sources of information. Notably, entomological collections continue to yield new species, even decades after the specimens were initially collected (see chapters 11, 14, 15, 16, and 17, and consult Appendixes 9, 12, 13, 14, and 15, respectively). Furthermore, the emergence of new geographical records and species owes much to the involvement of social media platforms and citizen science projects (see chapters 3, 6, 7, and corresponding Appendixes 1, 4, and 5, respectively). Moreover, the expansion of our knowledge base is driven by active entomological research efforts (refer to chapters 4, 5, 8, 9, 12, and 13, along with Appendixes 2, 3, 6, 7, 9, and 11, respectively). These research efforts continue to contribute science with new species and geographical records, nurturing a deeper understanding of Psychodidae.

Throughout history and continuing today, various factors have created obstacles in taxonomic research within the family Psychodidae. However, I am optimistic that in the near future, these impediments will significantly diminish, paving the way for numerous advancements in this field. The integration of cutting-edge technologies such as Next Generation Sequencing and other molecular techniques that will provide vast amounts of data, coupled with enhanced imaging systems like scanning electron microscopy and 3D modeling, that are poised to revolutionize our examination of morphological structures, will enhance our understanding of species and their intra- and interspecific relationships. This technological leap promises to furnish researchers with more comprehensive species concepts, fueled by the generation of vast datasets that can be integrated

and correlated with described and yet-to-be-found new species, thereby encouraging a deeper understanding of these organisms, and creating more robust species concepts.

In addition, the collaboration between citizen science initiatives and social media platforms will empower researchers to gather invaluable information concerning the distribution, behavior, and diversity of Psychodidae across broader geographical regions (see chapters 6 and 7, Appendixes 5 and 6 accordingly). This data, when incorporated into biogeographical studies utilizing Geographic Information Systems and spatial modeling, will refine our comprehension of distribution patterns, habitat preferences, and the ecological roles of various Psychodidae species. This enhanced information availability will enable exploration into the intricate ecological interactions between Psychodidae and their habitats. Delving into their involvement in nutrient cycling, pollination, and their position as integral components of ecosystems as food sources will become more feasible.

Moreover, study efforts will intensify their focus on understanding the profound influence of climate change on Psychodidae populations (and many other insect groups). This includes investigating alterations in their distribution, phenology, and the potential ramifications for disease transmission. By focusing efforts into these areas, we aim to strengthen our comprehension of the broader ecological implications of Psychodidae in a changing world.

In considering the near future, there is a contrasting duality that presents itself. On one side, there is a promising outlook illuminated by the arrival of innovative tools enabling the collection of extensive data through integrative taxonomy. These advancements expand our comprehension of moth flies, fostering an optimistic belief in the continuous flourishing of scientific inquiry and Psychodidae research. However, juxtaposed against this brightness is a looming grey area characterized by the alarming trend of insect decline, an issue compounded by numerous factors contributing to the taxonomic impediment and taxonomic research. Despite these challenges, I

hold onto the belief that the often-neglected field of taxonomy, once pivotal and now overshadowed, will resurface as the cornerstone of biological studies centered on species. I anticipate a resurgence of interest among new researchers in taxonomy, sparking fresh opportunities that will ultimately enrich the scientific community.

20.2 Overcoming the taxonomic challenges: Integrative Taxonomy within Psychodidae

The historical focus on morphological characters in Psychodidae is emblematic of alpha taxonomy, a discipline primarily centered on describing and classifying organisms based on their observable traits (in this case, morphology). Traditionally, much of the classification within Psychodidae relied on morphological features, notably in the male genitalia, with some attention given to female genitalia, larvae, and pupal characters. This approach has established a foundational understanding of Psychodidae taxonomy through the years. However, this approach has inherent biases and gaps, particularly regarding the comprehensive understanding of both sexes within the same species. The reliance on primarily the male genitalia for classification has led to a disparity in the available information between genders, a common issue across many insect groups studied through alpha taxonomy.

As research progresses, there is an increasing recognition of the limitations of solely morphological-based taxonomy. Therefore, integrative approaches where combining traditional morphological methods, molecular techniques (such as DNA barcoding), and other sources of information (i.e., distribution, ecology) are being increasingly adopted. These integrative methods aim to bridge the gaps in taxonomic knowledge, providing a more holistic understanding of Psychodidae species, addressing sex-specific morphological differences, and enhancing the accuracy and robustness of classification systems. Such integrative approaches represent a shift toward a more comprehensive and inclusive taxonomy beyond the confines of alpha taxonomy, allowing for a more robust exploration of Psychodidae diversity and classification.

The findings of this doctoral thesis support the efficacy of an integrative taxonomy approach in reducing the taxonomic knowledge gap within Psychodidae. For instance, in Chapter 3 (see Appendix 1), we published the first sequence of the 5'-end of the Cytochrome c Oxidase subunit I (COI or DNA barcode) for the genus *Alepia* Enderlein, 1937, alongside the new species *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 associating male and female sexes whitin the species. Similarly, in Chapter 4 (refer to Appendix 2), we successfully linked males and females of *Telmatoscopus advena* (Eaton, 1893), leading to the first-ever description of female specimens and their eggs. Likewise, Chapter 7 (see Appendix 5) marked a significant success in associating males and females across different species, facilitating the redescription of females in *Panimerus notabilis* (Eaton, 1893) *and P. goetghebueri* (Tonnoir, 1919). Moreover, Chapter 10 (refer to Appendix 8) presented the first DNA barcodes of the genus *Platyplastinx* Enderlein, 1937, matching the sexes of *Platyplastinx ibanezbernali* Jaume-Schinkel & Kvifte, 2022.

These studies lay the groundwork for future research by providing genetic information for both sexes, and discussing how integrating both, morphology and molecular characters can lead to a better understanding of species delimitation. The availability of DNA barcodes for species, preferably with both male and female specimens, holds immense importance in the rapidly changing framework of integrative taxonomy. Having information on both sexes facilitates the subsequent association of morphological characteristics, enabling researchers to distinguish females from different species, a critical step in identifying previously unknown or improperly associated sexes that have eluded proper classification to date.

As DNA barcoding continues to emerge as a powerful tool in species identification and classification theres is an increasing decline in the number of taxonomists worldwide. This decline, is attributed to various factors such as advancing age demographics, limited employment opportunities, insufficient funding, and shifting research priorities (Hochkirch et al., 2022). As traditional taxonomic practices face challenges and the pool of skilled taxonomists dwindles,

pertinent questions arise about the broader implications for the future of taxonomy and the preservation of taxonomic expertise in the scientific community, which make imperative to continue and to increase the support for taxonomic research.

As taxonomists struggle with escalating challenges, an increasing global crisis emerges, the decline in insect populations around the globe (Hallmann et al., 2017). This alarming trend exacerbates the discrepancy between our current taxonomic knowledge and the actual diversity of species in existence. The consequence is a disconcerting possibility: numerous undiscovered species may have succumbed to extinction, largely overlooked amidst this crisis. This crisis reiterates the need for an integrative taxonomic approach.

Integrative taxonomy, reliant on multiple sources of information beyond morphology and molecular data, harnesses modern tools such as social media and citizen science platforms across the globe. The findings from this dissertation shed light on this approach. For instance, Chapter 6 (refer to appendix 4) illustrates our ability to document a recently described species beyond its known geographical range through a citizen science project. Our publication explores the potential scenarios wherein species might be inadvertently transported globally, the ecological implications for local fauna, and notably, underscores the significance of citizen science and biomonitoring in employing an integrative approach to detect and monitor species distributions worldwide. Likewise, Chapter 7 (see appendix 5) elucidates new geographical records of Psychodidae for the Netherlands, highlighting that even in well-known fauna (like European Psychodidae) the integration of multiple tools is benefitial for the knowledge of moth flies.

Beyond the application of molecular tools such as DNA barcoding and the engagement of citizen science initiatives, the integration of high-quality imaging stands as a valuable asset in documenting the diverse array of Psychodidae species. The enhanced capability to scrutinize morphological structures in finer detail not only facilitates comprehensive observations but also

Reflecting on the Human Genome Project, a monumental endeavor aimed at mapping and sequencing the complete human genome, spanning over a decade and costing a total of 2.7 billion US dollars, starkly contrasts with today's technological landscape. Presently, sequencing the entire genome of various taxa can range from 1,000 to 5,000 US dollars, achievable within a matter of weeks. Undoubtedly, ongoing research stands to gain immeasurably from technological progress, with tools becoming increasingly accessible at reduced costs.

The concerns previously mentioned and the tools employed to address them merely scratch the surface, primarily based on observations derived from this doctoral dissertation. Undoubtedly, additional challenges will surface regarding the persistent taxonomic impediment. However, corresponding partial or comprehensive solutions will continue to emerge, prioritizing practicality and research enhancement. This ongoing pursuit remains integral to our overarching objective of preserving the environment and securing the survival of ecosystems for the benefit of future generations.

Setting the keystone in this technological/molecular advancements are projects like the “German Barcode of Life (GBOL) III: Dark Taxa”, which have assumed a pivotal role in enhancing our comprehension of the natural world. Within these projects, researchers are actively contributing to the development of a DNA Barcode reference library, a resource that promises to unveil new species and facilitate population monitoring while detecting the introduction of non-native species. Hugely tackling one of the issues revolving around the taxonomic impediment.

Moreover, if we extend the reach of such initiatives globally, the possibilities are boundless. Moreover, by acknowledging the biases in our knowledge, addressing the decline of taxonomists, and actively supporting taxonomy initiatives, we can hope to bridge the gaps in our understanding of these vital insects and, in doing so, contribute to the protection of our environment and the preservation of Earth's rich biodiversity for generations to come. I dare to

say, there is no coming back from integrative taxonomy and future studies within the family Psychodidae will heavily and increasingly realy on integrating all the available tools with the common goal or producing high quality research.

20.3 Molecular work

Methodological comments

Microscope slides with slide-mounted specimens remain fundamental in the taxonomic study of Psychodidae. Despite its historical reliability in observing morphological structures and preserving specimens over decades, this technique has limitations when pursuing an integrative approach. Specifically, the tissue maceration necessary for observing internal structures on slides results in irreversible DNA destruction. While suitable for morphology-based taxonomy, this method impedes subsequent access to specimens without damage.

Recent advancements in DNA extraction techniques offer non-destructive approaches that preserve specimens, enabling taxonomists to expand morphological studies. However, these extraction procedures often result in damage, particularly with soft-bodied and fragile specimens (just like mothflies). Addressing this challenge prompted the quest for a balanced approach between DNA extraction and preserving morphological structures (refer to chapter 18 and appendix 16). The emerging methodology, grounded in current DNA extraction and sequencing protocols, represents an initial step. Undoubtedly, ongoing technological progress may necessitate further refinements. Envisaging integrative approaches as the foundation for Psychodidae studies, the new protocol holds promise for successful implementation.

Moreover, with new generation sequencing (NGS) techniques, an unprecedented influx of data inundates researchers, surpassing the capacity of an individual to handle alone. This surge in data underscores the necessity for collaborative efforts within multidisciplinary teams. Collaborating in such teams offers an array of benefits, particularly in pooling diverse expertise for comprehensive problem-solving and accommodating the extensive data generated by NGS techniques. However, this collaborative dynamic can be a double-edged sword. While it presents opportunities for innovative solutions and shared insights, it also introduces challenges. Notably, within these multidisciplinary teams, individuals often harbor disparate goals and operate within distinct timeframes.

The amalgamation of different professional backgrounds and perspectives within a team can lead to enriched problem-solving strategies. Nonetheless, these varying perspectives might also clash, creating friction that stems from conflicting objectives and divergent timelines. The sheer complexity of coordinating efforts among individuals with dissimilar goals can impede the efficiency of the team's progress. To navigate this terrain effectively, it is crucial for team members to foster open communication channels and cultivate an environment that encourages mutual understanding. Finding common ground amid contrasting objectives and aligning timelines becomes imperative for ensuring cohesion within the team. While multidisciplinary collaboration remains instrumental for handling the overwhelming data landscape created by NGS techniques, addressing the challenges posed by differing goals and timelines is vital for harnessing the full potential of such teamwork.

General discussion about molecular work

To date, single-gene sequences at the molecular level serve as a prevalent method for species identification. Within the animal kingdom the cytochrome oxidase c subunit I gene fragment, commonly referred to as COI (Hebert et al., 2003), stands out as the most extensively

utilized for this purpose. Originally intended for specimen identification, DNA barcoding has expanded its applications over the years, delving into diverse areas such as epidemiology, ecology, agriculture, forensic sciences, conservation management, and pest control (Kvist, 2013). For instance, DNA barcoding has proven instrumental in tracking disease vectors, understanding ecological interactions, establishing food chain dynamics, and even aiding in criminal investigations.

Over the past few decades, the establishment of large-scale DNA barcode storage databases, notably the Barcode of Life Data System (BOLD), has emerged with rigorous curation (Ratnasingham & Hebert, 2007; Kvist, 2013). BOLD serves as an online database and informatics platform explicitly designed for storing, managing, and analyzing DNA barcode data. This platform empowers researchers and users to upload their DNA barcode sequences onto BOLD, enabling comparisons with the existing database to identify species. Leveraging a suite of analytical tools, algorithms, and diverse databases, BOLD not only aids in species identification but also provides crucial taxonomic information, geographical distribution data, and facilitates exploration into the relationships among species.

Nevertheless, the effectiveness of BOLD or any other barcoding database hinges upon robust data curation. Ideally, each uploaded DNA sequence should be linked with a voucher specimen, deposited in a collection accessible to the public for comparison. The accurate identification and labeling of species with up-to-date names are essential prerequisites. Without this meticulous curation, the reliability and usability of the database diminish significantly. Mislabeling, misidentification, or outdated taxonomy not only obstruct accurate species identification but also impede broader research efforts, leading to erroneous conclusions about biodiversity and potentially hindering conservation strategies.

As an example, the family Psychodidae encompasses nearly 3,500 described species worldwide. Astonishingly, only a fraction of these species (683 species precisely) currently possesses available DNA barcodes in BOLD. This means that merely around 20% of the global known fauna within this family is represented in this online repository. This significant lack of data highlights the extensive potential for growth and improvement in genetic data availability for these organisms.

For DNA barcoding to function optimally and comprehensively cover the species diversity within Psychodidae a more ambitious database is necessary. Ideally, achieving representative genetic data for each species across its distributional range would greatly enhance the accuracy and utility of DNA barcoding. This goal would necessitate the inclusion of multiple individuals from various geographic locations for each species, considering potential genetic variations within populations.

Expanding the genetic representation of Psychodidae species in repositories like BOLD to encompass a broader spectrum of this diverse family holds immense value. It could facilitate more precise species identification, aid in understanding population dynamics, uncover cryptic species diversity, and contribute significantly to various fields such as ecology, epidemiology, and conservation biology.

Projects like the German Barcode of Life play a crucial role in the global expansion of DNA barcode libraries. As previously mentioned, overcoming taxonomic obstacles necessitates an integrative strategy that incorporates DNA Barcoding, alongside other molecular tools. This approach is indispensable for achieving a comprehensive understanding of fauna, offering a more holistic perspective on species diversity and taxonomy.

While pursuing an integrative approach, the process of describing new species should incorporate multiple sources of information, including DNA Barcodes. Furthermore, generating new DNA barcodes for species already known should be prioritized, encompassing diverse localities and populations. This concerted effort aims to enrich our understanding of individual species and, consequently, contribute to a more comprehensive comprehension of the global Psychodidae fauna. Detailed information regarding this strategy can be found in chapters 3, 4, 7, 10, 12, 13, 14, and 15, along with appendices 1, 2, 5, 8, 10, 11, 12, and 13, respectively.

Molecular sequence data has evolved into a cornerstone not only for species identification and delineation but also for reconstructing phylogenetic relationships (e.g. Wiegemann et al., 2011; Call et al., 2023; Mengual et al., 2023). Initially, phylogenies relied on single-gene sequences with the advent of molecular techniques. As accessibility to these methods increased, additional gene fragments were incorporated. However, the landscape dramatically shifted with the introduction of next-generation sequencing (NGS) techniques. This revolutionary advancement exponentially augmented molecular data, encompassing multiple genes, transcriptomes, and even whole genomes. Consequently, the scope and resolution of phylogenetic analyses within taxa have substantially advanced (e.g. Call et al., 2023; Mengual et al., 2023).

Notwithstanding the advances made with NGS, the most recent phylogenetic reconstructions within Psychodidae predominantly relied on a limited number of genes (Curler & Moulton, 2012; Espíndola et al., 2012; Kvifte, 2018). In our research detailed in chapter 19 (appendix 17), we significantly expanded the scope of our phylogenetic analysis by incorporating a more extensive array of genes and molecular data. The progression in technological capabilities over the last decade empowered us to harness newer sequencing techniques, enabling the generation of substantially larger datasets for our analyses.

As previously discussed, technological advancements and the growing accessibility of tools at reduced costs have made the generation of extensive molecular data progressively easier. This trend is expected to persist, ensuring that molecular data becomes increasingly accessible worldwide. Consequently, online repositories will play a fundamental role in facilitating research centered around molecular information and species. Undoubtedly, forthcoming studies are poised to adopt an integrative approach, harnessing all available data sources. From my perspective, molecular data will remain an indispensable resource for species-related studies and their derivatives, consistently serving as a fundamental source of valuable information.

Psychodinae phylogeny

In our study focused on the Psychodinae phylogeny (see chapter 19, appendix 17), we designed 18,651 hybrid-capture baits to target 1,445 coding regions belonging to 1,161 ortholog groups. Our efforts successfully captured and targeted loci for 82 mothfly species from 46 genera, representing over 40% of the total genera included in Psychodidae. Our phylogenetic inference recovers a non-monophyletic Psychodidae, consistent with prior morphological and molecular studies. Based on our result, Psychodidae is divided into seven tribes: Brunettiini Vaillant, 1971, Maruinini Enderlein, 1937 **stat. rev.**, Mormiini Enderlein, 1937 **stat. rev**., Paramormiini Enderlein, 1937 **stat. rev.**, Pericomaini Enderlein, 1935 **stat. rev.**, Psychodini Newman, 1834, and Setomimini Vaillant, 1982 **stat. rev.**

While our dataset encompasses a broad array of genera and species within Psychodinae, our dataset lacks representation from two subfamilies, namely Horaiellinae and Brychomyiinae. Incorporating taxa from these subfamilies could enrich phylogenetic analyses and add robustness to future inferences. Nonetheless, using the exon-capture technique, supported by BAITFISHER, proved highly effective in capturing specified genetic regions and generating well-supported phylogenies within Psychodidae. Moreover, our bait kit serves as a reliable tool for advancing

research in phylogenomic analyses of moth fly relationships. Besides Psychodidae, our bait kit successfully captured CDS regions of three infraorders and several Diptera families, namely, Tipulomorpha [including Limoniidae and Trichoceridae], Psychodomorpha [Ptychopteridae, Blephariceridae, and Tanyderidae], and Culicomorpha [Culicidae and Chironomidae]. This result suggests that our bait kit can be used to infer phylogenies of medically important groups: i.e., biting midges (Ceratopogonidae, Corethrellidae), black flies (Simuliidae), and mosquitoes (Culicidae). Likewise, our kit is stands out as a reliable tool for phylogenetic analyses across various "nematoceran" taxa.

Our phylogenetic inference has the broadest molecular data set to date. Despite this, our dataset is limited to only molecular data. Integrating different data sources may be beneficial in understanding the evolution of the group. For instance, incorporating fossil data, could aid in calibrating molecular clocks and elucidating evolutionary relationships. Similarly, integrating ecological data (e.g., larval development habitat, adult feeding habits, activity patterns) and morphological data (e.g., genital morphology, larval and egg morphology) could provide deeper insights into group evolution.

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Appendix 1. (Publication chapter 3)

Chapter 3 – Publication

Jaume-Schinkel S, Kvifte GM, van der Weele R, Mengual X (2022) *Alepia viatrix* sp. nov. (Diptera: Psychodidae), a new species of a Neotropical genus found on the Azores Archipelago (Portugal). *Zootaxa,* 5128(3), 384–396. https://doi.org/10.11646/ZOOTAXA.5128.3.4

For copyright reasons, this article is not available in the appendix.

Appendix 2. (Publication chapter 4)

Chapter 4 – Publication

Jaume-Schinkel S, Morelli A, Kvifte GM, Mengual X (2022) What's inside the hole? A review of European dendrolimnetic moth flies (Diptera: Psychodidae: Psychodinae). *Diversity*, 14, 532. https://doi.org/10.3390/d14070532

diversity

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Article

What's Inside the Hole? A Review of European Dendrolimnetic Moth Flies (Diptera: Psychodidae: Psychodinae)

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Abstract: We conducted an extensive literature review in search of records of dendrolimnetic Psychodinae, with additional field sampling of the European species of Psychodinae associated with water-filled tree holes. After checking more than 100 publications, only 11 specific published records involving dendrolimnetic Psychodinae were found. Our results show that six genera, represented by 13 species of Psychodinae, are associated with 13 species of plant trees. As a result of our field sampling, we report *Lepiseodina latipennis* (Sarà, 1953) and *Telmatoscopus bartai* (Ježek, 2004) **comb. nov**. for the first time in Germany. Furthermore, we redescribe *L. latipennis* based on freshly collected material with a closer examination of the holotype. Derived from our findings, we review the genera *Lepiseodina* Enderlein, 1937 and *Telmatoscopus* Eaton, 1904, providing an identification key for the males of both genera. In addition, we synonymyze *Krivosheinoscopus* Ježek, 2001 **syn. nov.** under *Telmatoscopus*, changing combination of *Telmatoscopus ussuricus* (Ježek, 2001) **comb. nov.** and *Telmatoscopus bartai* (Ježek, 2004), additionally, we change combination and a sononymy of *Tematoscopus wagneri* (Salmanna, 1982) **comb. et syn. nov.** under *Telmatoscopus advena* (Eaton, 1893). Furthermore, we describe for the first time the female and eggs of *Telmatoscopus advena*. Moreover, we provide the first published DNA barcodes (COI) for *Telmatoscopus bartai*, *Lepiseodina latipennis* (Sarà, 1953), *Lepiseodina rothschildi* (Eaton, 1912), and *Lepiseodina tristis* (Meigen, 1830). Finally, we also discuss the taxonomy and ecology of the European dendrolimnetic species of the subfamily Psychodinae.

Keywords: water-filled tree holes; DNA barcoding; moth flies; new taxa; integrative taxonomy

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1. Introduction

Phytotelmata (singular phytotelma) derives from the Greek words "*phyto*", which means plant, and "*telma*", meaning pond, and was first proposed by Varga [1], based on his observations, to define the microhabitats product of water-filled bodies in plant surfaces [2]. Within the phytotelmata, we can find the dendrotelmata (singular dendrotelma), deriving from the Greek words "*dendron*", meaning tree, and "*telma*", which circumscribe the body waters only to water-filled tree holes. These dendrotelmata are considered an integral microhabitat inside forest ecosystems, as they provide a suitable space for development, prey, and water sources for many organisms ranging from invertebrates to vertebrates [3,4]. Species that relate to or inhabit these water-filled tree holes are known as dendrolimnetic, from the Greek words "*dendron*", and "*limnétēs*", which means relating to or inhabiting the open water of a body of fresh water.

Dendrotelmata are commonly found in old trees, dead or alive, and are a considered crucial component of ecosystems, especially with the modern forestry management where the number of mature and over-mature trees (commonly referred as old trees) has decreased [5]. The main sources of nutrients flowing in the tree holes consist of leaf litter and arthropod cadavers, while the quality and composition of these nutrients vary across species

and habitats [6,7]. The fact that these microhabitats have a small size, discrete boundaries, and are naturally replicated in nature, makes them attractive for studies of community structure and functionalities [7]. Researchers have reported the usage of dendrotelmata by amphibians during development, as a water source for reptiles and small mammals, and, as bathing sites for birds and bats [4]. Nonetheless, mainly invertebrates with aquatic development have been reported developing inside these microhabitats, with some reports of other invertebrates that use them as a water source [4,8]. Among them, the most common organisms found inside are immature stages of Diptera and Coleoptera [7–10].

Moth flies (Insecta: Diptera: Psychodidae) are commonly found in water bodies, as the majority of psychodid species develop in water during larval stages, with a few exceptions that develop in soil, dung, or fungi [11–13]. There are six subfamilies recognized worldwide, namely Bruchomyiinae, Horaiellinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyiinae; all of them except Horaiellinae are present in Europe [14,15]. Species in the subfamily Trichomyiinae develop inside tree holes or rotting wood and their larvae are considered xylophagous (from Greek "*xylon*", meaning wood, and "*faguein*", meaning eating) [5]. Species of Bruchomyiinae and Phlebotominae have been reported developing on the ground and leaf litter feeding on decaying organic matter [15]. The larval stages of Sycoracinae species have been found developing in aquatic mosses and leaf litter [15]. On the contrary, species of the subfamily Psychodinae are commonly reported developing in water bodies such as ponds and streams, but there are a few genera present in Europe whose larvae develop inside of tree holes, namely *Clogmia*, *Clytocerus*, *Lepiseodina*, *Pneumia*, *Psychoda*, and *Telmatoscopus* [13,16–25]. Although the larval development and habitat for some European species are known, the moth flies associated with water-filled tree holes have been poorly studied in Europe [25].

In the present study, we revise the available literature about the European species of the subfamily Psychodinae known to develop in dendrotelmata, with a special emphasis on the genera *Lepiseodina* and *Telmatoscopus.* Additionally, we study the dendrolimnetic moth flies collected in Germany resulting from the German Barcode of Life (GBOL) project [26] (www.bolgermany.de, accessed on 02 April 2022). As an outcome, we redescribe *Lepiseodina latipennis* (Sarà, 1953) from Germany (Nordrhein-Westfalen) and Italy (Sicily) based on new morphological and molecular data, and we synonymize *Krivosheinoscopus* Ježek, 2001 **syn. nov.** under *Telmatoscopus* Eaton, 1904. Furthermore, we change combination and synonymize *Telmatoscopus wagneri* (Salamanna, 1982) **comb. nov. et syn. nov.** under *Telmatoscopus advena* (Eaton, 1893), and we describe for the first time the female and eggs of *Telmatoscopus advena* (Eaton, 1893) based on morphological and molecular data. Moreover, we provide the first record of *Lepiseodina latipennis* (Sarà, 1953) and *Telmatoscopus bartai* Ježek 2004 **comb. nov.** from Germany. Furthermore, we provide the first COI (5′-end of the cytochrome c oxidase subunit I) sequences, also known as DNA barcode, for *Telmatoscopus bartai* (Ježek, 2001) **comb. nov.**, *Lepiseodina latipennis* (Sarà, 1953), *Lepiseodina rothschildi* (Eaton, 1912), and *Lepiseodina tristis* (Meigen, 1830).

2. Material and methods

2.1. Geographic Scope

We follow the proposed geographic boundaries of Europe by de Jong et al. [27] with the following limits: East: Ural (E 60°), West: Atlantic Ocean (W 30°), South: Mediterranean (N 35°), and North: Atlantic Islands (N 82°).

2.2. Literature Records

Literature search was conducted by tracking references from known literature with the help of search engines (e.g., www.scholar.google.com, accessed on 5 Jaunary 2022) and scientific databases (e.g., www.jstor.org, www.scopus.com, www.webofscience.com, 5 January 2022) using the search keywords "Psychodidae, Psychodinae, dendrolimnetic, Diptera, water-filled tree holes". Literature used for the study encompasses records since the description of the species to the most recent published works until the beginning of

2022, focusing on Psychodinae and dendrolimnetic studies. More than 100 items were analyzed; however, only 11 included records of dendrolimnetic Psychodinae (as listed in Table 1).

2.3. Sampling

Specimens were collected using Malaise traps during the years 2013–2021 as part of the German Barcode of Life (GBOL) project [26] (www.bolgermany.de, accessed on 2 April 2022). Specimens were preserved in 96% ethanol and stored at −20 °C until they were dissected for DNA extraction and preparing permanent slides. All sampled specimens are stored at the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). Further material was bred from organic matter sampled from dendrotelmata, and, occasionally collected with a Malaise trap and directly collected in Italy, during 2010–2022 (AM).

Additional examined material is deposited in the following natural history collections mentioned in the text using their acronyms:

AM: Alessio Morelli private collection, Pianella (PE), Italy; later to be deposited at the Natural History Museum of Genova.

BNHM: British National History Museum, London, United Kingdom.

MNGD: Museo di Storia Naturale Giacomo Doria, Comune di Genova, Genova, Italy.

NMP: Národní Muzeum, Prague, Czech Republic.

ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

2.4. Study of the Collected Material and Terminology

After lysis (see Genetics below), the specimens were cleared using NaOH 10%, dissected, and permanently mounted using Euparal (Waldeck GmbH & Co. KG, Division Chroma, Havixbecker Straße, Münster, Germany) as mounting medium, following the procedure detailed in Ibáñez-Bernal [28], with the modification that prior to the diaphanization process, we performed the dissection of the head, wings, and terminal abdominal segments to macerate them in NaOH 10% and continue with the procedure, while preserving the remaining tissue (thorax, legs, first abdominal segments) in ethanol for posterior DNA extraction. Additional material was macerated in KOH 10%, transferred in acetic acid 10%, and dehydrated in acetone 99%. Specimens were dissected in clove oil and parts mounted on microscope slides with Canada balm. For the additional material, some specimens were photographed to keep a quality image of the habitus.

We follow the general terminology proposed by Cumming et al. and Kvifte and Wagner [15,29], except for the male terminalia that we use the term "hypopods" for the posterior genitalic appendages, which have been treated in the literature as cercopodia or surstyli originating in the 9th abdominal segment, or 10th segment, or a combination of both, as proposed by Kvifte and Wagner [30]. For the female Terminalia we follow Kotrba [31]. Egg terminology follows De Almeida et al. [32].

In the examined material of each species, a code is provided for each examined specimen (e.g., ZFMK-DIP-000852020), and all the label information is found as Supplementary Material (Table S1).

2.5. Genetics

Specimens were processed at the ZFMK, where lysis and PCR were performed following the protocol and primers by [33,34]. After the PCR, samples were sent to Beijing Genomics Institute (BGI) for bidirectional sequencing. Raw data were curated manually using Geneious (v. 7.1.9). Final COI sequences were 658 bp long. All sequences will be publicly available at www.bolgermany.de. BOLD and genebank accession IDs can be found in the Supplementary Material.

3. Results

Based on our literature review, we report 13 species of Psychodinae associated with dendrotelmata belonging to six genera (Table 1), namely *Clogmia* [*C. albipunctata* (Williston, 1893)], *Clytocerus* [*C. xylophilus* Vaillant, 1983], *Lepiseodina* [*L. latipennis* (Sarà, 1953), *L. rothschildi* (Eaton, 1912) and *L. tristis* (Meigen, 1830)], *Pneumia* [*P. canescens* (Meigen, 1804) and *P. trivialis* (Eaton, 1893)], *Psychoda* [*P. alternata* Say, 1824, *P. cinerea* Banks, 1894 and *P. minuta* Banks, 1894] and *Telmatoscopus* [*T. advena* (Eaton, 1893), *T. bartai* (Ježek, 2004) **comb. nov.**, and *T. thuringicus* Beran, Doczkal, Pfister & Wagner, 2010]. Additionally, we list *Telmatoscopus bartai* (Jezek, 2004) **comb. nov.** as a potential dendrolimnetic species, giving arguments for this decision.

Table 1. Dendrolimnetic Psychodinae taxa reported for Europe, tree species where it was reported and references. Double asterisk (**) denotates a new record of tree species for the Psychodinae species.

Taxon	Tree Species	Reference
Clogmia albipunctata (Williston, 1893)	Oak (*Quercus* sp.), not specified	[35,36]
Clytocerus xylophilus Vaillant, 1983	Lime tree (*Tilia* sp.)	[37]
Lepiseodina tristis (Meigen, 1830)	Ash (*Fraxinus* sp.), beech (*Fagus* sp.), birch (*Betula* sp.), cherry (*Prunus* sp.), elm (*Ulmus* sp.), maple (*Acer* sp.), oak (*Quercus* sp.), mulberry (*Morus* sp.), lime (*Tilia* sp.), not specified, ** *Populus nigra* L.	[13,22,25,36,38]
Lepiseodina rothschildi (Eaton, 1912)	Maple (*Acer* sp.), oak (*Quercus* sp.), not specified	[9,13,38]
Lepiseodina latipennis (Sarà, 1953)	** Maple tree (*Acer* sp.), ** Oak (*Quercus* sp.)	
Pneumia canescens (Meigen, 1804)	Not specified	[18,25]
Pneumia trivialis (Eaton, 1893)	Not specified	[22,25]
Psychoda alternata Say, 1824	Not specified	[23]
Psychoda cinerea Banks, 1894	Apple (*Malus* sp.), oak (*Quercus* sp.), ** Hornbeam (*Carpinus* or *Ostrya* sp.)	[25]
Psychoda minuta Banks, 1894	Maple (*Acer* sp.), oak (*Quercus* sp.)	[25]
Telmatoscopus advena (Eaton, 1893)	Ash (*Fraxinus* sp.), birch (*Betula sp.*), elm (*Ulmus* sp.), oak (*Quercus* sp.), sycamore (*Platanus* sp.)	[13,22,25,38]
Telmatoscopus thuringicus Beran, Doczkal, Pfister & Wagner, 2010	Not specified (assumption of development)	[5]
Telmatoscopus laurencei Freeman, 1953	Lime tree (*Tilia* sp.)	[39]

3.1. Key to the Males of European Psychodinae Genera Found in Dendrotelmata

Differential diagnosis. Adults of the subfamily Psychodinae can be easily differentiated from the adults of the exclusively xylophagous subfamily Trichomyiinae, which can also be found in tree holes, by the presence of an eye bridge in Psychodinae (absent in Trichomyiinae) and wing vein R with five branches in Psychodinae, with two longitudinal veins between radial and medial forks (vein R with four branches in Trichomyiinae, with one longitudinal vein between radial and medial forks).

1. Antenna with at least flagellomeres 2–10 nodiform, divided into a basal nod and a distal neck (Figure 1B,D) . . . **3**

\- Antenna with flagellomeres cylindrical or fusiform (Figure 1A,C) . . . **2**

2. Cornicula (everted sac-shaped structures on the back surface of the head (presumed scent organs); also known as patagia) usually present; antennal scape more than three times longer than pedicel; flagellomere 1 with a distal brush of wavy setae (Figure 1C) . . . ***Clytocerus* Eaton, 1904**

\- Cornicula always absent; antennal scape less than three times the length of pedicel; flagellomere 1 without a distal brush of wavy setae (Figure 1A) . . . ***Pneumia* Enderlein, 1935**

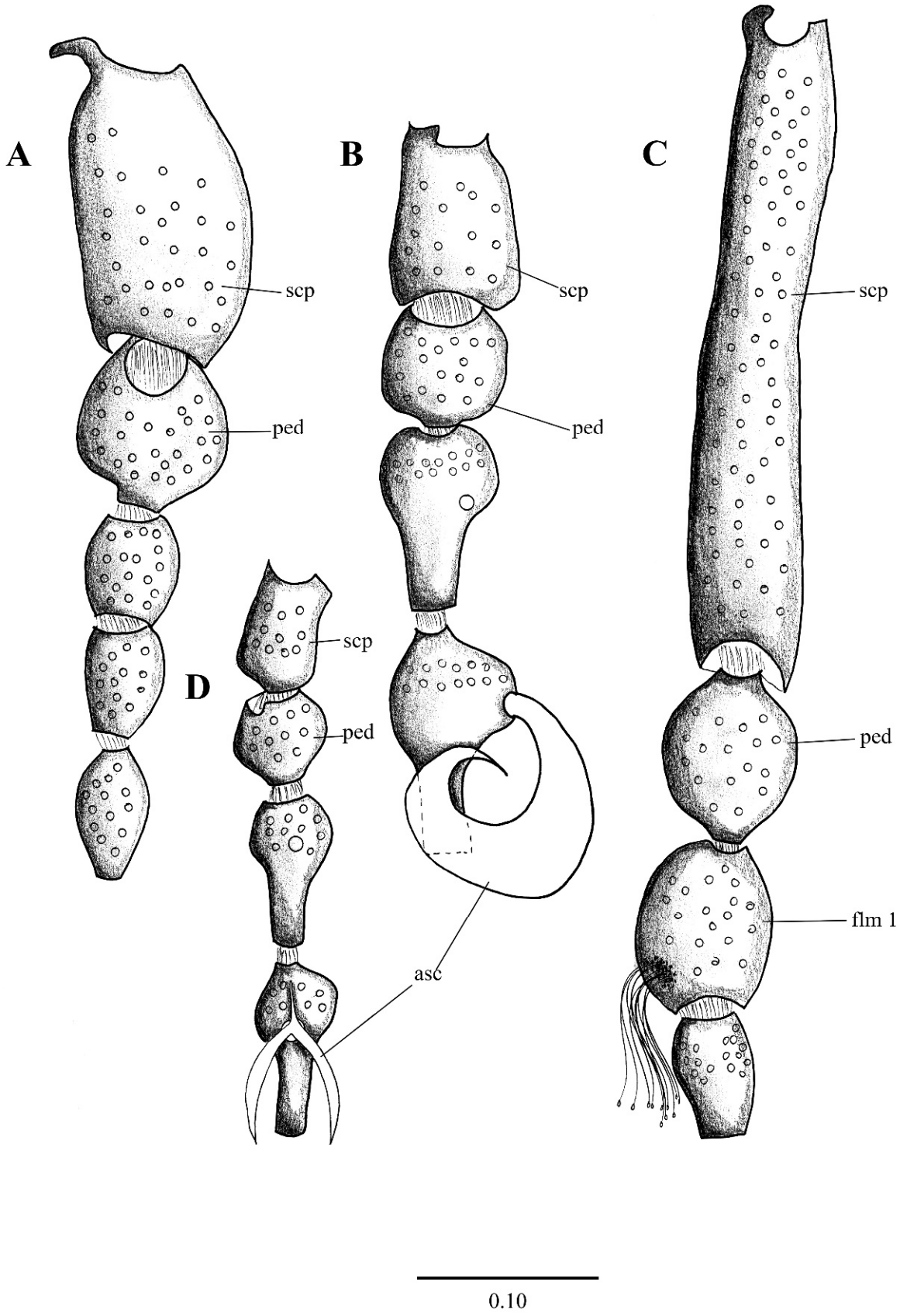

Figure 1. First antennal segments (left antenna) of: (**A**). *Pneumia trivialis* (Eaton, 1893), (**B**) *Telmatoscopus advena* (Eaton, 1893), (**C**). *Clytocerus ocellaris* (Meigen, 1818), (**D**). *Psychoda* sp. Abbreviations: asc = ascoids, flm = flagellomere, ped = pedicel, scp = scape. Scale (**A**–**C**) in millimeters (mm).

3. Ascoids with anterior and posterior branches (ascoids Y-shaped) (Figure 1D); hypopods of males with only one apical tenaculum . . . ***Psychoda* Latreille, 1797**

- Ascoids with a single curved branch or carrying only anterior branches (ascoids digitiform and not Y-shaped) (Figure 1B); hypopods of males with four or more apical tenacula . . . **4**

4. Ascoids bifurcate (as in Kvifte & Wagner [15], Figure 16; also in Ibáñez-Bernal [40], Figures 55 and 56); tenacula distally knife-shaped and shorter than basal width of hypopods (as in Ibáñez-Bernal [40], Figure 58) . . . ***Clogmia* Enderlein, 1937**

- Ascoids with a single digitiform or leaf-shaped branch, not bifurcate (Figure 1B); tenacula distally feathered and longer than the basal width of hypopods (Figures 2B, 3B, 4D, 5B, 6B, 7B, 8A,C, 9A–C, 10B and 11B) . . . **5**

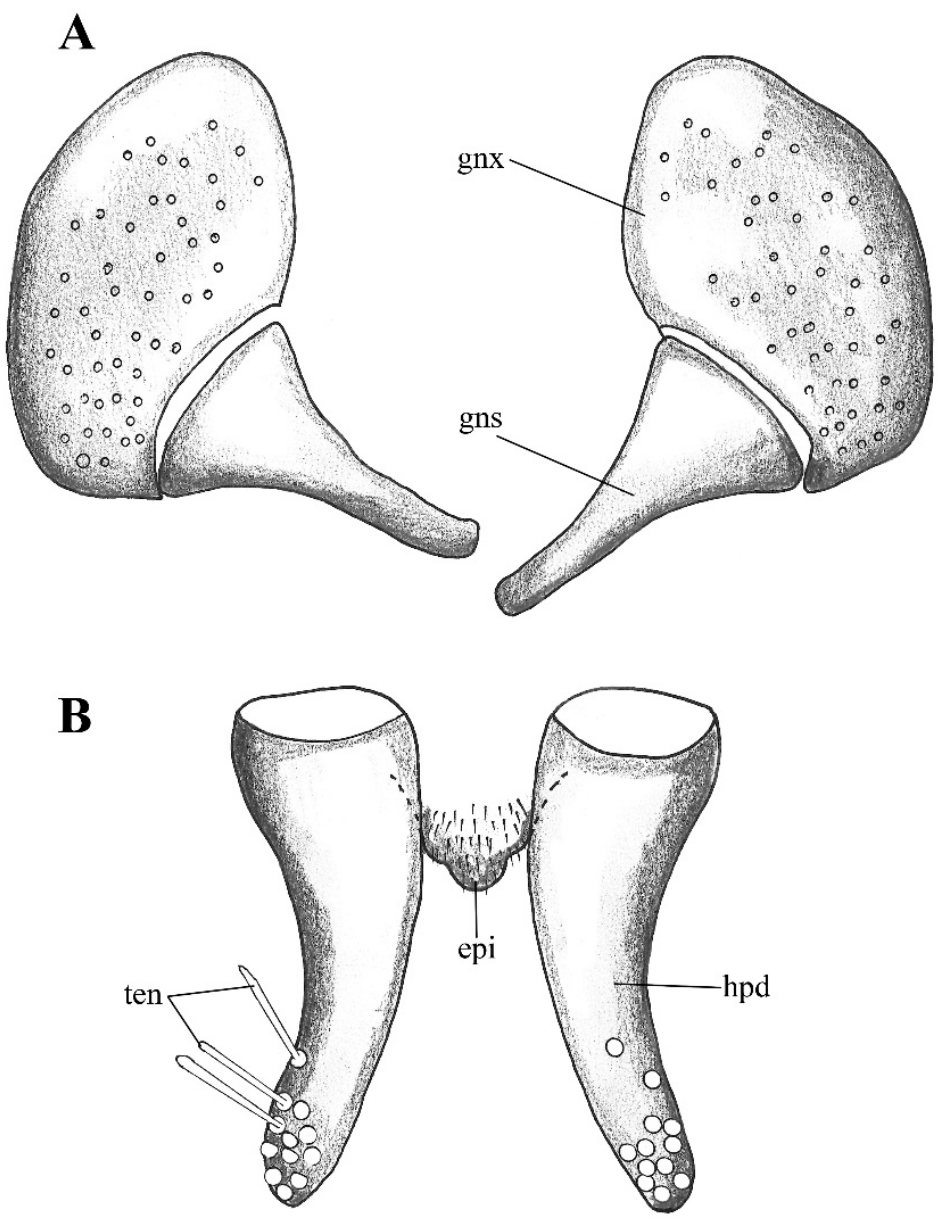

Figure 2. *Lepiseodina latipennis* (Sarà 1953), male genitalia adapted from the original description by Sarà (1953). (**A**). Gonocoxites and gonostyli. (**B**). Hypopods and hypoproct. Abbreviations: gnx = gonocoxite, gns = gonostyli, hpd = hypopod, epi = epiproct, ten = tenacula. No scale available based on original drawing.

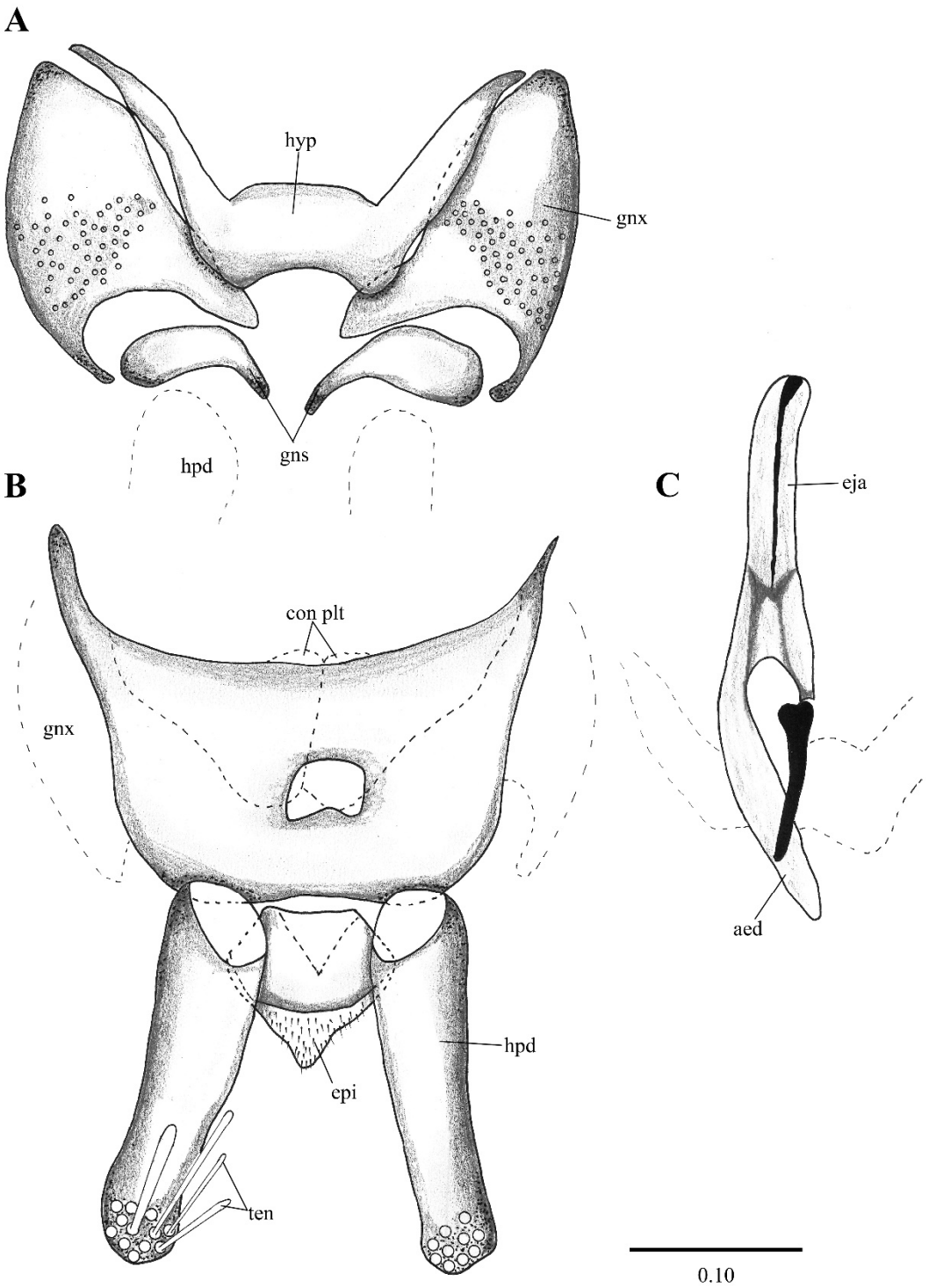

Figure 3. *Lepiseodina rothschildi* (Eaton, 1912), male genitalia. (**A**). Hypandrium, gonocoxites and gonostylus. (**B**) Epandrium, hypopods, tenacula, hypoproct. (**C**) Aedeagus. Abbreviations: aed = aedeagus, con plt = condyle plate-like, eja = ejaculatory apodeme, epi = epiproct, gnx = gonocoxite gns = gonostylus, ten = tenacula, hyp = hypandrium. Scale (**A**–**C**) in millimeters (mm).

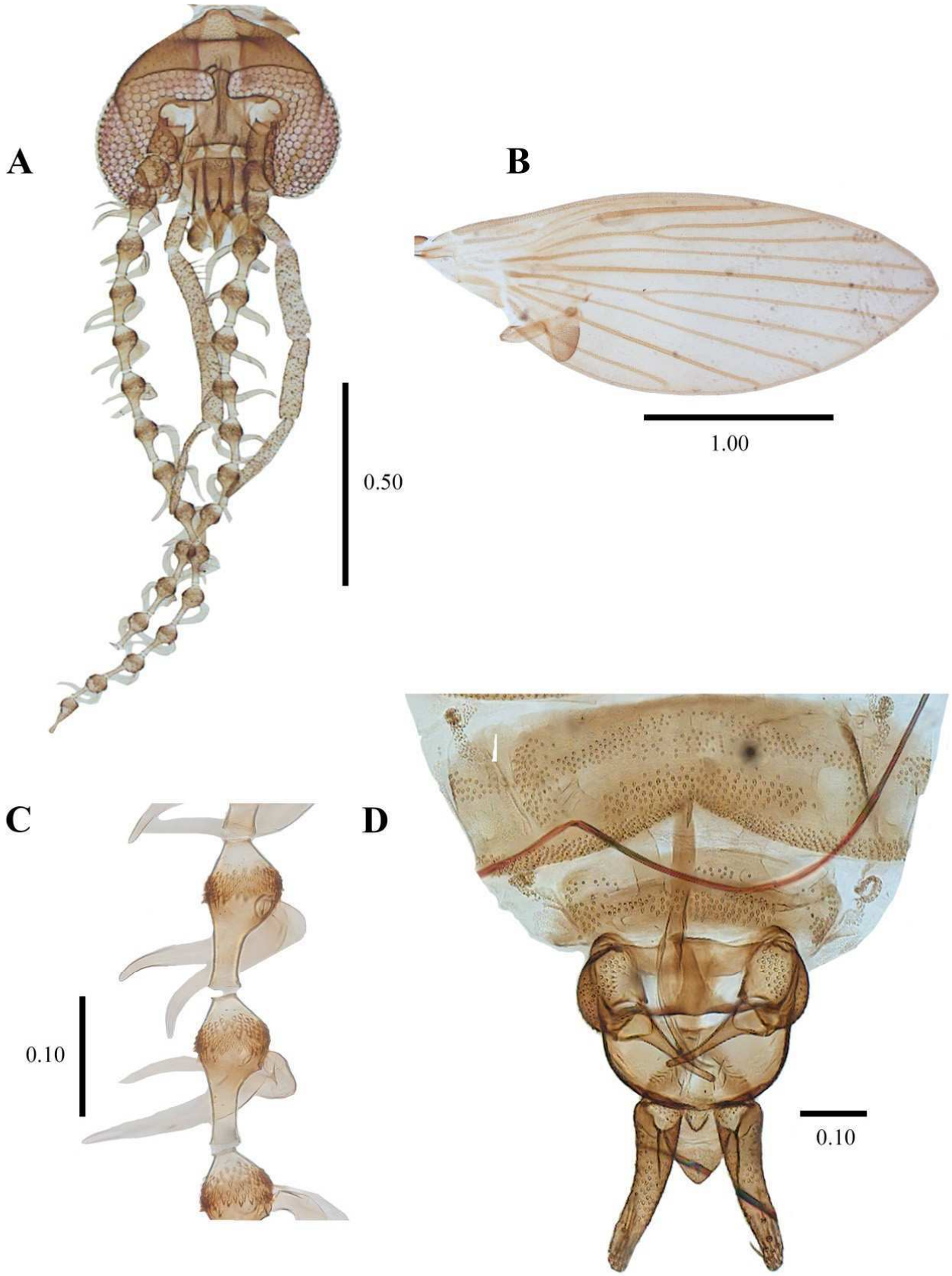

Figure 4. *Lepiseodina latipennis* (Sarà, 1953), male. (**A**) Head. (**B**) Wing. (**C**) Flagellomeres and ascoids. (**D**) Genitalia. Scale (**A**–**D**) in millimeters (mm).

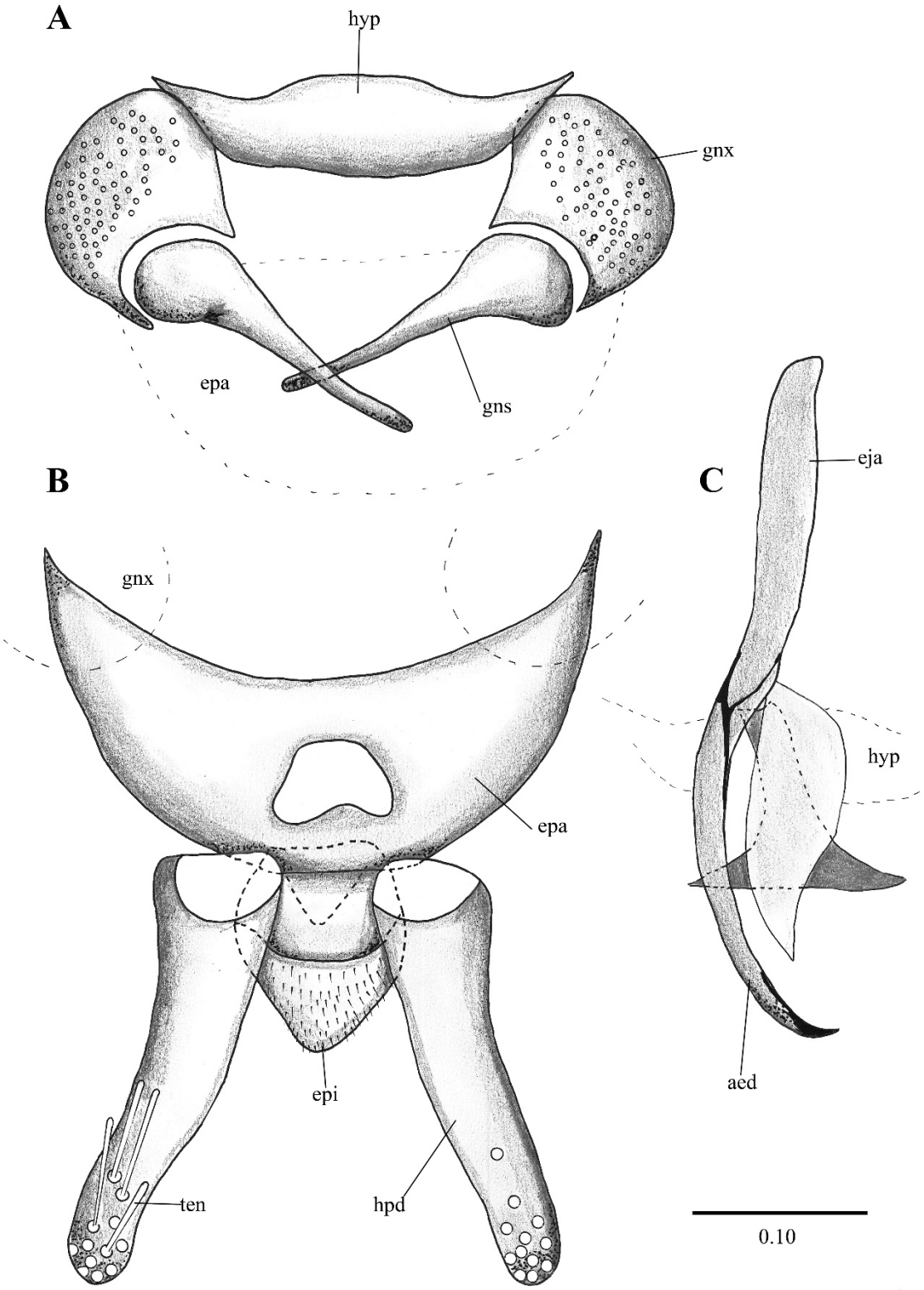

Figure 5. *Lepiseodina latipennis* (Sarà, 1953), male genitalia. (**A**) Hypandrium, gonocoxites and gonostylus. (**B**) Epandrium, hypopods, tenacula, hypoproct. (**C**) Aedeagus. Abbreviations: aed = aedeagus, epa = epandrium, eja = ejaculatory apodeme, epi = epiproct, gnx = gonocoxite, gns = gonostylus, ten = tenacula, hpd = hypopods, hyp = hypandrium. Scale (**A**–**C**) in millimeters (mm).

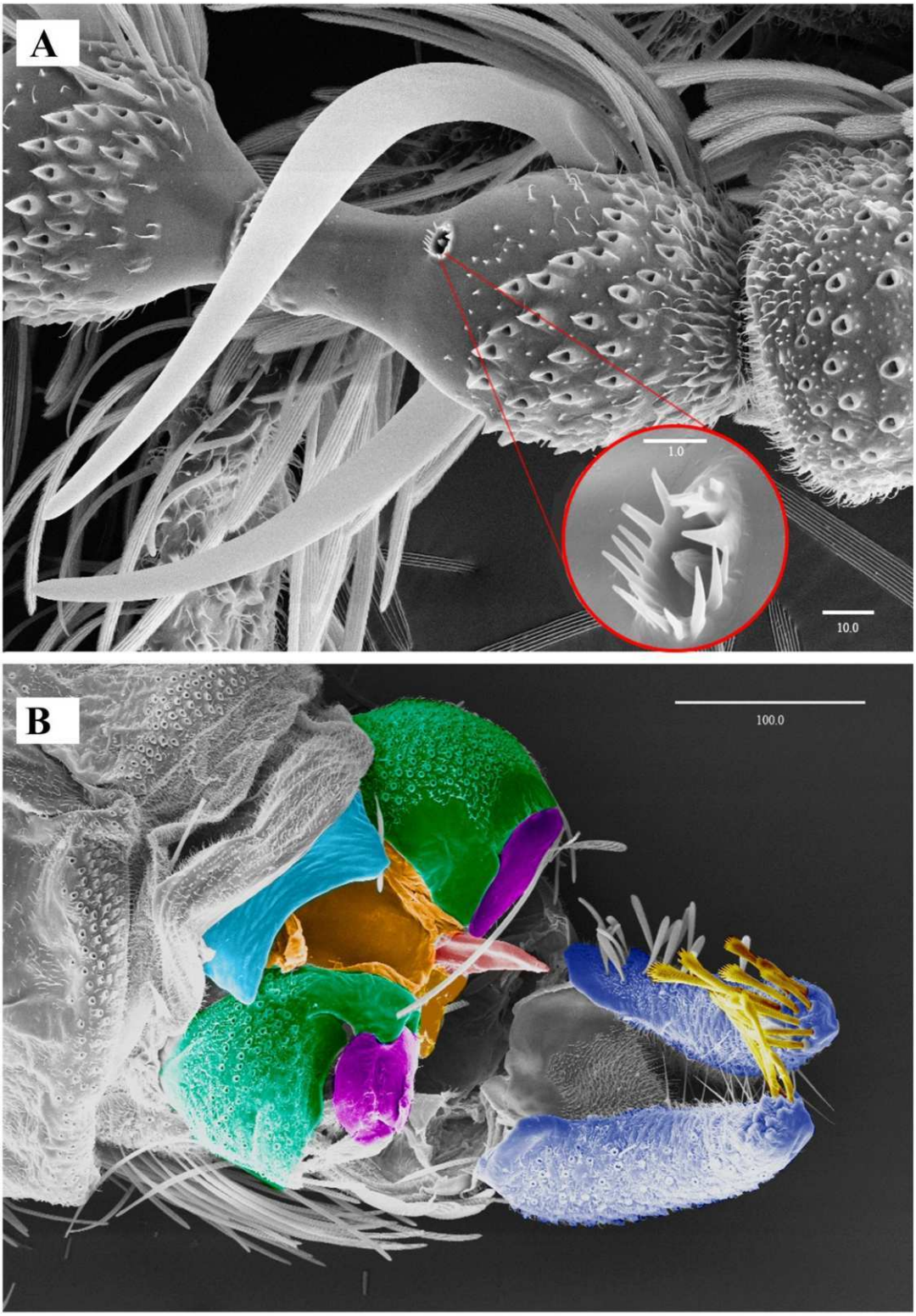

Figure 6. *Lepiseodina latipennis* (Sarà, 1953), male. SEM pictures. (**A**) First flagellomere (3rd antennal segment), red circle higher magnification of sensilla. (**B**) Genitalia, light blue = hypandrium, orange = aedeagal sheath, green = gonocoxites, purple = gonostyli, red = aedeagus, dark blue = hypopods, yellow = tenacula. Scale (**A**,**B**) in micrometers (μm).

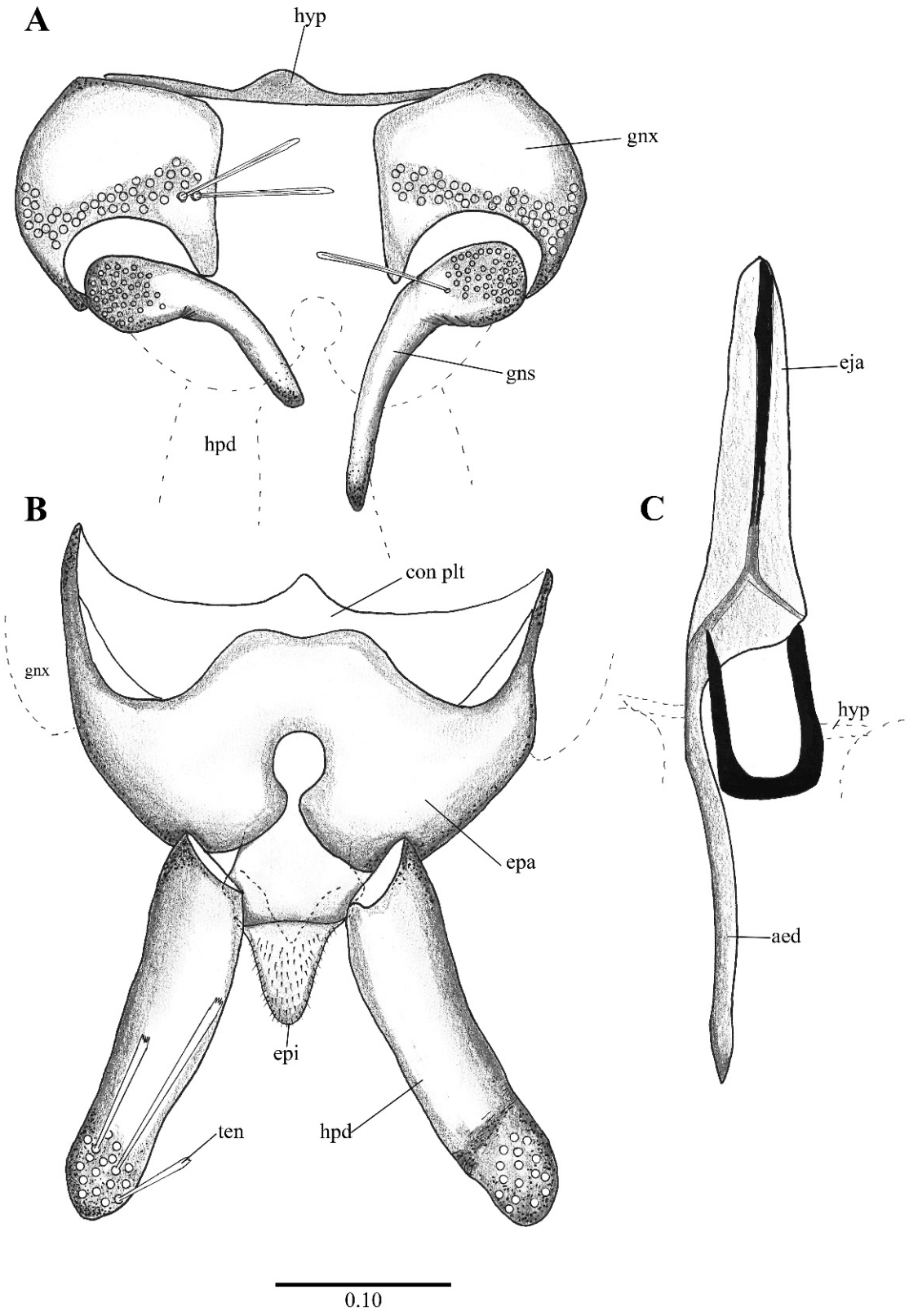

Figure 7. *Lepiseodina tristis* (Meigen, 1830), male genitalia. (**A**) Hypandrium, gonocoxites and gonostylus. (**B**) Epandrium, hypopods, tenacula, hypoproct. (**C**) Aedeagus. Abbreviations: aed = aedeagus, con plat = condyles plate-like, epa = epandrium, eja = ejaculatory apodeme, epi = epiproct, gnx = gonocoxite, gns = gonostylus, ten = tenacula, hpd = hypopods, hyp = hypandrium. Scale (**A**–**C**) in millimeters (mm).

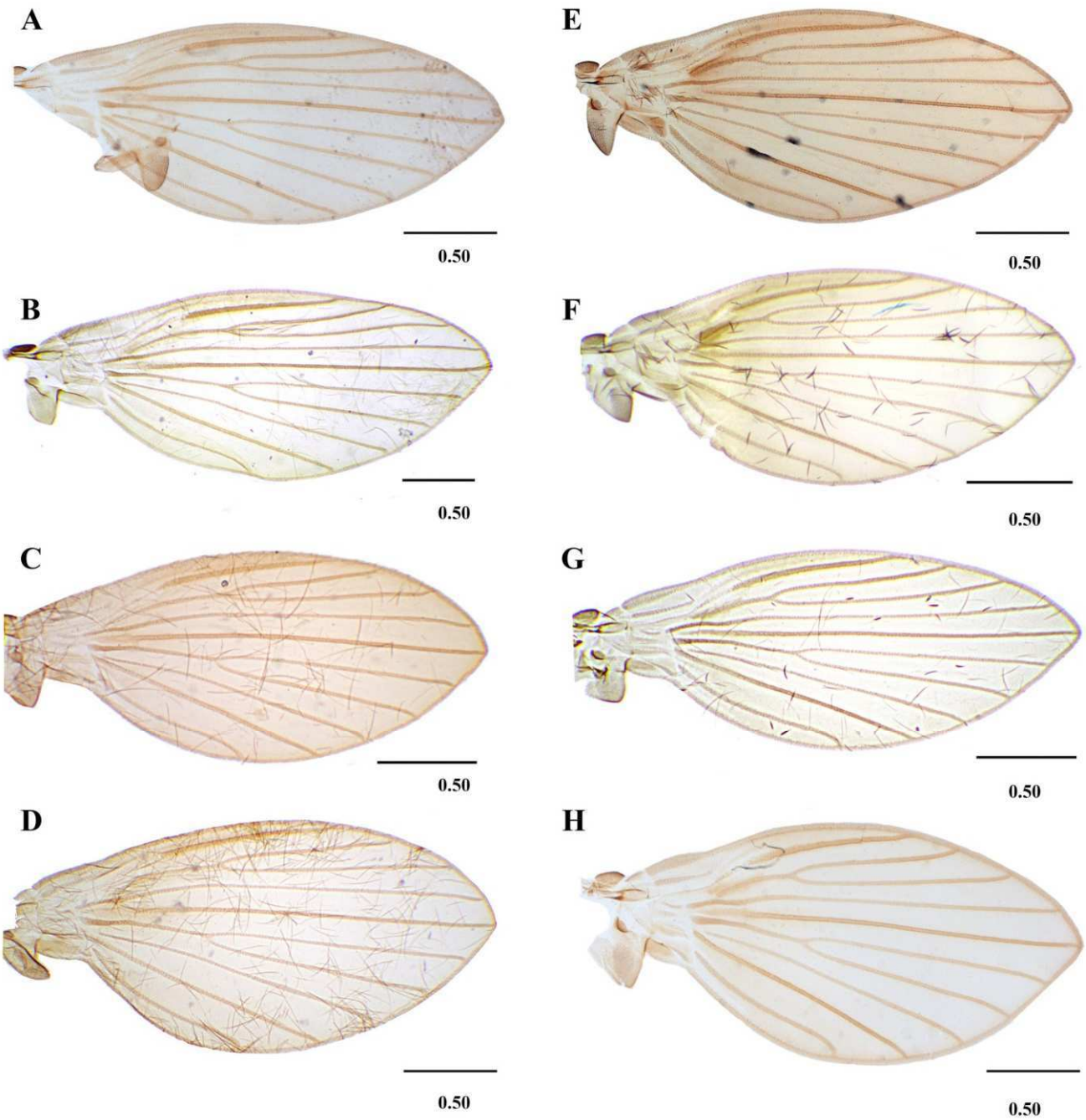

Figure 8. Wings variability of: (**A**–**D**) *Lepiseodina latipennis* (Sarà 1953). (**E**,**F**) *L. tristis* (Meigen, 1830), (**G**,**H**) *L. rothschildi* (Eaton, 1912). Scale (**A**–**H**) in millimeters (mm).

5. Aedeagus asymmetrical, with ejaculatory apodeme broader than distal elements (Figures 3C, 4D, 5C, 6B and 7C); ascoids digitiform or S-shaped … ***Lepiseodina*** **Enderlein, 1936**

- Aedeagus symmetrical, with ejaculatory apodeme narrower than distal elements (Figures 12C and 13C); ascoids leaf-shaped or digitiform … ***Telmatoscopus*** **Eaton, 1904**

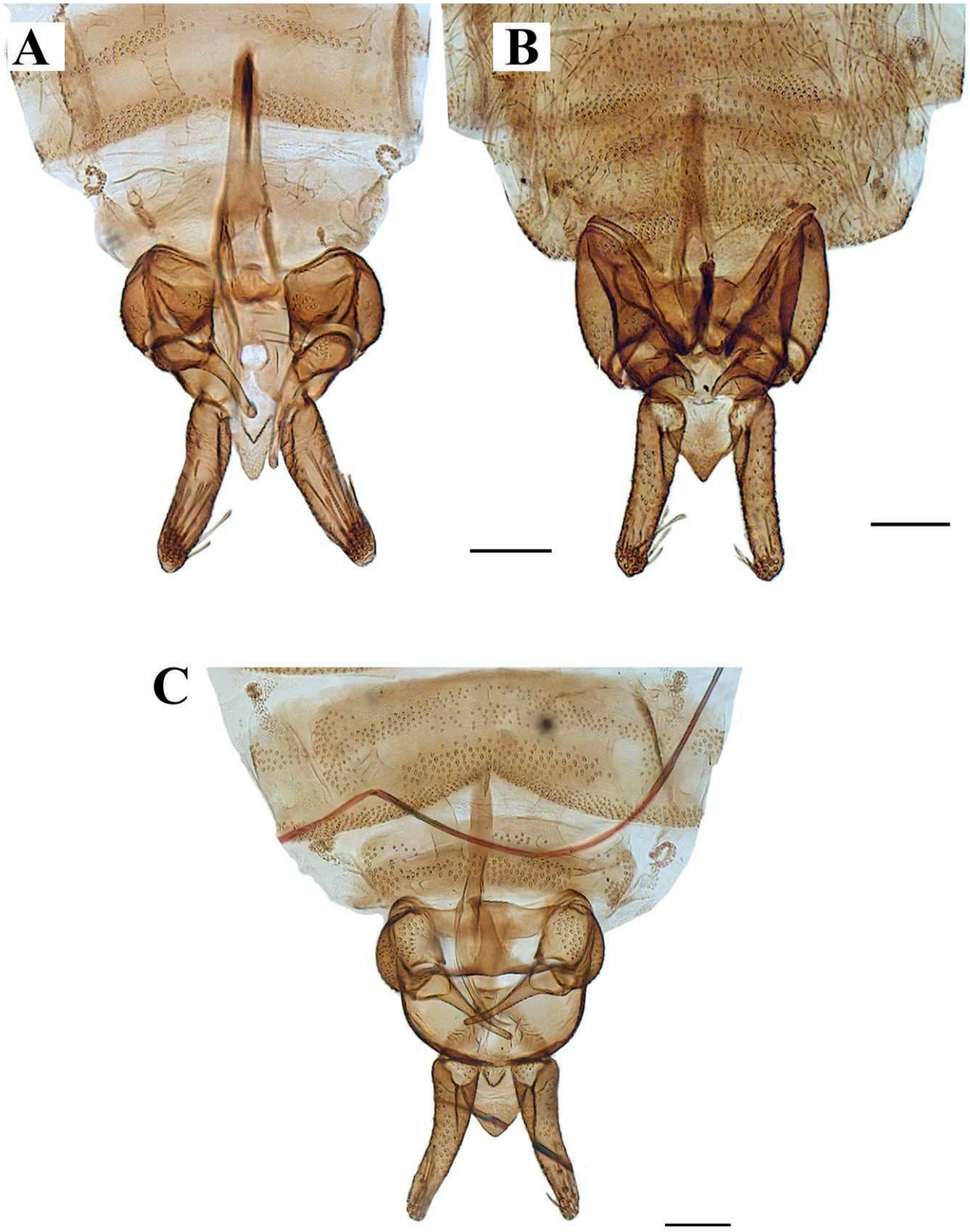

Figure 9. Genitalia of: (**A**) *Lepiseodina tristis* (Meigen, 1830), (**B**) *Lepiseodina rothschildi* (Eaton, 1912), (**C**) *Lepiseodina latipennis* (Sarà, 1953). Scale (**A**–**C**) in millimeters (mm), scale lines = 0.10 mm.

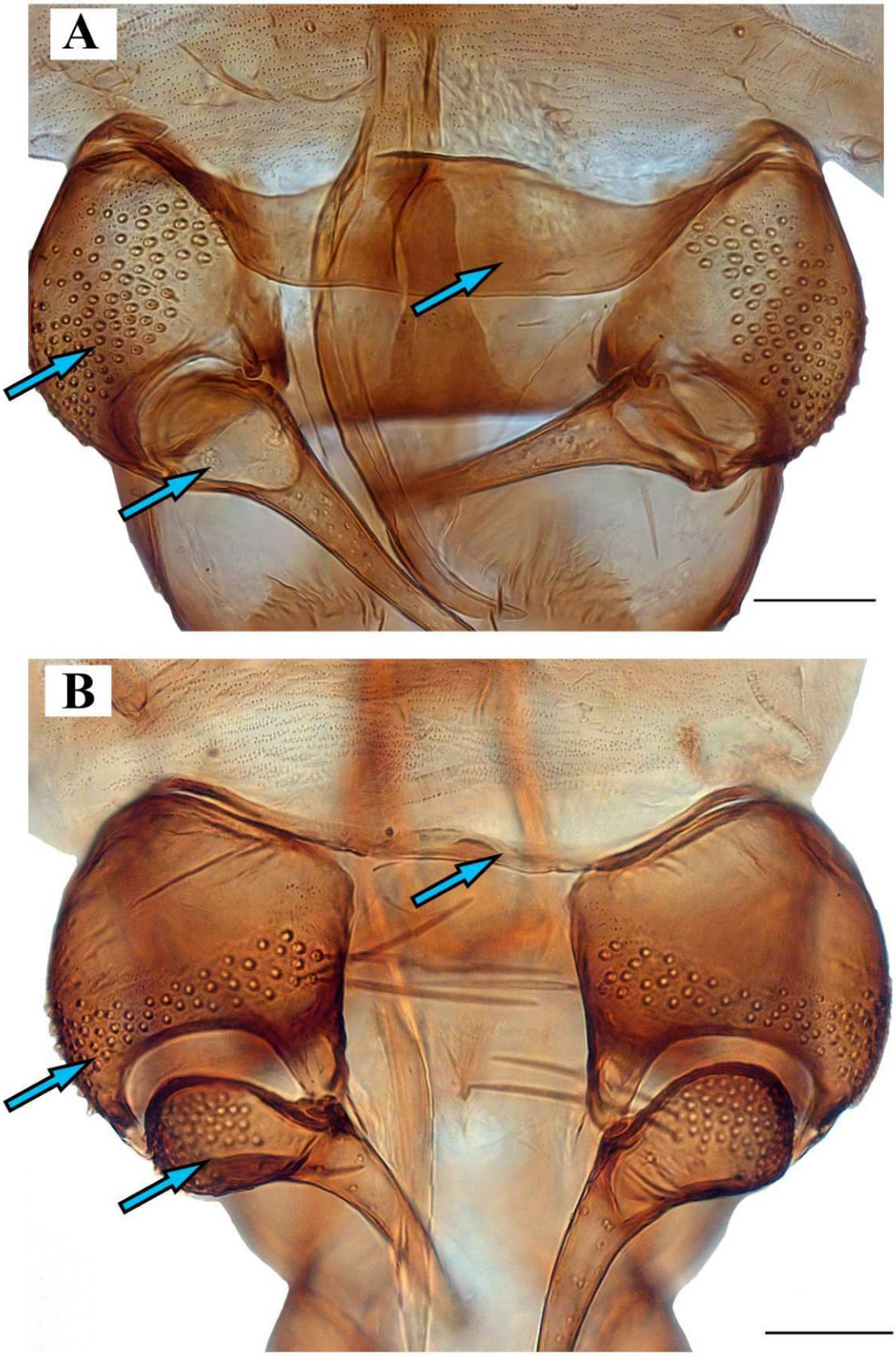

Figure 10. Genitalia of: (**A**) *Lepiseodina latipennis* (Sarà, 1953), (**B**) *Lepiseodina tristis* (Meigen, 1830). Blue arrows show differences in hypandrium, gonocoxite, and gonostyli. Scale (**A**,**B**) in millimeters (mm), scale lines = 0.10 mm.

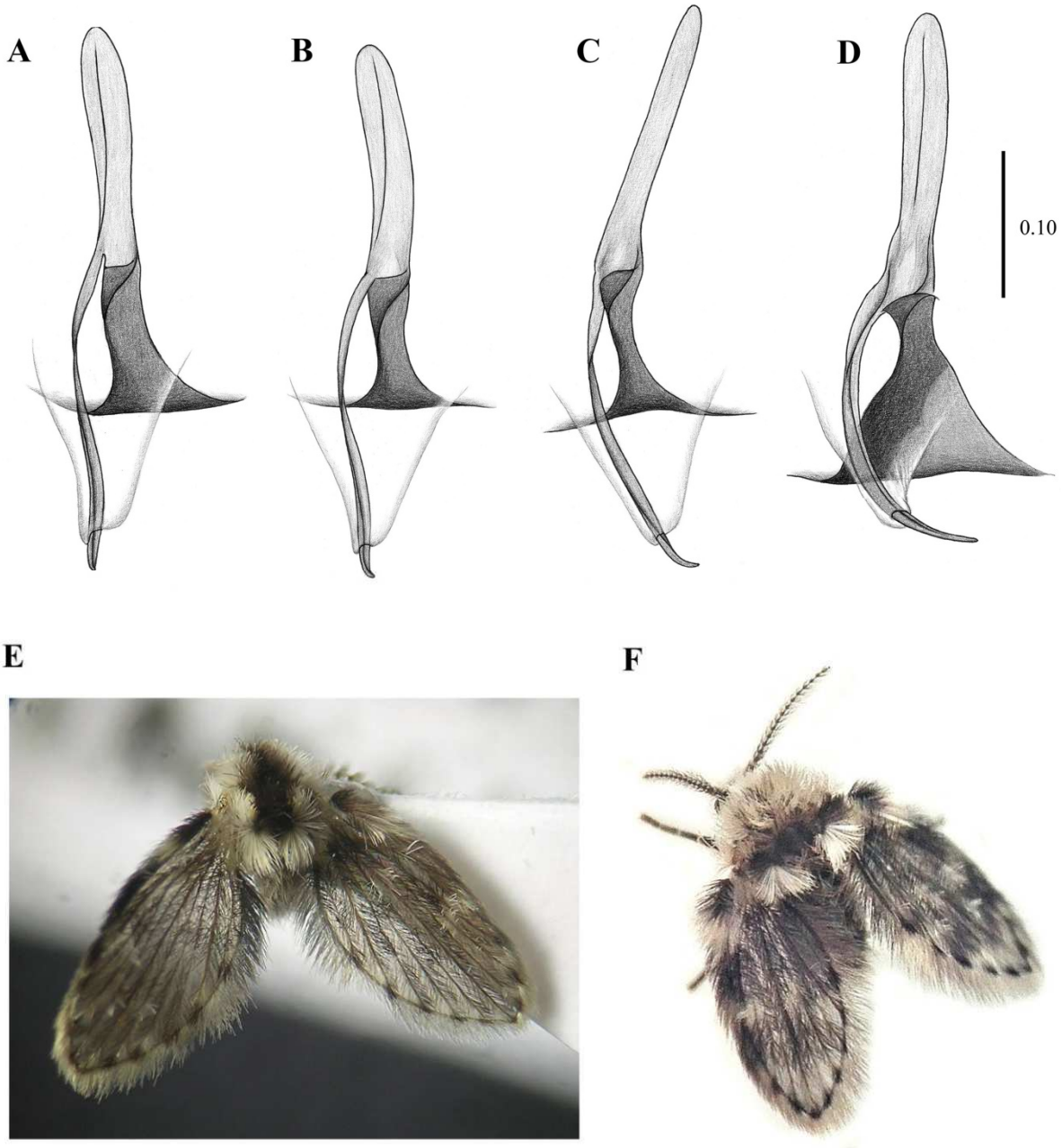

Figure 11. Genitalia variability of *Lepiseodina latipennis* (Sarà, 1953): (**A**–**C**) Aedeagal complex in dorsal view. (**D**) same in posterodorsal view. (**E**) Habitus of *L. latipennis* (Sarà, 1953), (**F**) Habitus of *L. rothschildi* (Eaton, 1912). Scale (**A**–**D**) in millimeters (mm); without scale (**E**,**F**).

3.2. Systematic Assessment

Genus *Clogmia* Enderlein, 1937

Clogmia Enderlein, 1937: 87. Type species: *Psychoda albipennis* Williston (=*albipunctata* Williston).

Diagnosis. Scape less than 2.5 times its width; flagellomeres symmetrically nodiform, with ascoids variable in shape; flagellomere 14 with elongated apiculus tapering towards apex; wing with radial fork basal to median fork; ejaculatory apodeme Y-shaped, narrow in dorsal view; aedeagus symmetrical in dorsal view.

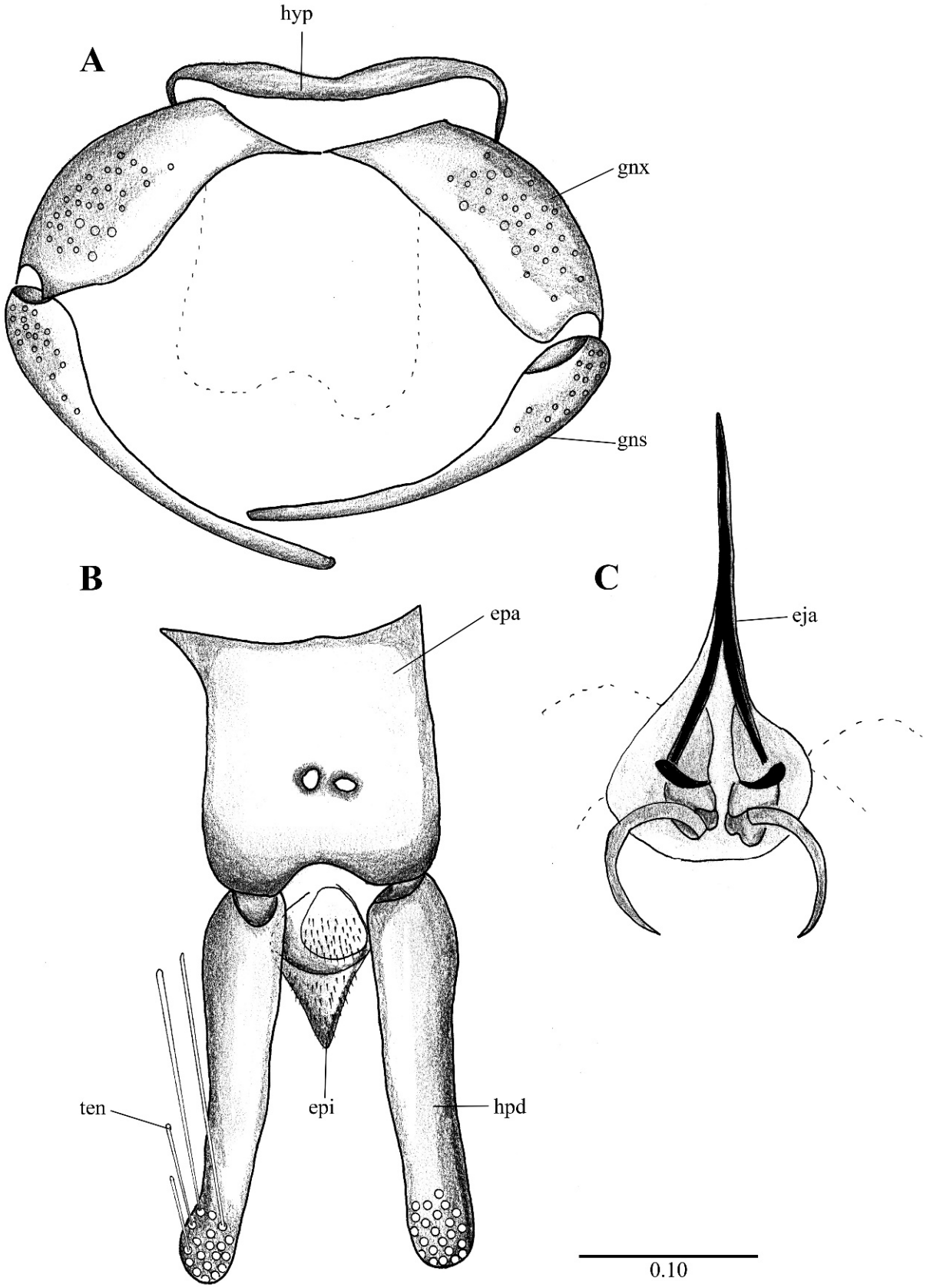

Figure 12. *Telmatoscopus advena* (Eaton, 1893), male genitalia. (**A**) Hypandrium, gonocoxites and gonostylus. (**B**) Epandrium, hypopods, tenacula, hypoproct. (**C**) Aedeagus. Abbreviations: epa = epandrium, epi = epiproct, eja = ejaculatory apodeme, gnx = gonocoxite gns = gonostylus, hpd = hypopod, ten = tenacula, hyp = hypandrium. Scale (**A**–**C**) in millimeters (mm).

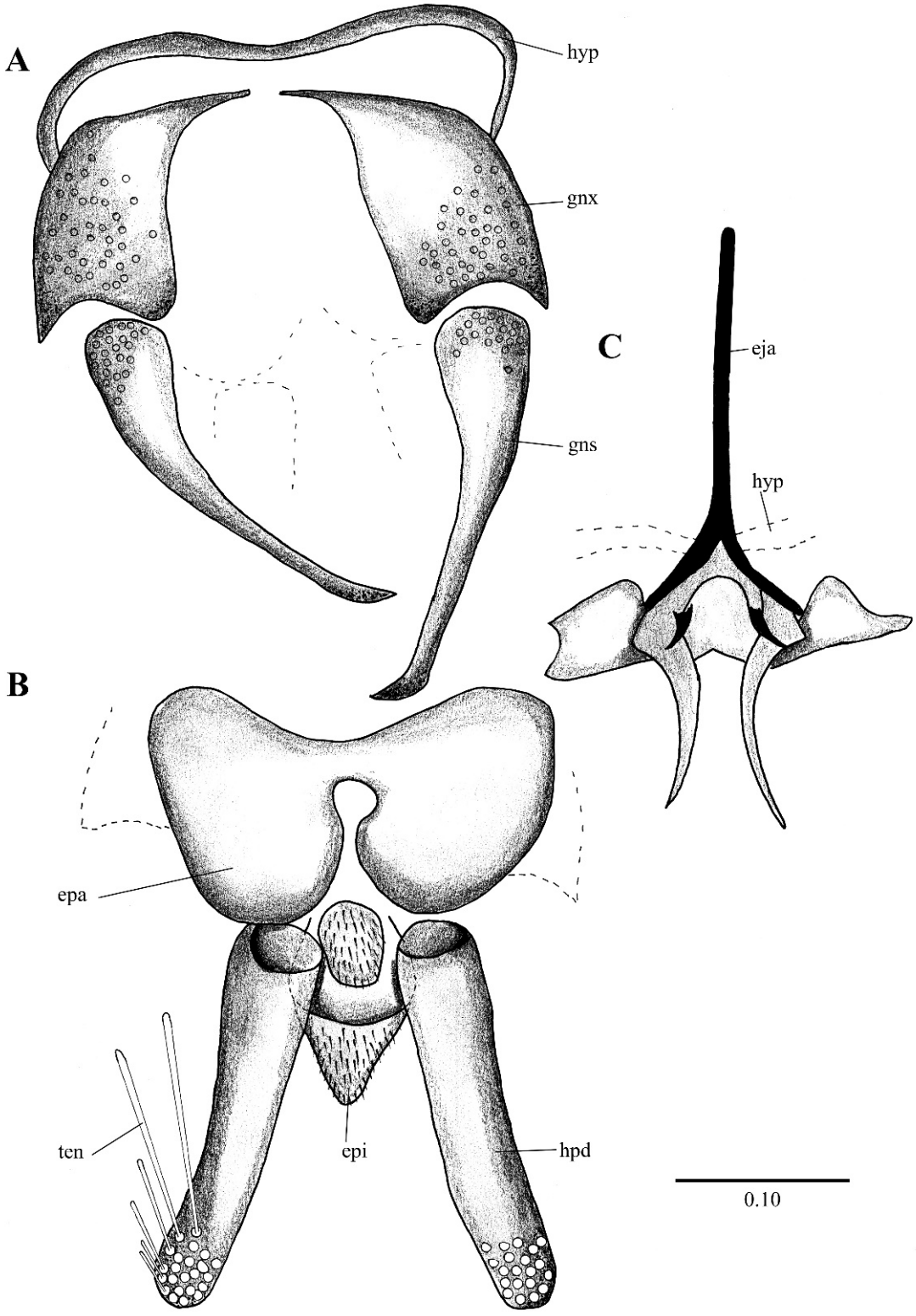

Figure 13. *Telmatoscopus bartai* (Ježek, 2004), male genitalia. (**A**) Hypandrium, gonocoxites and gonostylus. (**B**) Epandrium, hypopods, tenacula, hypoproct. (**C**) Aedeagus. Abbreviations: aed = aedeagus, epa = epandrium, epi = epiproct, eja = ejaculatory apodeme, gnx = gonocoxites, hpd = hypopod, hyp = hypandrium, ten = tenacula. Scale (**A**–**C**) in millimeters (mm).

Species present in Europe associated with dendrotelmata: *C. albipunctata* (Williston, 1893) [35,36] (Table 1).

***Clogmia albipunctata* (Williston, 1893)**

Psychoda albipunctata Williston, 1893: 113. Type locality: Cuba, La Havana.
Pericoma meridionalis Eaton, 1894: 194. Type locality: East Africa.
Psychoda snowii Haseman, 1907: 311. Type locality: USA, Texas, Galveston.
Psychoda legnothisa Spieser, 1909: 44. Type locality: Tanzania.
Psychoda erecta Curran, 1926: 102. Type locality: West Indies.
Pschoda nocturna Abreu, 1930: 115. Type locality: Not given, probably Canary Islands (see Ibáñez-Bernal [40]).
Psychoda nocturna var. *nigrithorax* Abreu, 1930: 115. Type locality: not given, probably Canary Islands.
Telmatoscopus haranti Mirouse, 1958: 93. Type locality: Midi de la France.
Telmatoscopus albipunctatus (Williston): [41] (p. 185).
Clogmia albipunctata (Williston): [42] (p. 42); 351 [11] (p. 351) (see Ibáñez-Bernal [40]).

Diagnosis. Antenna with symmetrically nodiform flagellomeres, each flagellomere with a bifurcate ascoids; eyes separated by 1 facet diameter, eye bridge with 4 facet rows; aedeagus symmetrical, ejaculatory apodeme straight and narrow in dorsal view; hypopods with five-six tenacula, tenacula decreasing in size towards the apex of hypopod.

Examined material: ZFMK-DIP-00081299, ZFMK-DIP-00081508, ZFMK-DIP-00081514, ZFMK-DIP-00081543, ZFMK-DIP-00081624, ZFMK-DIP-00081591, ZFMK-DIP-00082118 [ZFMK].

Distribution. This is one of the most widespread species of Psychodidae in the world, highly invasive and synanthropic. In Europe, it is recorded in Azores Archipelago, Belgium, Canary Islands, Corsica, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Italy, Luxemburg, Madeira Islands, Sardinia, Slovakia, Slovenia, Spain, Sweden and United Kingdom [9,43–47].

Genus *Clytocerus* Eaton, 1904

Clytocerus Eaton, 1904: 59. Type species: *Clytocerus ocellaris* (Meigen, 1804: 44) by subsequent monotypy of Malloch (1907) (see [48]).

Diagnosis: Cornicula (everted sac-shaped structures on the back surface of the head) are usually present; eye bridge with 3–6 facet rows, separated by 1–3 facet diameters; antennal scape elongate, three-six times longer than wide; flagellomere I and II fused to form a compound flagellomere, with a distal brush of wavy setae [14].

Species present in Europe associated with dendrotelmata: *C. xylophilus* Vaillant, 1983 [37] (Table 1).

***Clytocerus xylophilus* Vaillant, 1983**

Clytocerus xylophilus Vaillant, 1983: 355. Type locality: France.

Diagnosis. Eyes separated by 2 facet diameters, interocular suture absent. Antennal scape 4.75 times longer than wide. Hypopods with 6–7 tenacula.

Examined material. None.

Distribution. France [43].

Remarks. Vaillant [37] mentions under the description of *C. xylophilus* that the larvae of *Clytocerus pulvereus* Vaillant, 1983 can be found in the same substrate as *C. xylophilus*; however, it is referring to the "black humus" found near a river (same substrate mentioned by Krek [49]), and later Vaillant [37] clarifies that only *C. xylophilus* is known as a dendrolimnetic species.

Genus *Lepiseodina* Enderlein, 1937

Lepiseodina Enderlein, 1937: 91. Type species: *Psychoda tristis* Meigen, 1830: 272.

Diagnosis. Frons and clypeus separated and not protruding over eye margin; scape short, less than 2 times length of pedicel; flagellomeres symmetrically nodiform in dorsal view, with a pair of digitiform sinuous ascoids; flagellomere 14 with elongated apiculus; wing vein R_{2+3} not connected to R_4; apex of R_5 ending on, or below wing apex; gonocoxites short, with dorsal projection undeveloped; ejaculatory apodeme narrow in dorsal view; aedeagal complex asymmetric, with a parameral structure more or less sclerotized, connecting aedeagus to the gonocoxal apodemes; hypopods with indistinctly fringed tenacula; epandrium with a single foramen.

Species present in Europe associated with dendrotelmata: *L. rothschildi* (Eaton, 1912), *L. latipennis* (Sarà, 1953), and *L. tristis* (Meigen, 1830) [9,13,23,25,36,38] (Table 1).

***Lepiseodina latipennis* (Sarà, 1953)**

Figures 2, 4, 5, 6, 7A, 8C, 9C, 10 and 11E.

Telmatoscopus latipennis Sarà, 1953: 13. Type locality: Italy, Messina.

Telmatoscopus latipennis Sarà: [50] (p. 53).

Clogmia latipennis (Sarà): [51] (p. 60).

Lepiseodina latipennis (Sarà): [52] (p. 146).

Diagnosis. Male hypandrium broad along its entire length; gonocoxal alveoli distributed in the entire gonocoxal surface; gonostyli without alveoli on the base, twice the length of gonocoxites; epandrium not divided, with a single kidney-shaped foramen; aedeagus incurved; paramere not strongly sclerotized; hypoproct tongue-shaped.

Differential diagnosis. This species is closely related to *Lepiseodina tristis*, but it can be distinguished by the combination of the following characters: hypandrium broad along its entire length with convexity in the basal margin in *L. latipennis* (hypandrium narrow with a medial projection in the basal margin in *L. tristis*); gonocoxal alveoli distributed in the entire gonocoxal surface in *L. latipennis* (gonocoxal alveoli restricted to the apical margin in two or three irregular rows in *L. tristis*); gonostyli without alveoli on the base in *L. latipennis* (gonostyli with alveoli in the base in *L. tristis*); epandrium not divided in the apical margin, with a kidney-shaped foramen in *L. latipennis* (epandrium divided in the apical margin with a rounded foramen in *L. tristis*); aedeagus curved in *L. latipennis* (aedeagus straight in *L. tristis*); parameres not strongly sclerotized and almost not visible in *L. latipennis* (parameres strongly sclerotized in *L. tristis*).

Lepiseodina latipennis can be differentiated from *Lepiseodina rothschildi* by the gonostyli twice the length of gonocoxites in *L. latipennis* (gonostyli less than half of the length of gonocoxite in *L. rothschildi*), apical margin of hypandrium convex in *L. latipennis* (apical margin of hypandrium concave in *L. rothschildi*), and parameres not strongly sclerotized and almost not visible in *L. latipennis* (paramere strongly sclerotized in *L. rothschildi*).

Redescription. Measurements in mm (mean, SD = 0.005, n = 2). Wing length 2.53 (2.20–3.30), wing width 0.98 (1.02–1.32). Head length 0.50 (0.48–0.59), head width 0.63 (0.53–0.65). Antennal segments, scape length: 0.11 (0.09–0.11), pedicel: 0.08 (0.70–0.90), postpedicel: 0.10 (0.10–0.13), flagellomeres average length 0.11; Palpomeres 1: 0.10 (0.08–0.10), 2: 0.25 (0.20–0.27), 3: 0.23 (0.20–0.24), 4: 0.29 (0.24–0.29).

Male. Head: eye bridge with rows of four facets (rarely 3 facets); frontal patch undivided, with an irregular row of alveoli extending towards the interocular suture, the whole frontal patch together with the irregular row resemble the shape of a handbell; interocular suture as an inverted "v". No alveoli of supra ocular setae are present. Labella bulbous, longer than wide, with 10–12 small setae. Antenna with scape cylindrical, 1.34 times longer than its width, 1.43 (1.2–1.4) times the length of pedicel; pedicel spherical; 14 nodiform flagellomeres with basal bulb and distal neck, apical flagellomere with long apiculus, apiculus almost half the length of the flagellomere (Figure 4A); antennal ascoids slightly flattened, digitiform, sinous (S-shaped), total length about 1.88 the length of flagellomeres; flagellomeres with a sensilla (as shown in Figure 6A). Palpal segment proportions 1.0:2.63:2.37:3.03.

Thorax with no particular characters.

Wing: Length about 2.5 (2–2.5) times its width, variable in shape, with anterior margin more or less curved; membrane bare except on veins; hyaline with a slight infuscation on costal cell; Sc straight, ending at level of the origin of R_{2+3}; Origin of R_{2+3} not joining R_4, a little distal to the origin of M_{1+2}; origin of M_{1+2} broad and rounded; forks of R_{2+3} and M_{1+2} almost at the same level, the fork of R_{2+3} being slightly distal to M_{1+2}; R_5 ending at wing apex; CuA ending in wing margin at the level of R_{2+3} fork.

Abdomen. Without any particular characters.

Genitalia. Hypandrium plate-like, broad, basal margin with medial projection widely rounded, distal margin almost straight; gonocoxites longer than wide, covered in alveoli

on almost all the surface; gonostyli about 1.5 times the length of gonocoxites, tapering towards apex, almost straight; gonocoxal apodemes poorly distinguishable in dorsal view, in ventral view plate-like, strongly sinuous, fused and narrow at livel of the midline; parameres forming a single slight sclerotized structure, pyriform, resembling the upper half of a bowling pin connecting like a bridge aedeagus to gonocoxal apodemes (as in Figures 4D, 5C, 6B, 9C and 10A); epandrium subrectangular, about twice wider than long with a single kidney-shaped foramen; hypopods slightly out curved, apex rounded, with 9–13 tenacula (as in Figures 4B, 5C and 9C); epiproct short, triangular and covered in pilosity; hypoproct broad and tongue-shaped, covered in pilosity, extending towards mid of hypopods. Aedeagus with ejaculatory apodeme digitiform, narrow 7.5 times longer than its width, about 1.5 times the length of gonocoxites; distiphallus about the same length of ejaculatory apodeme, incurved, extending towards the apex of gonostyli, both ejaculatory apodeme and distiphallus form a single complex, jointed with parameral structure and encircles by a membranous parameral sheath.

Female. Unknown.

Remarks. The holotype (slide mounted) is quite dark, making the observation of structures difficult. The head is dissected, and quite difficult to see clearly, one antenna is dissected and the other antenna is missing, one complete palpus is dissected, the other palpus is missing. The hypopods are dissected and placed apart from each other, one gonocoxites and gonostylus are dissected and placed apart from the genitalia, the remaining parts of the genitalia (aedeagal complex, one gonocoxites and gonostylus) are found together. One wing is dissected and separated from the thorax. On the original description Sarà (1953) mentions the shape of the hypoproct being trilobed (Figure 2), after examination of the holotype in the preparation the hypoproct looks indeed trilobed; however, we consider this a malformation on the slide itself, and not the natural shape of the hypoproct, all other examined material present a broad tongue-shaped hypoproct. On the original description Sarà abstained to illustrate or describe the aedeagal complex; however, after examination we are sure all our specimens belong to the same species.

Biology. Some specimens (No. 0190-0193) emerged from organic matter sampled from an *Acer* sp. dendrotelma (Figure 14), in a mixed submontan forest, additionally, the specimen used for the SEM pictures was collected in a Malaise trap next to dendrotelmata of an oak (*Quercus* sp.) tree, suggesting that also *L. latipennis* is a dendrolimnetic species. In Germany altitude ranges from 250–280 m a.s.l. (meters above sea level), while in Italy it has a range from 40–800 m a.s.l.

Examined material. *Holotype* examined [MNGD], ZFMK-DIP-00081595, ZFMK-DIP-00081552 [ZFMK], Specimen No. 0039, 0488, 0487, 0439, 0190, 0191, 0192, 0193 [AM].

Distribution: Previously known only from the type locality in Messina (Sicily, Italy). The records here reported are the first from Germany (Rheinland-Pfalz) and for northern and central Italy.

***Lepiseodina rothschildi* (Eaton, 1912)**

Figures 3, 8G,H and 9B

Telmatoscopus rothschildii Eaton, 1912: 7. Type locality: England, London, Hyde Park.

Telmatoscopus rotschildi (Eaton): *Lapsus calami* of [50], followed by Salamanna in Dahl et al. [53] (see below).

Clogmia rothschildi (Eaton) [ICZN (1999): art. 33.3.1: incorrect subsequent spelling in prevailing usage is deemed to be a correct original spelling and maintained; see Ježek (2004)]: Vaillant (1982a): 298; (1982b): 206; Wagner (1990): 60; Bernotienė (2002): 7.

Clogmia rotschildi (Eaton): Dahl et al. [53] (p. 33).

Lepiseodina rothschildi (Eaton): Ježek [52] (p. 146).

Diagnosis. Male gonostyli are half the length of gonocoxites; paramere strongly sclerotized, overlapping to the lateral branch of aedeagus; hypandrium W-shaped, broad on the entire surface with the apical margin concave.

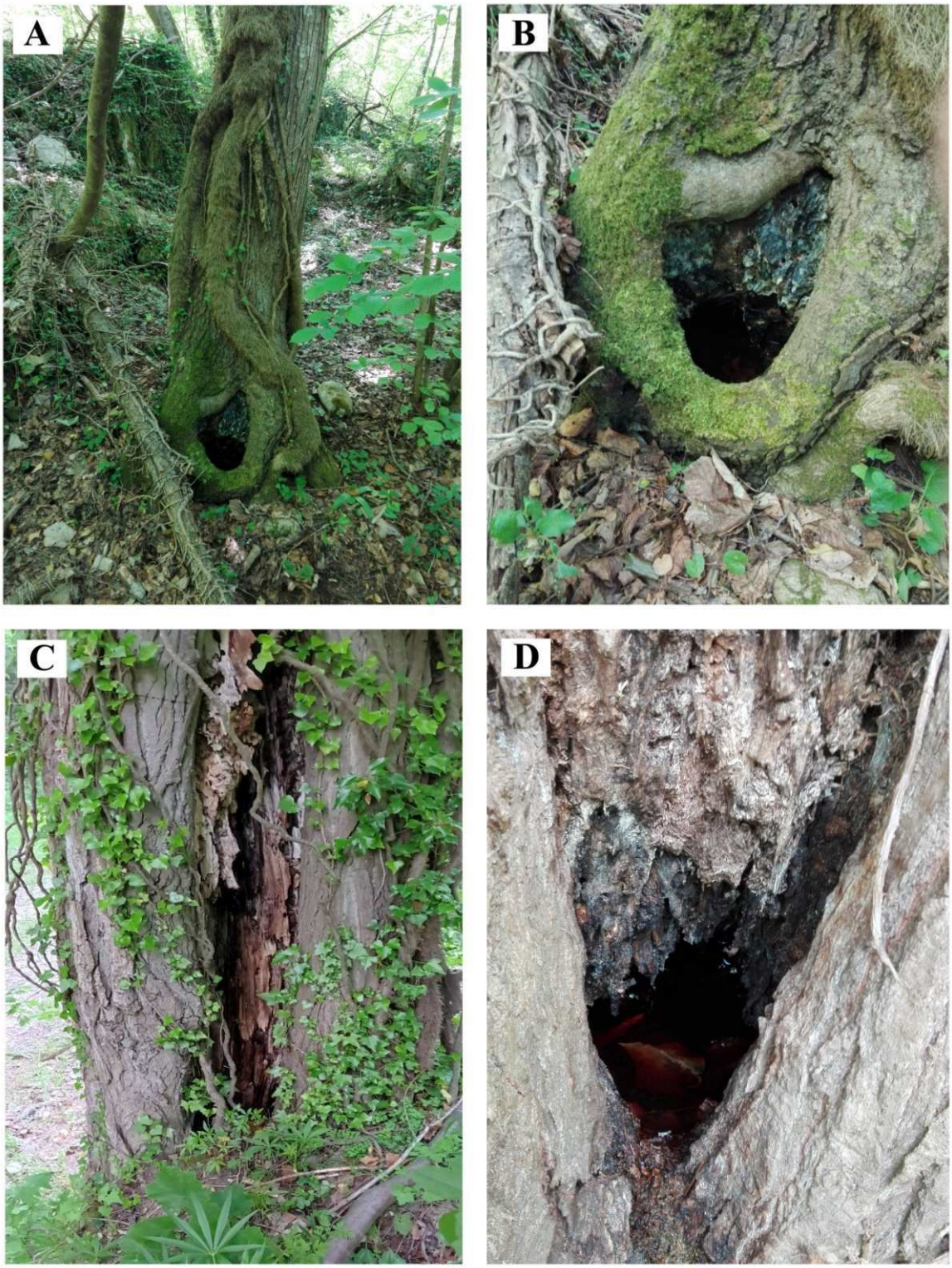

Figure 14. (**A**,**B**) Habitat of *Psychoda cinerea* Banks, 1894, dendrotelma of hornbeam (*Carpinus* or *Ostrya* sp.). (**C**,**D**) Habitat of *Lepiseodina tristis* (Meigen, 1830), rotting tree hole with basal dendrotelma of *Populus nigra*. All in a mixed submontan forest (Foro Valley, central Italy).

Examined material: ZFMK-DIP-00081311, ZFMK-DIP-00081322, ZFMK-DIP-00081323, ZFMK-DIP-00081324, ZFMK-DIP-00081327, ZFMK-DIP-00081328, ZFMK-DIP-00081329, ZFMK-DIP-00081330, ZFMK-DIP-00081510, ZFMK-DIP-00081537, ZFMK-DIP-00081551, ZFMK-DIP-00081558, ZFMK-DIP-00081559, ZFMK-DIP-00081567, ZFMK-DIP-00081574,

ZFMK-DIP-00081623, ZFMK-DIP-00082122, ZFMK-DIP-00082126, ZFMK-DIP-00082147, ZFMK-DIP-00082149 [ZFMK]. Specimens number: 13666, 13689, 13694, 13625, 13628, 13822, 18655, 18490, 18225, 19261, 19110, 16352, 16489, 18408, 19469, 17902, 21769, 12319, 12320, 21533, 21114 [NMP]. Specimens number: 0489 [AM].

Distribution. Austria, Belgium, Bulgaria, Czech Republic, Finland, France, Germany, Ireland, Italy, Lithuania, Netherlands, Slovakia, Spain, United Kingdom [9,13,54,55].

***Lepiseodina tristis* (Meigen, 1830)**

Figures 7, 8E,F, 9A and 10B

Psychoda tristis Meigen, 1830: 272. Type locality: Not specified, probably Belgium or Germany.

Psychoda (Pericoma) tristis (Meigen): [56] (p. 16).

Pericoma tristis (Meigen): [57] (p. 17).

Telmatoscopus tristis (Meigen): [58] (p. 170).

Lepiseodina tristis (Meigen): [42]) (p. 91); [52] (p. 146).

Clogmia tristis (Meigen): [53] (p. 33); [59] (p. 7).

Diagnosis. Male hypandrium narrow, with a small abrupt median projection; gonocoxal alveoli restricted to two irregular lines on the apical surface; gonocoxites with a patch of alveoli at base; two parameres strongly sclerotized.

Examined material: ZFMK-DIP-00081512, ZFMK-DIP-00081513, ZFMK-DIP-00081563, ZFMK-DIP-00081583, DIP-ZFMK-00081622, ZFMK-DIP-00082119, ZFMK-DIP-00082124, ZFMK-DIP-00082125, ZFMK-DIP-00082128, ZFMK-DIP-00082130, ZFMK-DIP-00082133, ZFMK-TIS-2628581, ZFMK-TIS-2628542, ZFMK-TIS-2628588, ZFMK-TIS-2628587, ZFMK-TIS-2628608 [ZFMK]. Specimen number: 24339, 13701, 13668, 13633, 13823, 17331, 17270, 20777, 20243, 20077, 20076, 20080, 20079, 20088, 20067, 3226, 33282, 34041, 34042, 34043, 34044, 20987, 21365, 23772, 24339 [NMP]. Specimen number: 0490, 0491 [AM].

Distribution. Algeria, Austria, Belgium, Croatia, Czech Republic, France (incl. Corsica), Germany, Ireland, Italy, Lithuania, Slovakia, and United Kingdom [25,36,43,50,53,59,60]. In Italy, the species is known only from two old and uncertain records for the northern and peninsular region [53,61]. The specimens here reported confirm the occurrence of this species in the Italian peninsula.

Key to the European adult males of *Lepiseodina*

1. Gonostyli longer than gonocoxites . . . **2**

\- Gonostyli about half the length of gonocoxites (Figure 3A) . . . ***L. rothschildi***

2. Hypandrium narrow, with a small medial projection on basal margin (Figure 7A); alveoli in gonocoxites restricted in two or three irregular rows in the apical margin (Figures 9A and 10B); gonostyli with alveoli on the base (Figures 9A and 10B); two parameres strongly sclerotized (Figure 7C) . . . ***L. tristis***

\- Hypandrium broad in its entire length, without medial projection (Figure 5A); alveoli in gonocoxites scattered in the whole surface (Figure 5A); gonostyli without alveoli on the base (Figure 5A); one parameral structure not strongly sclerotized (Figure 5C) . . . ***L. latipennis***

Genus *Pneumia* Enderlin, 1935

Pneumia Enderlein, 1935: 247. Type species: *Pericoma palustris* Meigen, 1804 (see [62]).

Diagnosis. Eye bridge with 6 facet rows. Antennal flagellomeres barrel-shaped, basal flagellomeres lacking spines or clusters of stiff setae; apical flagellomere with a digitiform apical protuberance about as long as or longer than the basal part of the flagellomere carrying it. Aedeagal complex symmetrical; gonostyli bulbose and elongate at the base, tapering towards the apex.

Species present in Europe associated with dendrotelmata: *Pneumia canescens* (Meigen, 1818) and *Pneumia trivialis* (Eaton, 1893) [18,23,25] (Table 1).

***Pneumia canescens* (Meigen, 1818)**

Trichoptera canescens Meigen, 1818: 45. Type locality: not given, probably Germany.

Pericoma canescens (Meigen): 20 (p. 156).

Pneumia canescens (Meigen): [63] (p. 124).

Diagnosis. Eye bridge with 5–6 facet rows: wing vein fork R_{2+3} with a backward projection (as in [11] (Figure 12)); hypopods with 10 tenacula; ejaculatory apodeme wider at the base than apex; aedeagal complex longer than gonocoxites, tapering towards apex.

Examined material. NMP-2687, NMP-2913, NMP-10787, NMP-17243 [NMP].

Distribution: Afghanistan, Armenia, Austria, Azerbaijan, Belgium, Bulgaria, China, Czech Republic, Denmark, France, Georgia, Germany, Greece, Hungary, Kyrgyzstan, Lithuania, Netherlands, Poland, Romania, Russia (Novosibirsk Region), Slovakia, Sweden, Turkey and United Kingdom [40,56,57].

Pneumia trivialis (Eaton, 1893)

Pericoma trivialis Eaton, 1893: 121. Type locality: Great Britain.

Pneumia trivialis (Eaton): [23] (p. 202); [63] (p. 116).

Diagnosis. Eye bridge with 7 facet rows; frons not extending beyond first palpal segment; all pal segments of similar width; fore tibia straight and not engrossed; wing vein fork R_{2+3} without a backward projection; hypopods with 7 or fewer tenacula; ejaculatory apodeme straight, not wider at base; aedeagal complex shorter than gonocoxites, not tapering towards apex, distal part of aedeagus smooth without wrinkles.

Examined material. ZFMK-DIP-00081529, ZFMK-DIP-00081536, ZFMK-DIP-00081541, ZFMK-DIP-00081576, ZFMK-DIP-00081601, ZFMK-DIP-00081602, ZFMK-DIP-00081603, ZFMK-DIP-00081604, ZFMK-DIP-00081605, ZFMK-DIP-00081606, ZFMK-DIP-00081607, ZFMK-DIP-00081610, ZFMK-DIP-00081999, ZFMK-DIP-00082001, ZFMK-DIP-00082002, ZFMK-DIP-00082003, ZFMK-DIP-00082006, ZFMK-DIP-00082007, ZFMK-DIP-00082008, ZFMK-DIP-00082009, ZFMK-DIP-00082010, ZFMK-DIP-00082011, ZFMK-DIP-00082012, ZFMK-DIP-00082013, ZFMK-DIP-00082014, ZFMK-DIP-00082015, ZFMK-DIP-00082016, ZFMK-DIP-00082017, ZFMK-DIP-00082029, ZFMK-DIP-00082031, ZFMK-DIP-00082032, ZFMK-DIP-00082033, ZFMK-DIP-00082034, ZFMK-DIP-00082035, ZFMK-DIP-00082036, ZFMK-DIP-00082037, ZFMK-DIP-00082039, ZFMK-DIP-00082040, ZFMK-DIP-00082041, ZFMK-DIP-00082042, ZFMK-DIP-00082043, ZFMK-DIP-00082044, ZFMK-DIP-00082045, ZFMK-DIP-00082046, ZFMK-DIP-00082047, ZFMK-DIP-00082048, ZFMK-DIP-00082060, ZFMK-DIP-00082061, ZFMK-DIP-00082062, ZFMK-DIP-00082063, ZFMK-DIP-00082064, ZFMK-DIP-00082065, ZFMK-DIP-00082066, ZFMK-DIP-00082068, ZFMK-DIP-00082069, ZFMK-DIP-00082070, ZFMK-DIP-00082071, ZFMK-DIP-00082072, ZFMK-DIP-00082073, ZFMK-DIP-00082074, ZFMK-DIP-00082076, ZFMK-DIP-00082077, ZFMK-DIP-00082078, ZFMK-DIP-00082079, ZFMK-DIP-00082080, ZFMK-DIP-00082081 [ZFMK].

Distribution: Austria, Azerbaijan, Belgium, Bosnia–Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Hungary, Ireland, Netherlands, Norway, Poland, Portugal, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine and United Kingdom [43,55,64,65].

Genus *Psychoda* Latreille, 1797

Psychoda Latreille, 1796: 152. Type species: *Tipula phalaenoides* Linnaeus, 1758 by subsequent designation of Quate [41] (p. 191).

Diagnosis. Species with short vertex; labellum flattened and carrying digitiform setae; eyebridge without interocular suture; antenna with 12–14 flagellomeres, those beyond 11th always reduced in size and showing different types of fusion; flagellomeres nodiform with a neck (except apical flagellomeres); ascoids usually with three branches, two anterior and one posterior branch, Y-shaped or with four branches, shaped like a plus sign (+); aedeagus often asymmetrical; hypopods with a single apical tenaculum.

Species present in Europe associated with dendrotelmata: *P. alternata* Say, 1824, *P. cinerea* Banks, 1894 and *P. minuta* Banks, 1894. [23,25] (Table 1).

***Psychoda alternata* Say, 1824**

Psychoda alternata Say, 1824: 358. Type locality: USA, Pennsylvania, Philadelphia.

Psychoda tripunctata Macquart, 1838: 85. Type locality: not given, probably France.

Psychoda marginepunctata von Roser, 1840: 50. Type locality: not given.

Psychoda sexpunctata Phillipi, 1865: 631. Type locality: not given.

Psychoda schizura Kincaid, 1899: 21. Type locality: USA, Seattle, Washington.
Psychoda nocturnala Haseman, 1907: 319. Type locality: USA, Missouri, Columbia.
Psychoda floridica Haseman, 1907: 324. Type locality: USA, Florida.
Psychoda bengalensis Brunetti, 1908: 371. Type locality: India, Calcutta and Simla.
Psychoda albimaculata Welch, 1912: 411. Type locality: USA, Illinois.
Psychoda dakotensis Dyar, 1926: 109. Type locality: USA, South Dakota.
Psychoda alternata var. *marmosa* Abreu, 1930: 123. Type locality: not given.
Psychoda alternata var. *floridica* Haseman: Johannsen, 1934: 25.
Tinearia alternata (Say): [66] (p. 142); [63] (p. 114) [67] (online catalogue); [51] (p. 46); [68] (p. 96); [69] (p. 89); [70] (p. 107);
Psychoda (Tinearia) alternata Say: Bravo et al. [71] (p 5, 11).
Psychoda alternata Say: [40] (p. 97). [41] (p. 218) [72] (p. 216); [73] (p. 195); [74] (p. 16); [75] (p. 12); [76] (p 238); [77] (p. 67); [78] (p. 21); [79] (p. 48).

Diagnosis. Eyes separated by 1–3 facet diameters, without interocular suture; antenna with 13 flagellomeres, last three flagellomeres small, flagellomere 11–12 fused, flagellomere 13 smaller and partially fused to 12; labellum with one short and four long teeth and four setae; aedeagus asymmetrical, with on paramere thicker than aedeagus, ejaculatory apodeme broad posteriorly, longer than aedeagal complex; hypopods 2.3 times longer than gonostyli. [40].

Examined material. None.

Distribution. Cosmopolitan [40,55]. In Europe, it is present in Austria, Balearctic Islands, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Dennmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Sardinia, Slovakia, Slovenia, Spain, Sweden, Switzerland, and United Kingom [55].

***Psychoda cinerea* Banks, 1894**

Psychoda cinerea Banks, 1894: 331. Type locality: USA, New York, Sea Cliff, L. I.
Threticus compar Eaton, 1904: 57. Type locality: Algeria, England, Ireland, and Maderia.
Psychoda prudens Curran, 1924: 219. Type locality: Canada, Alberta.
Psychodocha cinerea (Banks): [66] (p. 135); [80] (p. 100).

Diagnosis. Eyes separated by 1–2 facet diameters, without interocular suture; antenna with 14 flagellomeres, last 3 separated not fused; labellum with four terminal digitiform setae and up to 5 setiform setae; medial fork with basal swelling; aedeagal complex slightly S-shaped, asymmetrical, with unpaired bent paramere; aedeagus subtriangular, covering a squarish structure; ejaculatory apodeme triangular at base in dorsal view; hypandrium trapezoidal; hypopods as long as gonostyli, gradually narrowing toward the apex, but without basal swelling.

Examined material. Specimens number: No. 0480-4081 [AM] Reared from dendrotelmata of a hornbeam (*Carpinus* or *Ostrya* sp.).

Distribution. Cosmopolitan [71]. In Europe, it is present in Austria, Azores archipelago, Belgium, Bosnia–Herzegovina, Bulgaria, Canary Islands, Corsica, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxemburg, Madeira Islands, Netherlands, Norway, Poland, Romania, Sardina, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, and United Kingdom [43,49].

***Psychoda minuta* Banks, 1894**

Psychoda minuta Banks, 1894: 331. Type locality: USA, New York, near Sea Cliff.
Psychoda marylandana Del Rosario, 1936: 111.
Psychoda spreta Tonnoir, 1940: 57.
Psychodula minuta (Banks): [52] (p. 56).

Diagnosis. Eyes separated by 1 facet diameters, without interocular suture; antenna with 16 flagellomeres, last 4 flagellomeres reduced in size and fused; labellum with four terminal digitiform setae and two trichiform setae; aedeagus asymmetrical, flanked by two large triangular plates of morphologically unknown origin; gonostyli narrowing to tapered point in apical fifth; hypandrium narrow, not trapezoidal; hypopods much longer than gonostyli, with basal swelling.

Examined material. None.

Distribution. Holarctic. In Europe, it is present in Austria, Balearic Islands, Belgium, Bulgaria, Corsica, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Madeira Islands, Netherlands, Norway, Romania, Sardinia, Slovakia, Slovenia, Spain, Sweden, Switzerland, and United Kingdom [25,43,59,81].

Genus *Telmatoscopus* Eaton, 1904

Telmatoscopus Eaton, 1904: 58. Type species: *Pericoma advena* Eaton, 1893, by designation of Quate [82].

Sciria Enderlein, 1935: 247. Type species: *Pericoma advena* Eaton, 1893, by original designation (see [83]).

Krivosheinoscopus Ježek, 2001: 57 Type species: *Telmatoscopus ussuricus* (Ježek, 2001) **syn. nov.**

Diagnosis (modified from Kvifte, [83]). Frons and clypeus separated and not protruding over eye margin; flagellomeres asymmetrically nodiform, with paired leaf-shaped to digitiform ascoids; flagellomere 14 with elongated apiculus; wing veins R_{2+3} not connected to R4; apex of R5 ending at wing apex; ejaculatory apodeme narrow in dorsal view and distally ending in two short branches with membranous connection to aedeagal complex; aedeagal complex symmetrical; aedeagal complex encapsulated in a parameral sheath; hypopods with indistinctly fringed tenacula; epandrium with a single foramen.

Remarks: Ježek [84] described *Krivosheinoscopus* based on five male specimens of the type species *K. ussuricus* Ježek, 2001 (now *Telmatoscopus ussuricus* **comb. nov.**) from the Russian Far East. Later, he described *K. bartai* Ježek, 2004 (now *Telmatoscopus bartai* **comb. nov.**) from the Czech Republic (Ježek 2004). Ježek [84] provided a diagnostic table of closely related telmatoscopoid genera, namely, *Lepiseodina*, *Sciria* (=*Telmatoscopus*), *Iranotelmatoscopus*, *Krivosheinoscopus*, *Telmatoscopus* auctt. (=*Seoda*) [84] (p. 60).

In the table, seven morphological characters were provided to differentiate *Krivosheinoscopus* from *Sciria* (=*Telmatoscopus*), they are numbered and discussed below.

1. The scape/pedicel proportion is 2:1 in *Kvirosheinoscopus* (1:1 in *Sciria*); however, even while the proportion of scape/pedicel length proposed by Ježek [84] is 2:1 in *T. ussuricus*, this is not the case for *T. bartai* where it is 1:1.
2. The ascoids are very long, thin and coiled in *Krivosheinoscopus* (large, flat, leaf or hood-shaped in *Sciria*). This character is contradicted by *Telmatoscopus laurencei* [38], which has digitiform ascoids and coiled, and these character states are polymorphic in *Vaillantodes* Wagner, 2001 and *Panimerus* Eaton, 1913. Intermediary forms also occur in some genera of Telmatoscopoids. Due to widespread polymorphism of this character we do not consider this a reliable genus-level character unless supported by other, independent lines of evidence.
3. The first palpal segment very short and keg-shaped in *Krivosheinoscopus* (long and cylindrical in *Sciria*). This character is variable inside the genus *Telmatoscopus* (e.g., long in *Telmatosocpus advena* and shorter in *Telmatoscopus thuringicus* Beran, Doczkal, Pfister & Wagner, 2010), therefore it is here considered as intrageneric variability, and thus not a diagnostic character.
4. The position of the radial and medial forks of wing venation, at the same distance in *Krivosheinoscopus* (medial fork distad to radial fork in *Sciria*). This is another character presenting as variable within the genus *Telmatoscopus* (e.g., medial fork distal in *T. advena*, medial fork basal in *T. thuringicus*, forks at the same level in *Telmatoscopus bartai*).
5. Two pairs of protuberances in the aedeagal complex in *Krivosheinoscopus* (one pair in *Sciria*). According to the homologization of [83], the absence of protruding parameres in *Telmatoscopus* are not due to absence of parameres, they are present as transverse sclerites within the aedeagal–parameral complex. The difference between *T. advena*, *T. bartai* and *T. ussuricus* in the parameres is a question of degree of development rather than a clear-cut presence/absence question, and intermediate cases occur in Nearctic species (e.g., *T. patibulus* Quate).

6. The hypoproct has a terminal projection, long and narrow in *Krivosheinoscopus* (without a terminal projection, triangular in *Sciria*). This character is contradicted by *Telmatoscopus bartai* **comb. nov.** where the hypoproct is triangular, and not thin and elongated.

With three shared characters between *Krivosheinoscopus* and *Telmatoscopus* (=*Sciria sensu* Ježek) including the wing vein R_5 ending at wing apex, vein Cu ending distal to M_{1+2} and, the ejaculatory apodeme laterally compressed (=basal apodeme of the aedeagal complex in Ježek) [84].

Furthermore, Ježek listed seven (five are commented here) morphological characters separating *Krivosheinoscopus* from *Seoda* Enderlein (considered as *Telmatoscopus* auctt. In Ježek [84]), including:

1. The scape/pedicel proportion, being 2:1 in *Krivosheinoscopus* (3-4:1 in *Seoda*); this remains a diagnostic difference.

2. First palpal segment short and keg-shaped in *Krivosheinoscopus* (long and cylindrical in *Seoda*). This character is variable inside *Telmatoscopus*, and is thus not a diagnostic character.

3. Vein Cu ending distal to M_{1+2} in *Krivosheinoscopus* (samel level or distad to M_{1+2} in *Seoda*) yet again, a variable character;

4. Wing vein R_5 ending at wing apex in *Krivosheinoscopus* (ending below wing apex in *Seoda*), this character is diagnostic between *Seoda* and *Telmatoscopus* as pointed out in [83].

5. The ejaculatory apodeme laterally compressed in *Krivosheinoscopus* (dorso-ventrally compressed in *Seoda*) also a diagnostic character pointed out in [83].

Diagnostic morphological characters to separate *Telmatoscopus* from *Seoda* presented in [83] include single pair of digitiform to filiform ascoids (an additional ring of small setiform ascoids + the main pair of digitiform ascoids in *Seoda*); frons clearly separated from clypeus not protruding over the mesal margin of eyes in *Telmatoscopus* (frons fused with clypeaus and protruding over the mesal margin of eyes in *Seoda*); parameral sclerites not fused in *Telmatoscopus* (parameral sclerites fused in *Seoda*).

Our currently reformulated conscription of *Telmatoscopus* includes 10 species globally, namely *T. advena* (Eaton, 1893), Palaearctic; *T. bartai* (Ježek, 2004) **comb. nov.**, Europe; *T. dendrophilus* Vaillant, 1983, USA; *T. frondeus* Tokunaga & Etsuko, 1955, Japan; *T. laurencei* Freeman, 1953, Europe; *T. pappi* (Wagner, 1979), Afghanistan; *T. patibulus* Quate, 1955, USA; *T. ussuricus* (Ježek, 2001) **comb. nov.**, Russia; *T. tanegashimensis* (Ježek & Mogi, 1995), Japan; *T. thuringicus* Beran, Doczkal, Pfister & Wagner, 2010, Europe.

Notes. Previously, Kvifte, [83] included *Panimerus wagneri* Salamanna, 1982 as *Seoda*, however the inclusion of *T. wagneri* (=*T. advena*) **comb. nov. et syn. nov.** inside of *Telmatoscopus* is based on the male genital morphology presented in the original description, therefore the new combination from *Seoda* to *Telmatoscopus*. Additionally, the characters separating *T. wagneri* (=*T. advena*) **syn. nov.** and, *T. advena* are quite inconspicuous, however, we did not examine the holotype of *T. wagneri* (=*T. advena*) **syn. nov.,** nonetheless, based on the original description and figures, we did not find any morphological characters that support them as separates species, and we treat *T. wagneri* as a new synonym of *T. advena*.

Additional characters and closer examination of the species *T. pappi*, *T. frondeus,* and, *T. tanegashimensis* is desirable as they share some characters with the genus *Lepiseodina*, however, these species are outside the geographical scope of this work, and therefore are included as *Telmatoscopus* following [83] until a broader revision of the genus is available.

Species present in Europe associated with dendrotelmata: *T. advena* (Eaton, 1904), *T. bartai* (Ježek, 2004) **comb. nov.**, *T. laurencei* Freeman, 1953, *T. thuringicus* Beran, Doczkal, Pfister & Wagner, 2010 [5,13,25,38,39] (Table 1).

***Telmatoscopus advena* (Eaton, 1893)**

Figures 12–14

Pericoma advena Eaton, 1893: 127. Type locality: Great Britain.

Telmatoscopus advena (Eaton): [81] (pp. 205–209)

Sciria advena (Eaton): [85] (p. 87).

Telmatoscopus advenus (Eaton): [86] (p. 86).

Panimerus advenus (Eaton): [50] (p. 80).
Panimerus havelkai Wagner, 1975: 1; (see [83]).
Panimerus wagneri Salamanna, 1982 **syn. nov.**
Telmatoscopus seguyi Vaillant, 1990: 378; (see [83]).

Diagnosis. Male. Ascoids broad, leaf-shaped and coiled; phallomeres incurved, symmetrical, less than half the length of ejaculatory apodeme; hypopods with tenacula restricted to apex.

Female description (Figures 15 and 16) based on the description of the male in [83] the female is similar to the male except: Head slightly longer than wide (0.5 mm wide, 0.6 mm length). Eye bridge with four facet rows, separated by 1.5 facet diameter; only first palpal segment present in examined material; apical antennal flagellomeres absent in examined material, ascoids s-shaped, coiled, thin (not broad leaf-shaped as in male). Wing length, wing width, length x times its width.

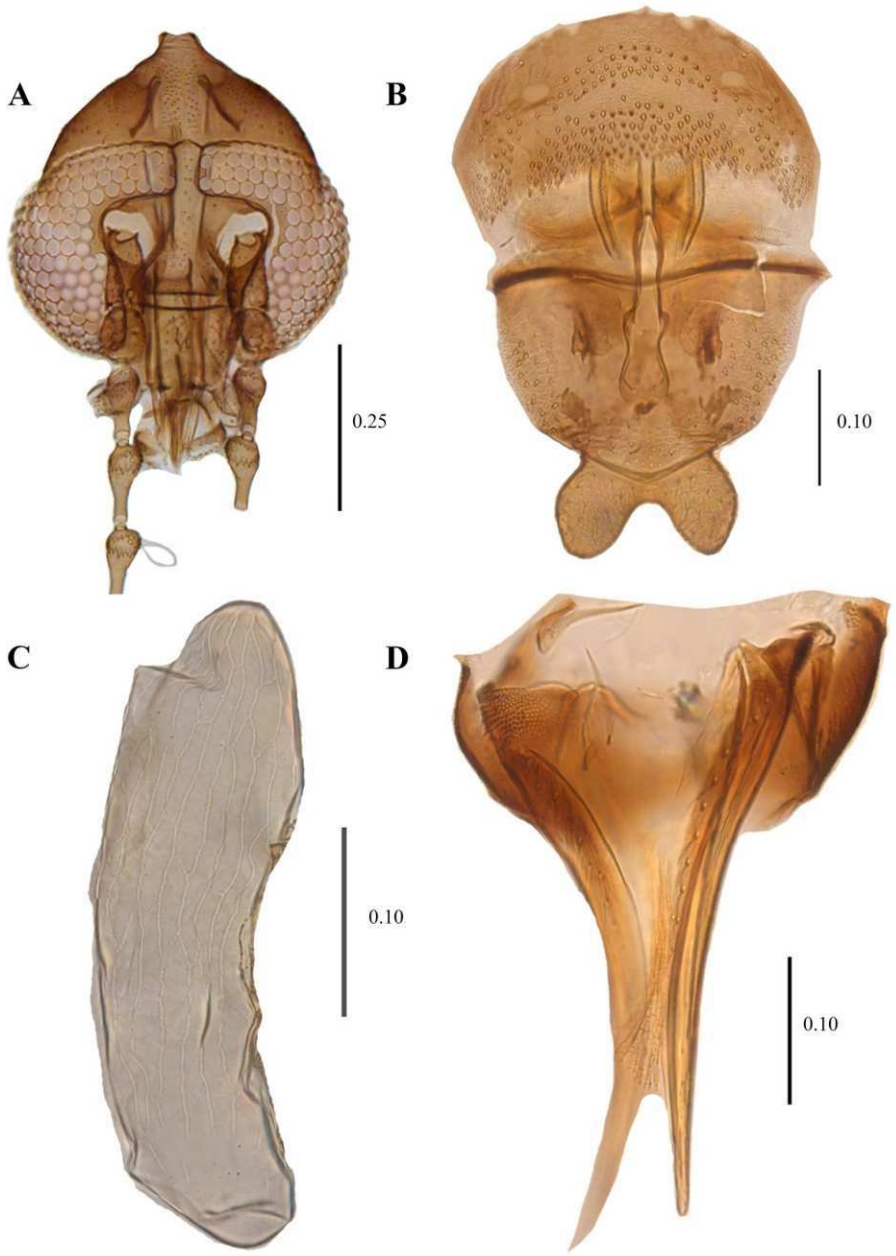

Figure 15. *Telmatoscopus advena* (Eaton, 1983), female. (**A**) Head. (**B**) Genital chamber. (**C**) Egg. (**D**) Cerci. Scale (**A**–**D**) in millimeters (mm).

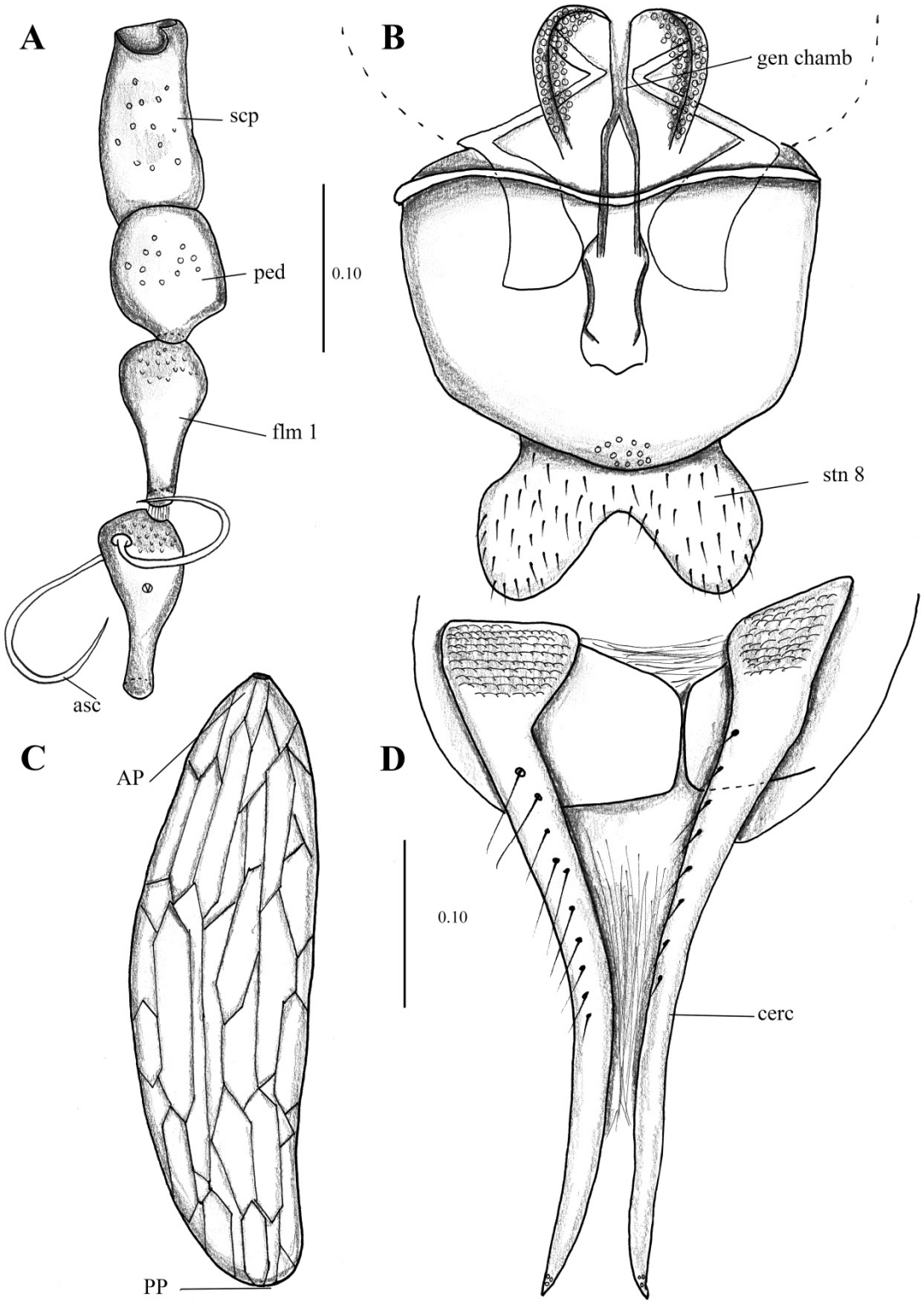

Figure 16. *Telmatoscopus advena* (Eaton, 1893), female. (**A**) First antennal segments. (**B**) Sternite 8 and genital chamber. (**C**) Egg. (**D**) Cerci. Abbreviations: AP = anterior pole, asc = ascoids, cerc = cerci, flm = flagellomere, gen chamb = genital chamber, ped = pedicel, PP = posterior pole, scp = scape, stn = sternite. Scale (**A**–**D**) in millimeters (mm).

Wing 2.7 times longer than wide (1 mm wide, 2.7 mm long), Sc long, ending at junction of R_4 + R_{2+3}. Infuscated in area between C and R1.

Sternite 8 (Subgenital plate) about the same length and width (0.25 mm wide, 0.24 mm long), waisted in the middle; basal margin straight, apical margin covered in small setae and strongly concave in the middle. Cerci long (0.4 mm length), 1.6 times the length of the sternite 8, with 10 setae on the dorsal surface, the surface in the base of the cerci presents multiple plate-shaped protuberances, resembling the texture of crocodile skin. Subgenital lobes rectangular.

Egg description. (Mean length 0.32 mm, width 0.1 mm, n = 15) Ovoid, longer than wide (0.1 mm wide, 0.32 long). No Micropyle structure is visible in the anterior pole; however, it does seem to be a circular aperture where the micropyle could be located. Exochorion with both longitudinal and transversal single, flattened ridges that when joined, form cells (mainly rectangular or hexagonal) across the exochorion.

Material examined. Holotype examined: no. 235444 [BMNH], ZFMK-DIP-00081296, ZFMK-DIP-00081297, ZFMK-DIP-00081298, ZFMK-DIP-00081306, ZFMK-DIP-00081307, ZFMK-DIP-00081308, ZFMK-DIP-00081309, ZFMK-DIP-00081310, ZFMK-DIP00081313, ZFMK-DIP-00081314, ZFMK-DIP-00081315, ZFMK-DIP-00081316, ZFMK-DIP-00081317, ZFMK-DIP-00081318, ZFMK-DIP-00081319, ZFMK-DIP-00081325, ZFMK-DIP-00081320, ZFMK-DIP-00081331, ZFMK-DIP-00081571, ZFMK-DIP-00081572, ZFMK-DIP-00081573, ZFMK-DIP-00081581, ZFMK-DIP-00081582, ZFMK-DIP-00081620, ZFMK-DIP-00081621, ZFMK-DIP-00081312, ZFMK-DIP-00081500, ZFMK-DIP-00081501, ZFMK-DIP-00081502, ZFMK-DIP-00081509, ZFMK-DIP-00081511, ZFMK-DIP-00081517, ZFMK-DIP-00081520, ZFMK-DIP-00081522, ZFMK-DIP-00081538, ZFMK-DIP-00081545, ZFMK-DIP-00081546, ZFMK-DIP-00081547, ZFMK-DIP-00081549, ZFMK-DIP-00081550, ZFMK-DIP-00081553, ZFMK-DIP-00082120, ZFMK-DIP-00082121, ZFMK-DIP-00082123, ZFMK-DIP-00082127, ZFMK-DIP-00082129, ZFMK-DIP-00082131, ZFMK-DIP-00082132, ZFMK-DIP-00082134, ZFMK-DIP-00082135, ZFMK-DIP-00081570, ZFMK-DIP-00081579, ZFMK-DIP-00081596 [ZFMK]. Specimen number: 10047, 13667, 13602, 13527, 12687, 18493, 13193, 18215, 18237, 20784, 20750, 20823, 0, 11356, 11264, 11357/34235, 21212, 21315, 22648, 22649, 22650, 22651, 22652, 22653 [NMP].

Distribution. Aegan Islands, Belgium, Finland, France, Germany, Ireland, Norway, Slovakia, United Kingdom [13,43,87].

***Telmatoscopus bartai* (Ježek, 2004) comb. nov.**

Figure 13

Krivosheinoscopus bartai Ježek, 2004: 117, Type locality: Czech Republic: Bohemia or., Železné hory Mts Protected landscape area.

Diagnosis. Male. Ascoids digitiform, thin, long, and coiled; phallomeres symmetrical, out curved, more than half the length of ejaculatory apodeme; hypopods with tenacula restricted to apex.

Examined material. Holotype examined: Cat. No. 34243 [NMP]. ZFMK-DIP-00081598, ZFMK-DIP-00081597, ZFMK-DIP-00081599 [ZFMK].

Distribution. Czech Republic [88], Germany. Our three reported specimens are the first record of this species in Germany.

***Telmatoscopus laurencei* Freeman, 1953**

Telmatoscopus laurencei Freeman, 1953: 71. Type locality: Great Britain. Herts, Harpenden, Rothamsted Experimental Station.

Diagnosis. Male. Ascoids digitiform, thin, coiled; phallomeres coiled, less than half the length of ejaculatory apodeme; hypopods with tenacula restricted to apex.

Examined material. None.

Distribution. Only known from Great Britain [13,39,43].

***Telmatoscopus thuringicus* Beran, Doczkal, Pfister & Wagner, 2010**

Telmatoscopus thuringicus Beran, Doczkal, Pfister & Wagner, 2010: 63. Type locality: Germany, Thuringia, National Park Hainich, Weberstedt, Birkensee.

Diagnosis. Male. Ascoids are broad, leaf-shaped, tapering towards the apex and coiled; phallomeres out curved, less than half the length of ejaculatory apodeme; hypopods

with a cluster of approximately 30 tenacula at apex, and more tenacula scattered along the entire surface of hypopods.

Examined material. None.

Distribution. Only known from Germany [5].

Key to the European adult males of *Telmatoscopus*

1. More than 30 tenacula; tenacula not restricted to the apical portion of hypopods, scattered in all the hypopods length (as in [5] (Figure 10)) . . . ***T. thuringicus***

\- Less than 30 tenacula; tenacula restricted to the apical portion of hypopods and not scattered in the hypopods length . . . **2**

2. Ascoids broad, almost as broad as the basal node of flagellomere carrying them (Figure 1B); aedeagus with parameres not coiled and strongly incurved (Figure 12C) . . . ***T. advena***

\- Ascoids narrow, never broader than 1/3 of the width of the basal node of flagellomere carrying them; parameres coiled or out curved (Figure 13C) . . . **3**

3. Parameres coiled with strong incurvation after the coil and apical tips hook-out curved (as in [38] (Figure 2D)); hypopods with less than 20 tenacula . . . ***T. laurencei***

\- Parameres not coiled and out curved (Figure 13C); hypopods with more than 20 tenacula . . . ***T. bartai***

4. Discussion

All of the above-mentioned species (also in Table 1) are recorded to be associated with dendrotelmata to a certain degree, whether they are specialized to complete their life cycle in this ecosystem or they are only opportunistic is a different matter. As mentioned by Oboňa and Ježek [25] the extreme variation of environmental conditions such as pH, temperature, frequent water loss with rapid flooding and, oxygen deficit can cause the death of non-specialized species, especially in larval stages that are using dendrotelmata as an irregular breeding/developing site, while specialized species can endure these harsh 822 environmental variations thriving through all their life cycle.

The occurrence of *Clogmia albipunctata*, *C. xylophilus*, *Pneumia canescens*, *P. trivialis*, *Psychoda alternata*, *P. cinerea*, and *P. minuta* in dendrotelmata is rather incidental or highly understudied [23,25]. In other words, the presence of these species could be an extension of their regular development sites (e.g., small water bodies, streams, etc.) and adults happen to find water-filled tree holes that are a potential development site for their offspring, thus, they could develop in Dendrotelmata and survive, but their long-term usage of this site is not well documented. Further studies in different habitat could provide key information to better understand the ecological relationship between water-filled tree holes and the species of Psychodinae that develop in them. To the date, only a handful of studies specifically targeted moth flies in dendrotelmata.

Clogmia albipunctata is broadly distributed both in Europe and worldwide and is the most synanthropic species inside the Psychodidae fauna, it can be commonly found inside buildings, and sewage treatment plants in almost every city. The high synanthropy is shared with some species of the genus *Psychoda*, which are also often found in cities, including *P. alternata* and *P. cinerea*, this species can certainly adapt and develop in a wide range of habitats, including dendrotelmata, therefore, these species could be classified as generalists when it comes to sites for larval development and not bound to develop in water-filled tree holes. Some further *Psychoda* species may be found in dendrotelmata, but the development of most species is still unknown.

Lepiseodina tristis, *L. rothschildi*, *Telmatoscopus advena* and *T. laurencei* are well-documented species that develop inside dendrotelmata (Table 1) with multiple records of both adults and larvae. On the contrary, *Telmatoscopus thuringicus* and *T. bartai* **comb. nov.**, are only assumed to be dendrolimnetic based on observations of the closely related species *T. advena* [5]. In the case of the herein described species *Lepiseodina latipennis*—some specimens collected in Italy

were reared from decaying organic matter collected in a dendrotelmata from a maple tree (*Acer sp.*), further specimens collected in Germany came from a Malaise Trap placed next to a water-filled tree whole in an Oak tree, thus proving that this species develop inside dendrotelmata as congeneric species.

5. Conclusions

In Europe, only 13 species of Psychodinae are known to develop inside water-filled tree holes, after our extensive record search we report that the Psychodinae species develop in 13 different tree species, including two new tree species in which no previous record of larval development was reported. We redescribed *Lepiseodina latipennis* through holotype and new material examination, and we report it for the first time in Germany. We also report *Telmatoscopus bartai* **comb. nov.** for the first time in Germany, and we provide a generic discussion of *Telmatoscopus*. Water-filled tree holes (dendrotelmata) are usually associated with old trees which are commonly endangered through the forest management strategies applied in European countries, and they are becoming rare to find inside forests. To summarize, there is a gap in the knowledge of the ecological interactions inside the Psychodinae and their environment, further studies can potentially provide new information, new records and new interactions that would be beneficial to better understand the ecosystems. Furthermore, old tree individuals remain a key component in the forests, as they harbor high biodiversity that is still understudied and they should remain untouched until the natural decomposition takes place.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14070532/s1, Table S1: Examined material collection Data.

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Appendix 3. (Publication chapter 5)

Chapter 5 – Publication

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PENSOFT

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Revisionary notes on *Feuerborniella* Vaillant, 1971, with the first record of the genus from the Afrotropical region (Diptera, Psychodidae)

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Abstract

We establish a diagnosis for *Feuerborniella* Vaillant, 1971, based on a re-description of its type species, *Feuerborniella obscura* (Tonnoir, 1919) and comment on earlier diagnoses. *Feuerborniella sinefurcata* Kvifte & Jaume-Schinkel, **sp. nov.** is described, based on material from Tanzania, representing the second Afrotropical species of the genus following *Psychoda morogorica* Wagner & Andersen, 2007 which we treat as *Feuerborniella morogorica* **comb. nov.** We furthermore review earlier combinations, transferring *Philosepedon ensiger* Quate, 1996 and *Philosepedon longistylus* Quate, 1996 to *Feuerborniella* **comb. nov.**, and briefly discuss generic limits with *Quatiella* Botosaneanu & Vaillant, 1970 and *Nielseniella* Vaillant, 1971.

Key Words

moth flies, moth fly, Psychodinae, Psychodini

Introduction

The genus *Feuerborniella* was named by Vaillant (1971), with the European species *F. obscura* (Tonnoir, 1919) as the type species. The original circumscription of the genus also comprised two species from Brazil and one from the Malay Archipelago, but the definition remained unclear for many years due to confusion with the closely-related *Quatiella* Botosaneanu & Vaillant, 1970 and different opinions amongst specialists regarding generic limits in Psychodini/Trichopsychodina (see discussions in, for example, Kvifte (2015); Ježek and Le Pont (2016); Kvifte et al. (2018); Kvifte (2019)).

Feuerborniella was redefined by Ibáñez-Bernal (2004), Cordeiro et al. (2014) and Cordeiro et al. (2015); the latter definition was not recognised by Ježek & Le Pont (2016) or in the classifications used in Brown et al. (2018) and Borkent et al. (2018). This is due to many characters within the diagnosis being polymorphic within the genus concept and one (the fusion of flagellomeres 11 and 12) is even polymorphic within species of Psychodini (see, for example, Quate (1955), p. 231). Furthermore, *Feuerborniella obscura*, the type species of the genus, differs from their diagnosis in at least two characters: flagellomere 11 and 12 are separated (e.g. Vaillant (1974), fig. 267) and the female cerci are equal or slightly shorter in length than the width of the female genital plate (Gunnar Mikalsen Kvifte, pers.obs.).

In the present paper, we thus follow the diagnoses given by Ježek (1985) and Ibáñez-Bernal (2004); however, in order to expand upon these, we also provide a supplementary re-description of the type species of the genus, *Feuerborniella obscura* from Europe. We also describe *Feuerborniella sinefurcata* sp. nov. from Tanzania and transfer *Psychoda morogorica* Wagner & Andersen, 2007, *Philosepedon ensiger* Quate, 1996 and *Philosepedon longistylus* Quate, 1996 to *Feuerborniella*, comb. nov.

Materials and methods

The studied specimens are deposited at the Royal Belgian Institute of Natural Sciences (**RBINS**), the Department of Natural History, University Museum of Bergen, Bergen, Norway (**ZMBN**) and the personal collection of Rüdiger Wagner (**RW**). In the material examined section, the holding institution is indicated at the end of each record and between square brackets ([]).

Measurements were made with an ocular micrometer in a microscope Leitz model Dialux 20, measurements are given in millimetres (**mm**). Head width was taken at the widest part, approximately above the insertion of the antennal scape, whereas the length was taken from the vertex to the lower margin of the clypeus; wing length was measured from the base of the wing at the start of the costal node to the apex of the wing-tip, while the width was taken approximately at an imaginary vertical line crossing the radial and medial forks; palpal proportions are given considering the length of the first palpal segment as a unit (1.0).

Morphological terminology is according to Kvifte and Wagner (2017).

Taxonomy

Feuerborniella Vaillant, 1971

Feuerborniella Vaillant, 1971: 119. Type species: *Psychoda obscura* Tonnoir, 1919.

Diagnosis (modified from Vaillant (1974) and Ibáñez-Bernal (2004)). Eyes separated; flagellomeres 11–14 reduced in size, fused or separated; labellum of both sexes fleshy, carrying spines and hairs, but not blunt teeth; wing membrane without pilosity; R5 ending in wing apex; male genitalia with gonocoxites widely separated; aedeagus and parameres symmetrical; surstylus longer than epandrium and carrying single terminal tenaculum on the surstylus.

Remarks. Apart from *Feuerborniella*, two other genera of Psychodini taxa have a fleshy labellum and a single terminal tenaculum of the surstylus. *Nielseniella* Vaillant, 1971 has asymmetric aedeagus and parameres, setae on the wing membrane and often one of the posterior branches of the ascoids reduced (as in *Threticus* Eaton, 1904). The Neotropical/Nearctic *Quatiella* Botosaneanu & Vaillant, 1970 is separated from *Feuerborniella* on the gonocoxites touching medially and the surstyli being as short as or shorter than the tenacula they carry.

Species included. *F. amblytes* (Quate, 1999), *F. ancepitis* (Quate, 1996), *F. bicuspis* (Quate, 1996), *F. ensiger* (Quate, 1996) comb. nov., *F. hamata* (Quate, 1996), *F. longistylus* (Quate, 1996) comb. nov., *F. morogorica* Wagner & Andersen, 2007 comb. nov., *F. obscura* (Tonnoir, 1919), *F. pandiculata* (Quate, 1996), *F. plaumanni* (Duckhouse, 1968), *F. retusus* (Quate, 1996), *F. sinefurcata* sp. nov., *F. spathipenis* (Duckhouse, 1968), *F. veracruzana* Ibañez-Bernal, 2004.

Feuerborniella obscura (Tonnoir, 1919)

Fig. 1

Psychoda obscura Tonnoir, 1919: 140.
Psychoda eximia Feuerborn, 1923: 200.
Philosepedon uniretinacleum Krek, 1971: 92.

Material examined. ***Lectotype*** female. "Uccle Av. Defré, 21. Mai 1917, A.Tonnoir". Designated by J. Ježek (1985). 3 female paralectotypes, one with the same data as holotype, two from "Forêt Soignes, 3 Juin 1918, A. Tonnoir". All in coll. [RBINS]. Belgium: "Linkebeek, 26.V.[19]20, A.Tonnoir", 2 females (RBINS). "Falaën, Juin [19]21, A.Tonnoir", 1 male [RBINS]. "Ohain, 23.V.[19]20, R. Mayné", 2 females [RBINS]. Germany: Hessen, Vogelsbergkreis, Schlitz, 13.VI.1971, R. Wagner leg. 1 male. [RW]

Diagnosis. *Feuerborniella obscura* can be separated from other described species of *Feuerborniella* by the following combination of characters: Wing forks complete, ejaculatory apodeme narrow, parameres dorsally connected, with elongate projections reaching apical ⅕ of aedeagus, aedeagus without subapical constriction.

Re-description. Measurements in mm (n = 1). Wing length 1.63, width 0.65; Head length 0.30, width 0.32; Antennal segments, scape: 0.06, pedicel: 0.04, flagellomere 1-9 0.10; Palpomeres 1: 0.06, 2: 0.08, 3: 0.10, 4: 0.12.

Male. Head about the same length as width, vertex about ⅓ of head length; eyes separated by 2 facet diameters, eye bridge with four facet rows, interocular suture as an inverted Y, length of suture about 2 facet diameters. Antennal scape about the same length as width, subquadrate; pedicel spherical, about the same length of scape, flagellomeres vaguely asymmetrical and nodiform, nodes progressively decreasing in size and internode increasing in length up to apical flagellomeres which are reduced in size and globular, not fused; ascoids with one anterior branch and to posterior branches, Y-shaped; frontal alveoli patch undivided, anterior margin extending almost to interocular suture reaching the second facet; labella bulbous, setose, without teeth. Palpal segments sclerotised, palpal proportions 1.0:1.4:1.7:2.1.

Wing hyaline, except costal cell which is infuscated; wing length 2.8 times its width; Sc short ending at the base of R_1; Radial fork apical to Medial fork, M_2 not connected to M_1; R_5 looks more sclerotised than the rest of wing veins, ending at wing apex.

Terminalia. Hypandrium sclerotised and plate-like; gonocoxites about the same length of gonostylus, cylindrical; gonostylus simple, tapering towards apex, incurved. Aedeagus symmetrical, extending towards the apex of gonostylus, parameres with a broad triangular base, tapering towards the apex, connected by a bridge morphologically ventral to the aedeagus, out-curved, ejaculatory apodeme narrow. Epandrium about the same length as its width, with both anterior and posterior margins concave; surstylus conical, tapering towards the apex

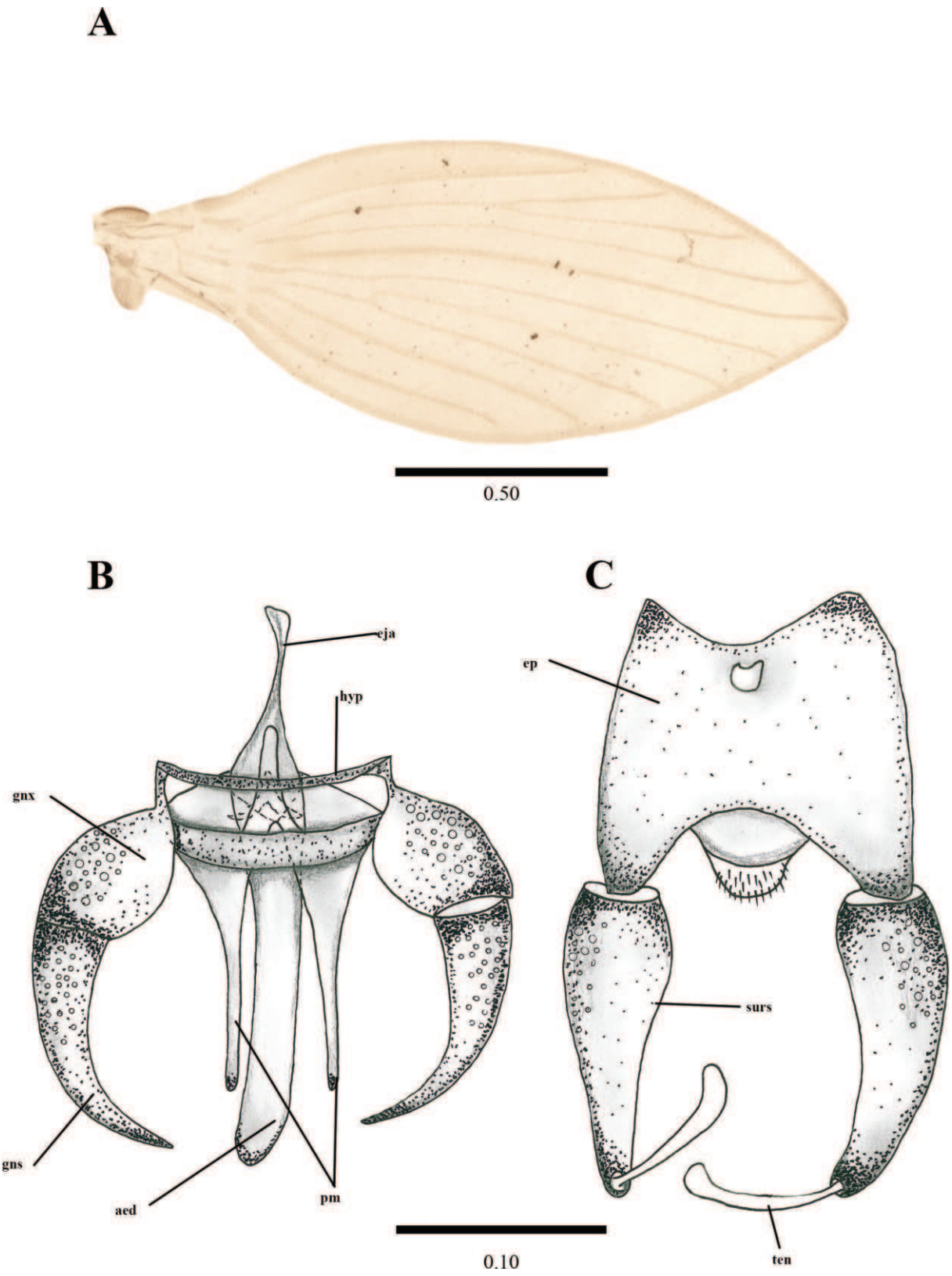

Figure 1. *Feuerborniella obscura* **A.** Wing; **B.** Genitalia, aedeagus, gonocoxites, gonostyli; **C.** Genitalia, epandrium and surstyli. Scales in millimetres (mm). Abbreviations: aed: aedeagus; eja: ejaculatory apodeme; ep: epandrium; gns: gonostyli; gnx: gonocoxite; hyp: hypandrium; pm: paramere; surs: surstyli; ten: tenaculum.

with a single spatulate tenacula, about half the length of surstylus; Hypoproct with posterior margin rounded, subquadrate, covered in small setulae.

Remarks. The description is based on the male from Germany; the other specimens listed under "material examined" have been consulted only to confirm the ge-

neric diagnosis. Previous re-descriptions of *F. obscura* by Vaillant (1971), Ježek (1985) and Krek (1999) show some discrepancies in whether the ascoids have a posterior branch or not. Vaillant (1974) describes the ascoids as Y-shaped (i.e. three branches) on the first ten flagellomeres and as either Y-shaped or V-shaped on the following flagellomeres. Ježek (1985) describes and figures the ascoids as possessing two branches, similar to Vaillant's (1974) illustration of the V-shaped condition. Krek (1999) figures three branches and lists the ascoids as Y-shaped, not mentioning any V-shaped ascoids on the distal flagellomeres at all. We deem it likely that this is a variable character as described by Vaillant (1974).

Feuerborniella morogorica (Wagner & Andersen, 2007), comb. nov.

Psychoda morogorica Wagner & Andersen, 2007: 293.

Diagnosis. *Feuerborniella morogorica* can be separated from all other *Feuerborniella* species by the following combination of characters: Forks complete, ejaculatory apodeme narrow, parameres not reaching beyond gonocoxites, aedeagus without subapical constriction.

Remarks. *Feuerborniella morogorica* is transferred to *Feuerborniella*, based on the labellum bulbous, aedeagus symmetric with symmetric parameres, gonocoxites widely separate and surstylus with a single tenaculum.

Feuerborniella sinefurcata sp.nov.

https://zoobank.org/1D5795FC-48BB-4ECF-9022-C81AB1F8B304
Fig. 2

Type material. ***Holotype*** male. Tanzania: Tanga Region, West Usambara Mountains, Mazumbai Forest Reserve, "Loc G & F". 2–6.XI.1990 (Malaise trap), "ZMBs Tanzania Expedition" leg. [ZMBN].

Diagnosis. *Feuerborniella sinefurcata* can be separated from all other *Feuerborniella* species by the following combination of characters: Wing forks incomplete, ejaculatory apodeme narrow, parameres separated, reaching apical ⅕ of aedeagus, aedeagus without subapical constriction.

Description. Measurements in mm (n = 1). Wing length 1.50, width 0.63; head length 0.28, width 0.29; Antennal segments, scape: 0.06, pedicel: 0.04, flagellomere 1-3: 0.09; Palpomeres 1: 0.04, 2: 0.08, 3: 0.08, 4: 0.11.

Male. Holotype. Head about the same length as width, vertex about ⅓ of head length; eyes separated by approximately 1.5 facet diameters, eye bridge with 4 facet rows, interocular suture angular arch-shaped. Antennal scape about the same length as width, subquadrate; pedicel spherical, about the same length of scape, flagellomeres asymmetrical and nodiform, apical flagellomeres missing in revised material. Ascoids are absent in the revised material. Frontal alveoli patch undivided, anterior margin extending almost to interocular suture reaching the second facet; labella bulbous, setose, without teeth. Palpal segments sclerotised, palpal proportions 1.0:1.6:1.7:2.5.

Wing hyaline, except costal cell which is infuscated; wing length 2.6 times its width; Sc short ending at the base of R_1; Radial fork apical to Medial fork, R_2 not connected to R_1, M_2 not connected to M_1; R_5 looks more sclerotised than the rest of wing veins, ending at wing apex.

Terminalia. Hypandrium narrow, sclerotised, and plate-like; gonocoxites about half the length of gonostylus, cylindrical; gonostylus simple, tapering towards apex, incurved. Aedeagus extending towards the apex of gonostylus, parameres triangular-broad base, tapering towards the apex, weakly curved laterad, not connected by a bridge; ejaculatory apodeme narrow, about half the length of aedeagus. Epandrium about the same length as its width, with both anterior and posterior margins concave; surstylus cylindrical, slightly tapering towards the apex with a single spatulate tenaculum, about half the length of surstylus; Hypoproct with posterior margin rounded, tongue-shaped, covered in small setulae.

Etymology. From Latin *sine*, meaning without and, *furca*, meaning fork – referring to the reduced radial and medial forks in the new species.

Remarks. *Feuerborniella sinefurcata* sp. nov. is the 19th species of Psychodidae to be described from the West Usambara Mountains (see Kvifte (2022)) and the second Afrotropical species of *Feuerborniella*.

Discussion

Vaillant (1974) recognised four species in his first review of *Feuerborniella*, spanning the Neotropical, Palearctic and Oriental Regions. Ježek (1985) considered *Psychoda plaumanni* Duckhouse, 1968 and *Psychoda spathipennis* Duckhouse, 1968 to belong to his genera *Psycha* Ježek, 1983 and *Psychomora* Ježek, 1983 and considered *Trichopsychoda malayensis* Satchell, 1955 to belong to an undescribed genus. Neither these changes nor his transfer of the Oriental *Psychoda nigripennis* Brunetti, 1908 to *Feuerborniella* were supported by mention of specific morphological characters and they have generally not been considered in subsequent works.

Ibáñez-Bernal (2004) followed Vaillant's (1974) initial species list and added an additional Neotropical species, lifting the world total to five and Cordeiro et al. (2014) recognised six species with their description of *F. paramuna*. Many additional combinations were made by Cordeiro et al. (2015), but since their diagnosis is not consistent with the type species of *Feuerborniella*, the validity of these combinations must be considered on a case-by-case basis.

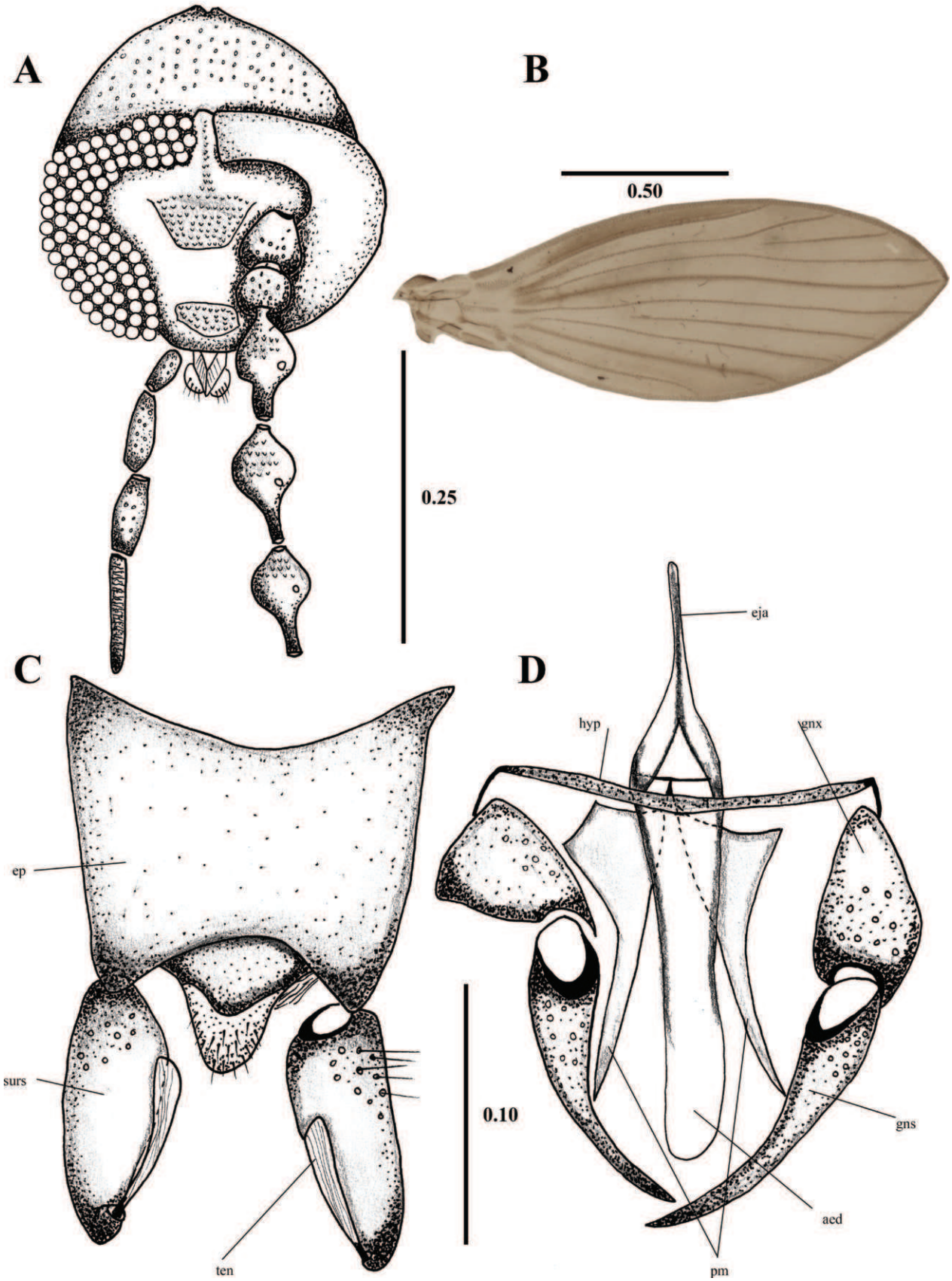

Figure 2. *Feuerborniella sinefurcata* sp. nov. **A.** Head; **B.** Wing; **C.** Genitalia, epandrium and surstyli; **D.** Gentalia, aedeagus, gonocoxites, gonostyli. Scales in millimetres (mm). Abbreviations: aed: aedeagus; eja: ejaculatory apodeme; ep: epandrium; gns: gonostyli; gnx: gonocoxite; hyp: hypandrium; pm: paramere; surs: surstyli; ten: tenaculum.

Our current diagnosis adds the criteria of wing membranes without pilosity and symmetry in the male genitalia to previously-published diagnoses. This excludes the Oriental *Trichopsychoda malayensis* Satchell, 1955 and the Neotropical *Feuerborniella pilosella* Cordeiro & Bravo, 2015 and *F. paramuna* Cordeiro, 2014, which would all be *Feuerborniella* species, based on the diagnoses of Ibáñez-Bernal (2004) and Cordeiro et al. (2014). These species all have pilose wing membranes and (based on illustrations) an asymmetric aedeagus; in the case of *F. pilosella*, the parameres are asymmetric as well. According to the differential diagnosis given above, these may belong to *Nielseniella*; however, it would be premature to transfer the species to that genus until the generic limits of *Nielseniella* are re-assessed, based on study of the type species. *Psychoda nigripennis* Brunetti, 1908, which was placed in *Feuerborniella* by Ježek (1985), was insufficiently described in its original description and only a damaged collection of females remained of the type series when it was revised by Quate (1962). That species can, therefore, not be confidently placed in any genus. Of the 14 described species considered by us to form part of *Feuerborniella*, eleven are Neotropical, one is Palearctic and two are Afrotropical. With the high number of undescribed *Feuerborniella* species encountered in the Zurquí project, we suggest the genus to have its highest diversity in the Neotropical Region (Borkent et al. 2018; Brown et al. 2018).

The present paper represents the first mentions of *Feuerborniella* from the Afrotropical Region, which means existing keys will have to be emended. In Kvifte's (2015) key to Afrotropical genera of Psychodini, *Feuerborniella* keys to couplet 6 where neither of the two options work (surstylus with two tenacula vs. surstylus with three or more tenacula). The same is the case for Kvifte and Wagner (2017) where *Feuerborniella* keys to couplet 29 which uses the same character. *Feuerborniella* can be separated from all other Afrotropical Psychodini by the labellum being bulbous rather than flat and the surstylus carrying one tenaculum only.

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Appendix 4. (Publication chapter 6)

Chapter 6 – Publication

Jaume-Schinkel S, Mengual X, Howe A, Fagan-Jeffries EP (2023) The Hitchhiker's Guide to Australia: The 18,000-km-long journey of *Alepia viatrix* (Diptera: Psychodidae) discovered through citizen science, *CheckList* 19(4), 589–597. https://doi.org/10.15560/19.4.589

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PENSOFT

The hitchhiker's guide to Australia: the 18,000-km-long journey of *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 (Diptera, Psychodidae) discovered through citizen science

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Abstract. The Neotropical genus *Alepia* Enderlein, 1937 (Diptera, Psychodidae) is newly recorded in Australia. We present new geographical records for *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022, extending the range of this species by 18,000 km. We attribute these new Australian records to the likely unintentional introduction of *A. viatrix* through international bromeliad trade. This moth fly was found by school children working with insect taxonomists through an Australian citizen-science project, Insect Investigators. We describe and present for the first time high-resolution SEM pictures of the eggs of the genus *Alepia*.

Keywords. Moth flies, introduced species, dark taxa, DNA barcoding, taxonomy, new distribution, new record, community science, school project

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Introduction

Introduced species, also known as non-native, casual, or alien species, overcome geographical barriers in their native range, increasingly as an unintentional consequence of human-mediated international trade and travel pathways (Lockwood et al. 2005; Minchin 2009; Simberloff et al. 2013). Upon reaching new localities, an introduced species must successfully reproduce to become established at which point it may be considered naturalized. Only when a species has detrimental impacts on at least one aspect of the environment, human health, or economy is it considered "invasive" (Blackburn et al. 2011). Invasive species are key drivers of global declines in biodiversity (Simberloff et al. 2013; Wagner 2020). Furthermore, introduced species usually exist unnoticed by the scientific community during the establishment phase, especially during the initial period of low abundance (Encarnação et al. 2021), and insect species that successfully mount incursions are often not intercepted at international borders (Caley et al. 2015; Turner et al. 2021). One of the most effective options to detect introduced species is through citizen science (Encarnação et al. 2021), which is defined as "active involvement of citizens in scientific inquiry generating new knowledge or understanding" (Wiggins and Crowston 2011). The involvement of the general public in scientific research allows early monitoring and tracking of species' dispersal over a large geographical area at a relatively low cost (Eitzel et al. 2017; Encarnação et al. 2021; Epanchin-Niell et al. 2021) and simultaneously benefits participants by increasing

environmental awareness (e.g. Schreck-Reis et al. 2013; Payne et al. 2023).

Invasive species can be found among Diptera, the two-winged insect order that comprises gnats, midges, mosquitos, and true flies. The natural history of dipterans is very diverse and include predation, phytophagy, parasitism, and saprophagy among the common feeding strategies. Taxa with aquatic or semi-aquatic larvae can develop in artificial water bodies, such as canals, reservoirs, dams, or containers, and they can be unintentionally spread by human activities. There are well-documented examples of invasive dipterans that breed in containers, such as mosquitoes and moth flies (Reiter 1998; Zielke et al. 2015; Kvifte 2023).

Among the moth flies (Insecta, Diptera, Psychodidae), *Alepia* Enderlein, 1937 is considered one of the most diverse Neotropical genera with 60 described species, the majority of which are naturally distributed in the Neotropics (Tonnoir 1920; Quate 1963, 1999; Duckhouse 1974; Wagner 1993; Bravo et al. 2004; Quate and Brown 2004; Wagner and Hibrar 2004; Wagner and Svensson 2006; Bravo 2008; Wagner et al. 2008, 2010; Jezek et al. 2011; Omad and Rossi 2012; Cordeiro et al. 2015, 2021; Tkoč et al. 2017; Duran-Luz et al. 2018; Jaume-Schinkel et al. 2022). To date, three species have been described beyond the expected Neotropical distributional range of the genus, and are henceforth considered as introduced species, namely, *Alepia symmetrica* Wagner & Hibrar, 2004, which was described based on specimens collected in Florida, USA and reared from a bromeliad plant; *Alepia vaga* Wagner & Svensson, 2006, described from specimens reared from a bromeliad in Sweden; and *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022, described based on specimens collected in a botanical garden on the Azores (Portugal).

In the present study, we report new *Alepia* records from Australia originating from an Australian citizen-science project, Insect Investigators. In addition, we describe and present for the first time high-resolution SEM pictures of the eggs of the genus *Alepia*.

Methods

The examined material used for the study was collected using a Malaise trap, as part of the Insect Investigators project (https://insectinvestigators.com.au/). Insect Investigators partnered with 50 schools in the Australian states of Queensland, South Australia, and Western Australia, with each school monitoring a Malaise trap on or near school grounds for four weeks in March 2022. Schools sent their weekly trap samples to the University of Adelaide where samples were sorted to order level, prior to the selection of 285 specimens for DNA barcoding.

Specimens were collected into 80% propylene glycol as an initial preservative medium and transferred into 100% ethanol within a few weeks of collection. DNA extraction and sequencing of the standard 5′-end of the cytochrome c oxidase subunit I (COI), also known as DNA barcode (Hebert et al. 2003), were performed at the Canadian Centre for DNA Barcoding at the University of Guelph, Canada, following the in-house protocols. Data were uploaded to the open-access Barcode of Life Database (BOLD; https://www.boldsystems.org/). After DNA extraction, specimens were preserved in ethanol. Morphological characters were observed using a Zeiss Scope A1. Specimens are deposited in the Queensland Museum (QM), Brisbane, Australia, and in Museum Koenig (previously known as Zoologisches Forschungsmuseum Alexander Koenig) (ZFMK), Bonn, Germany.

As an extra exercise, we asked the young citizen scientists (students of year 4, now year 5) to draw and write their interpretation of insect adaptation to their environment focusing on our results (available as Supplementary file, Figs S1, S2).

Terminology. Egg poles follow the definition of Dutra et al. (2011). The anterior pole is defined as the end of the egg that bears the pedicel or a projection, on the contrary, the posterior pole is usually round and smooth and lacks external structures or openings. Additionally, we follow the recategorization of the exochorionic sculpture patterns and terminology of de Almeida et al. (2004).

Results

Alepia viatrix Jaume-Schinkel, Kvifte, Weele & Mengual, 2022

Alepia viatrix Jaume-Schinkel et al. 2022: 385. Type locality: Portugal, Azores Archipelago, Terceira Island, botanical garden.

Figures 1–3

New records (Fig. 1). AUSTRALIA – **Queensland** • Brisbane, Yeronga State School; –27.519, 153.022; 8–15.III.2022; Yeronga State School students leg.; Insect Investigators week 1, Malaise trap, 1–8.III.2022; BOLD accession number ASMII2842-22, 1 ♀ (QM T259470) • same locality and collector; 8–15.III.2022; Insect Investigators week 2, Malaise trap, 8–15.III.2022; BOLD accession number ASMII2894-22, 1 ♀ (QM T259471).

Identification. A preliminary identification of the collected specimens was firstly based on blasting the DNA COI barcodes in BOLD. The COI sequence of the two female Australian specimens are very similar to each other, with a difference of only 0.46%. Moreover, both sequences are also quite similar to previously published sequences of *A. viatrix* with a difference of 0.05–5.22%. COI sequences can be accessed in BOLD under the Dataset DS-DTALEPIA (https://doi.org/10.5883/ds-dtalepia). Furthermore, the identification was supported comparing external morphology and female genitalia of the Australian specimens with the type series and, additionally, by comparison with the original description and detailed figures published by Jaume-Schinkel et al. (2022). No morphological differences were noticed between the Australian specimens and the type series individuals.

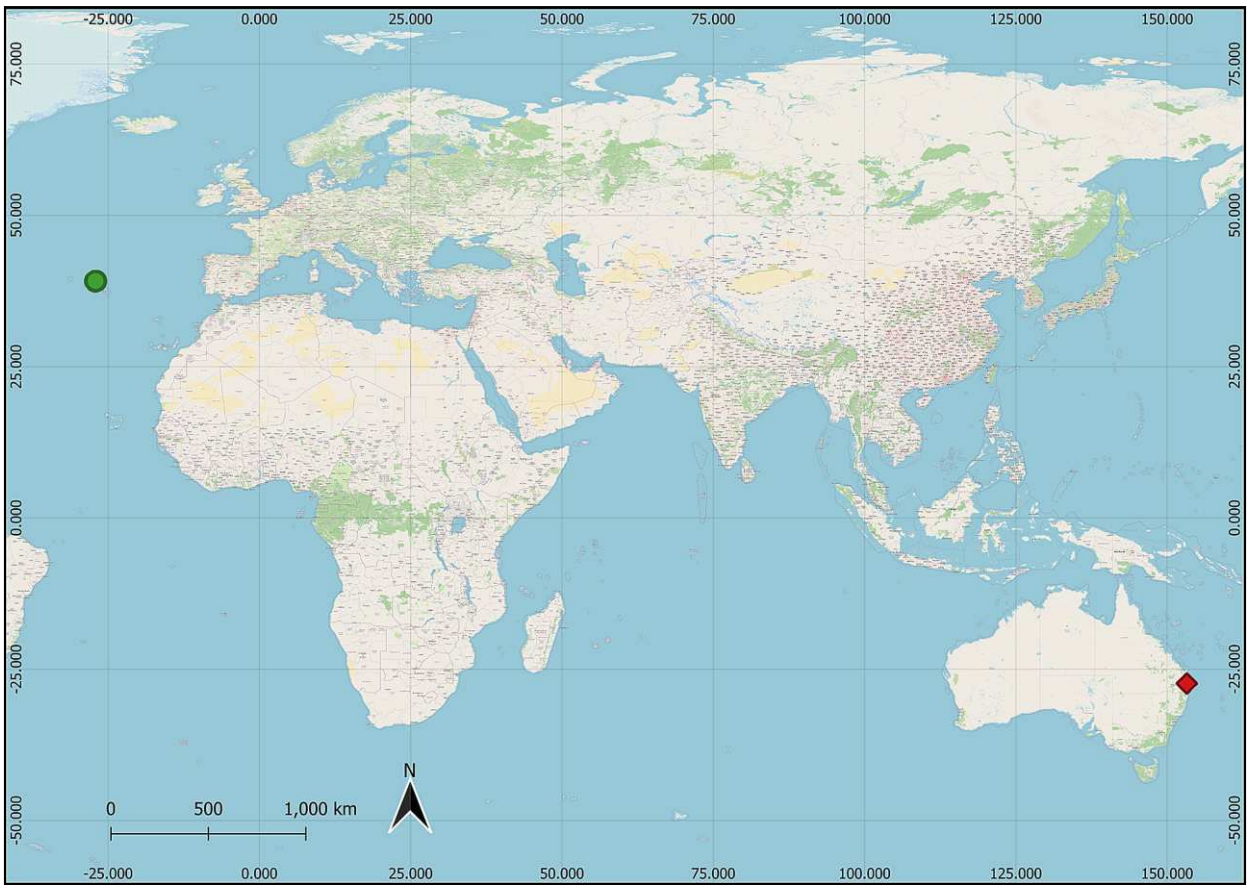

Figure 1. Records of *Alepia viatrix*. Green circle indicates the type locality of the species; red rhombus indicates our new record from Australia. The map was built using QGIS v. 3.28.6 with the Quick-MapServices function.

Remarks. Australian female individuals are morphologically and genetically congruent with the type series specimens of *A. viatrix* (see Discussion). Nevertheless, the diagnostic characteristics to distinguish *Alepia* species are all found in the male genitalia (see Jaume-Schinkel et al. 2022), as the female sex is not known for all the described *Alepia* species, especially for the morphologically closely related taxa to *A. viatrix*. This knowledge gap, together with the lack of DNA barcodes for any *Alepia* species except for *A. viatrix*, limits our determination. Although the likelihood is difficult to assess, we cannot rule out that females of other *Alepia* species share external and internal morphology with *A. viatrix*, as well as their COI sequence.

Egg description. Length 0.436 ± 0.018 mm. Width 0.104 ± 0.036 mm (n = 15) (Fig. 2A–E). The exochorion sculptures form a distinct polygonal pattern, forming elongated hexagons running transversal along the long axis of the egg (Fig. 2A–C). The basal lamina inside the hexagons is generally smooth, with some irregularly distributed protuberances. No aeropiles were observed in the posterior pole (Fig. 2D). The anterior pole presents a conical projection of about 0.015 mm, with the apex rounded, without exochorion sculptures (Fig. 2E).

Genital chamber. The genital chamber is a complicated three-dimensional structure, usually portrayed in two dimensions because of the common process of preparing permanent microscope slides as the most accepted way to study morphology in Psychodidae. The original description of the genital chamber by Jaume-Schinkel et al. (2022: figs 12–15) can be better understood while observing Figure 3A–C. With the aid of an SEM microscope, lateral (Fig. 3A) and dorsal (Fig. 3B, C) views of the genital chamber can be observed. However, the genital chamber is surrounded by membranous tissue, which hinders the observation of the internal structures; thus, only the outer structure is discernible.

Discussion

Our findings have greatly expanded the known distribution range of *Alepia viatrix* (Psychodidae, Psychodinae) by over 18,000 km (Fig. 1) and suggest international trading of bromeliads as the origin for the unintentional introduction of this Neotropical genus in other regions. We have discovered a new population in Australia, Queensland (–27.519, 153.022), which is a considerable distance from the species' type locality in the Azores (38.6526, –27.2190). These new observations are particularly noteworthy as they constitute the first documented occurrence of the *Alepia* genus in Australia and the entire Australasian Region.

Almost nothing is known about the biology of the adults and little is known about the immature stages of the genus *Alepia* aside from the species reared from bromeliads and the reported larvae of *A. longinoi* Quate & Brown, 2004 collected in ant colonies of the genus

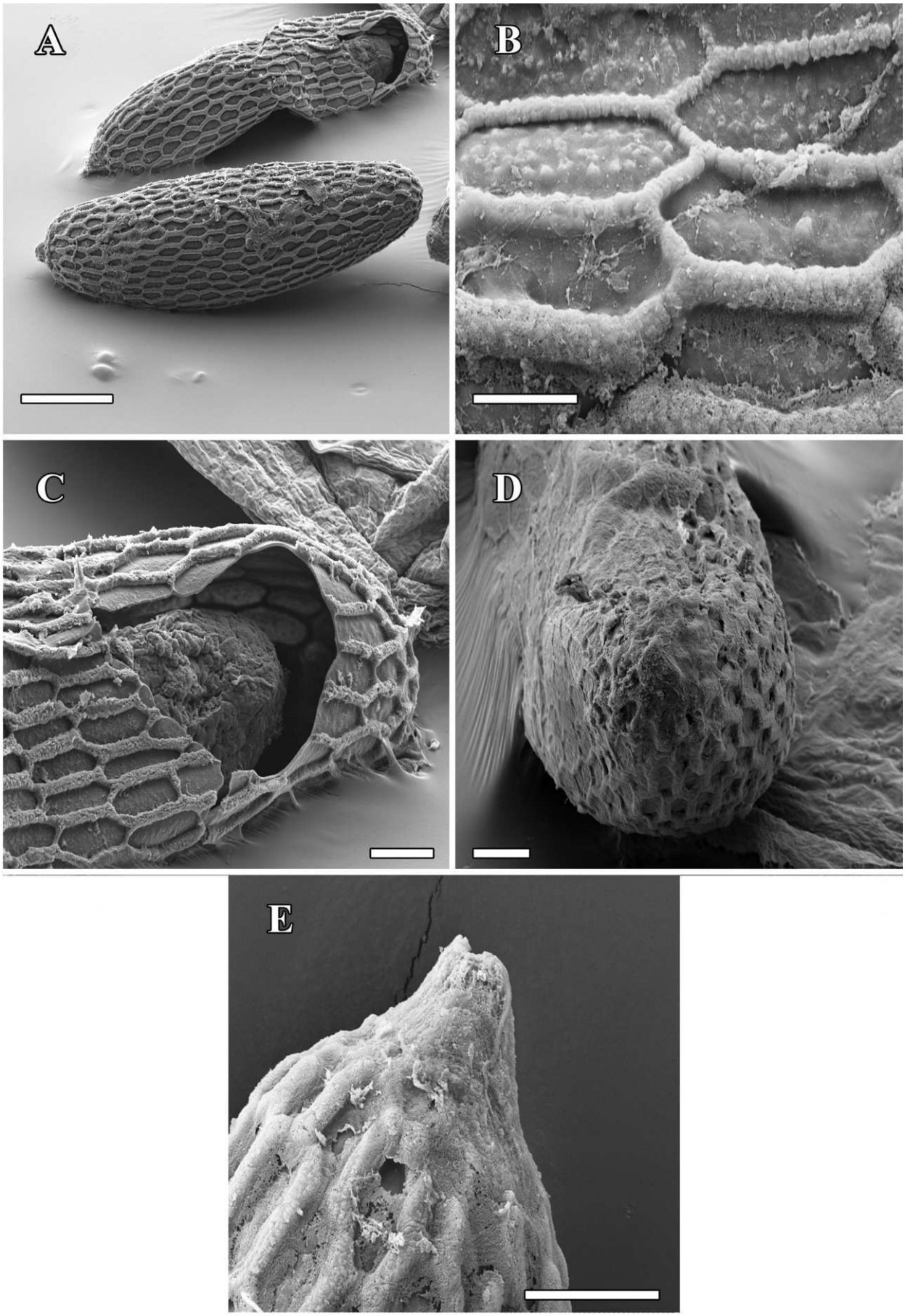

Figure 2. Eggs of *Alepia viatrix*. **A.** Lateral view. **B.** Exochorion sculptures forming elongated hexagons and showing protuberances in the basal lamina. **C.** Inside of the egg. **D.** Posterior pole of the egg. **E.** Anterior pole of the egg. Scale bars: A = 100 µm, B = 10 µm, C–E = 20 µm.

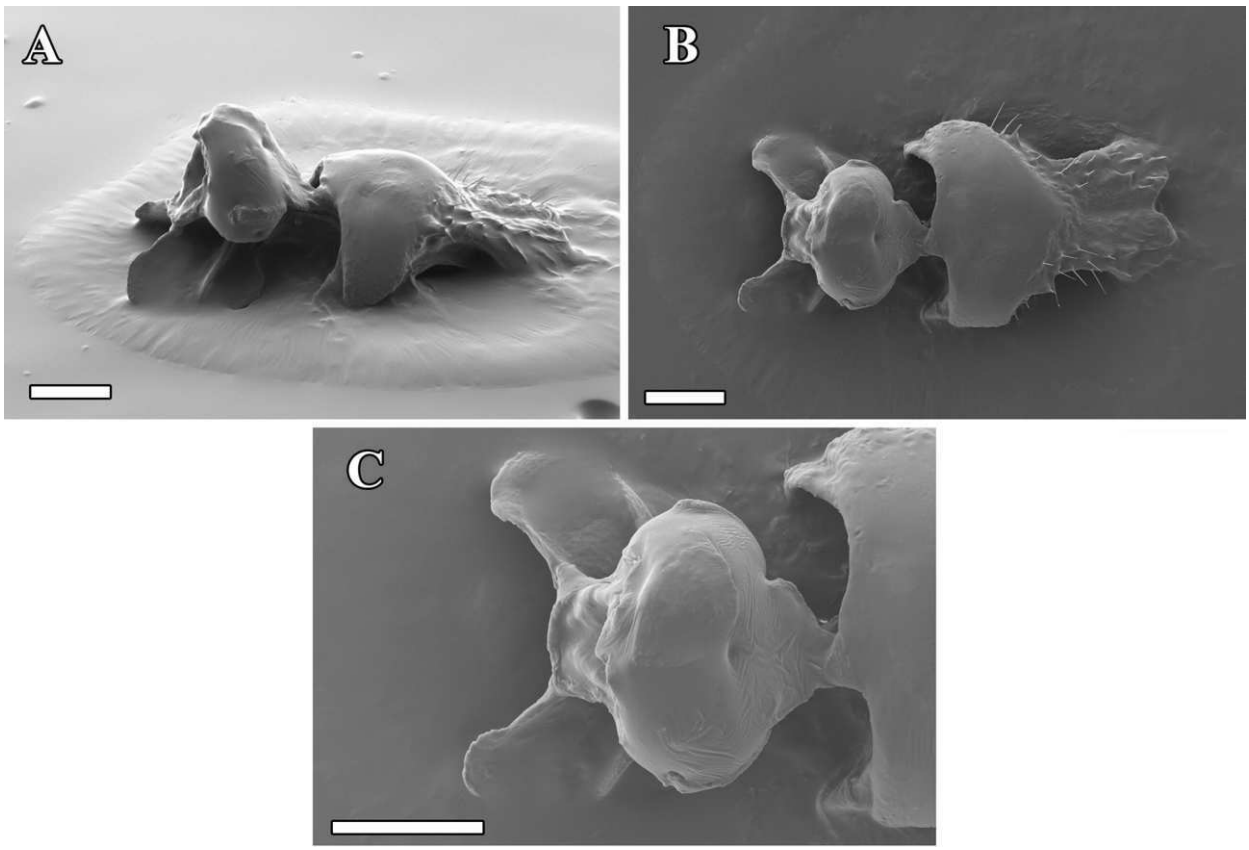

Figure 3. *Alepia viatrix*. **A.** Lateral view of genital chamber and subgenital plate. **B.** Dorsal view of genital chamber and subgenital plate. **C.** Dorsal view of genital chamber and subgenital plate. Scale bars = 100 µm.

Azteca Forel, 1878 (Quate and Brown 2004). The previously published COI sequences plus the two new sequences from this study are the only published DNA barcodes for the genus *Alepia*, and no further molecular data are available for the remaining species. This fact becomes even more relevant when we know that *Alepia* is one of the most diverse Neotropical genera. All DNA barcodes of *A. viatrix* in BOLD cluster into two Barcode Index Numbers or BINs (Ratnasingham and Hebert 2013); the first BIN has one female specimen that differs 5.05–5.47% from all other specimens, and the second BIN comprises four specimens, including the male holotype, one male paratype, and the two females collected in Australia (with differences between 0.0% and 0.46%). Jaume-Schinkel et al. (2022) associated males and females on the basis of general morphology of both sexes and their co-occurrence at the same locality, as *A. viatrix* was the only species of *Alepia* present. We may provide arguments to justify the significant higher difference of the female paratype (5.05–5.47%) from the other sequenced specimens of *A. viatrix*, but they would be speculative. Despite this, we are confident that all of the specimens in the two BINs belong to the same species, based on the co-distribution of the specimens described, sequenced, and sex-associated by Jaume-Schinkel et al. (2022), the specimens sequenced herein, and the morphological comparison with the previously known female. Consequently, we believe that the relatively small uncorrected pairwise distance between the two previously sequenced males and the herein sequenced females indicates that sex association in the genus *Alepia* can be done through DNA barcoding. However, as mentioned under Remarks, a broader representation with males and females of other *Alepia* species is necessary to understand the dissimilarity threshold for intra- and interspecific variability in this moth-fly genus.

Exochorionic structures in the eggs are species-specific and can help in the delimitation of species inside Psychodidae, and even delimit locality-specific populations (Almeida et al. 2004; Montes de Oca-Aguilar et al. 2017). We provide the first SEM images of the eggs for the genus *Alepia* and, as detailed in Figure 2A–C, the exochorion sculptures are quite distinctive with elongated hexagons along the long axis of the eggs. These egg structures may be a morphological characteristic to differentiate eggs of different species of the genus *Alepia*, but we do not have material of additional species for comparison. The only other Psychodinae exochorion description is of *Clogmia albipunctata* (Williston, 1893) (Rocha et al. 2011). Eggs of *C. albipunctata* present exochorion sculptures that continuous and discontinuous along the long axis, while eggs of *A. viatrix* form clear hexagonal-shaped sculpture along the long axis; thus, both species eggs can be easily differentiated by the shape of the exocorion sculpture.

Although species in the subfamily Psychodinae are not considered harmful to humans and/or domestic

including the University of Adelaide, Queensland Museum and the University of the Sunshine Coast. The Yeronga State School Malaise trap was run by Clare Triggell accompanied by teachers Katie McDermott, Ryan Henry, Josh Newby, Wendy Wooton, Kathy Lyons, and the enthusiastic participation of the remarkable young citizen scientists and students in year 4 (2022). Santiago Jaume-Schinkel's work on European Psychodidae is supported by the Bundesministerium für Bildung und Forschung, Berlin, Germany, the project "German Barcode of Life III: Dark Taxa" (FKZ 16LI1901A). We are thankful to Alessandre Pereira, Danilo Cordeiro, and Gunnar Kvifte for their comments that improved the final version of our publication.

Author Contributions

Conceptualization: SJS. Data curation: XM, EPFJ, AGH, SJS. Formal analysis: XM, SJS. Funding acquisition: AGH, EPFJ. Investigation: SJS. Methodology: XM, SJS, AGH, EPFJ. Project administration: EPFJ, AGH. Resources: SJS, XM, EPFJ, AGH. Validation: XM, SJS. Visualization: SJS. Writing – original draft: SJS. Writing – review and editing: AGH, XM, EPFJ.

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animals, there is virtually no information on potential effects of the species on local native taxa. With the exception of a few species, there is no evidence of adult feeding in Psychodinae (Kvifte and Wagner 2017), and the ecological niche in which *A. viatrix* larvae develop is highly specific, i.e., water reservoirs inside bromeliad plants (phytotelmata). Bromeliads are native to the Neotropical Region (Breviglieri and Romero 2017), and some species can form phytotelmata, or water reservoirs, through the imbrication of the leaves; these water reservoirs are capable of maintaining small ecosystems with associated biota (Tsuda and Castellani 2016; Lopes-Filho et al. 2023). Introduced ornamental bromeliads (Kolicka et al. 2016; Wilke et al. 2018; Poniewozik et al. 2020) are implicated in facilitating establishment of viable populations in Florida of the non-native mosquito *Aedes albopictus* (Skuse, 1894) (Lounibos et al. 2003; Wilke et al. 2018), the vector for the transmission of several viral pathogens; thus, bromeliad phytotelmata are a good mechanism for passive invertebrate dispersal (Kolicka et al. 2016).

Australian specimens of *A. viatrix* were collected in an area where bromeliads are grown in residential gardens for decorative purposes. Moreover, records in the Atlas of Living Australia (ALA 2023) suggest bromeliads are widespread in the Brisbane area and evidently widely available for purchase from nurseries and garden centers (Howe pers. obs.). In addition, there are 28 records of bromeliads in the Brisbane area in the iNaturalist website (iNaturalist 2023), represented by six species, namely *Aechmea fasciata* (Lindl.) Baker, *Aechmea gamosepala* Wittm., *Ananas comosus* (L.) Merr., *Billbergia* sp. 1821; *Neoregelia* sp. 1934, and *Tillandsia usneoides* (L.) L. All species present on iNaturalist are non-native. It is noteworthy that, according to Arteaga et al. (2020: supplementary material 1), the two species present in the type locality of *A. viatrix* in the Azores are *Aechmea fasciata* and *Neoregelia carolinae* (Beer) L.B.Sm., which are also found in the surroundings of the new distributional records according to the iNaturalist records. Both species *Aechmea fasciata* and *Neoregelia carolinae* are naturally endemic to the Atlantic Forest in Brazil, primarily in the state of Rio de Janeiro (Martinelli et al. 2008). However, cultivated specimens may come from beyond their natural range, complicating the identification of the natural distribution of *A. viatrix*. Yet, it provides valuable insights into potential habitats for this species of moth fly.

The locality of the specimens described here (Yeronga State School; –27.519, 153.022) is 5 km from the nearest iNaturalist record of *A. fasciata* (–27.4935, 153.0439) and 3 km from the nearest record of *Neoregelia* sp. (–27.5135, 153.0039), and it is likely there are other bromeliads distributed in the area but not recorded on the Atlas of Living Australia or iNaturalist. In fact, 30% of the 80 student citizen scientists indicated they had bromeliads growing in their garden (distance to school = approximately 200 m to 7 km). Incidentally, an approximately 40 m^2 patch of false bromeliad plants (*Callisia fragrans* (Lindl.) Woodson) was located (flowering) within 5 m of the Malaise trap at Yeronga State School (Howe pers. obs.). Although we do not have evidence that *A. viatrix* was imported to Australia through the commercialization of bromeliads, it is a likely scenario explaining the presence of this species and the gigantic gap between the new records, the known distribution of this species, and the hypothesized biogeographic origin of the genus *Alepia*.

Wagner and Hribar (2004) hypothesized that bromeliads containing larvae of *A. symmetrica* were imported from the Neotropics for decorative purposes, and, in the same way, bromeliads containing larvae of *A. vaga* were imported from Brazil to Sweden (Wagner and Svensson 2006). Similarly, Jaume-Schinkel et al. (2022) reported the presence of two bromeliad species, which were imported from Brazil, in the botanical garden in which the species *A. viatrix* was collected, and they argued these bromeliads are likely the cause of this *Alepia* species in the Azores. In summary, the international trade of bromeliads may be the cause of three *Alepia* species, hitchhikers which were unintentionally introduced to other countries.

Citizen-science projects have been successful in detecting and monitoring non-native species as well as providing new distributional data on native species (e.g. Gardiner et al. 2012; Johnson et al. 2020; Mengual and de Soto Molinari 2020; Feldman et al. 2021; Barahona-Segovia et al. 2022; Howard et al. 2022; Kvifte 2023; Jaume-Schinkel et al. in press). Whilst Insect Investigators did not primarily aim to act as a biosecurity surveillance program, the discovery of *Alepia* specimens in a Malaise trap in a primary school in a capital city is evidence of the diversity of scientific outcomes similar citizen-science projects can generate.

Finally, the involvement of youth in citizen-science projects can have a profound impact on species conservation efforts. By engaging young individuals in scientific research and data collection, they develop a deeper understanding and appreciation for the natural world around them. Citizen-science projects provide an opportunity for children to actively participate in real-world conservation efforts, fostering a sense of ownership and responsibility towards the environment. Through their involvement, children not only contribute valuable data that can aid in species monitoring and management, but also become advocates for biodiversity conservation in their communities. Additionally, participation in citizen science can spark curiosity and inspire future generations to pursue careers in science and conservation, ensuring the continuation of efforts to protect and preserve our planet's habitats and ecosystems.

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Supplemental Files

Figure S1. Examples of Year 4 (now 5) students' drawings of insects during an exploration of ecosystems and animal adaptions to their environment, including features and characteristics and how these suit particular environments.

Figure S2. Examples of Year 4 (now 5) students' drawings of insect adaptations to their environment and comparisons with introduced species, and negative effects of invasive species. Students were asked to hypothesize as to how *Alepia viatrix* was introduced to Australia.

Appendix 5. (Publication chapter 7)

Chapter 7 – Publication

Jaume-Schinkel S, Kvifte GM, Njunjić I, Schilthuizen M (2023) New records of moth flies (Diptera, Psychodidae) for the Dutch Fauna. *Biodiversity Data Journal*, 11, e108636. https://doi.org/10.3897/BDJ.11.e108636

Biodiversity Data Journal 11: e108636
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Taxonomy & Inventories

New records of moth flies (Diptera, Psychodidae) for the Dutch Fauna

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Abstract

Background

Prior to this study, the moth flies in The Netherlands were represented by 61 species. Our findings derive from a citizen-science expedition in the Vondelpark in Amsterdam, one of the oldest public parks and best known parks in The Netherlands. The combination of citizen science and the exploration of a well-known urban park has allowed us to contribute to the knowledge of moth fly species present in The Netherlands. The findings from this study provide valuable insights into the distribution, taxonomy and genetic resources of *Psychoda* and *Panimerus* species, enhancing our understanding of insect biodiversity and promoting future research in this field.

New information

Our study provides two new geographical records of the moth flies in The Netherlands, namely, *Psychoda uniformata* Haseman, 1907 and *Panimerus maynei* (Tonnoir, 1920) elevating the total number of species to 63. Furthermore, we provide re-descriptions of the females of *Panimerus notabilis* (Eaton, 1893) and *P. goetghebueri* (Tonnoir, 1919). Additionally, we make available for the first time, the sequence of the 5'-end of the cytochrome c oxidase subunit I (*COI*) gene or *COI* Barcodes for *Panimerus notabilis*, *P. goetghebueri* and *P. maynei*. These *COI* Barcodes serve as valuable tools for future species identification within the genus.

Keywords

citizen science, Psychodinae, Taxon Expeditions, dark taxa, COI barcoding

Introduction

Moth flies (Diptera, Psychodidae) exhibit a global distribution and have been classified into approximately 3,000 documented species (Pape et al. 2011, Curler et al. 2019). This widespread family showcases remarkable diversity, particularly in tropical regions, while Europe alone has documented more than 500 species (Wagner 2013). Despite the relatively extensive taxonomic knowledge of European Psychodidae, ongoing efforts continue to reveal new species and new geographical records (e.g. Wagner and Kvifte (2015), Omelková and Ježek (2017), Morelli (2018), Kvifte et al. (2020), Jaume-Schinkel et al. (2022)Jaume-Schinkel et al. (in press)). However, there are gaps in the species distributions and knowledge remains limited; regional surveys consistently provide previously undocumented distributional records (e.g. Kvifte (2019), Jaume-Schinkel et al. (2022), Morelli and Biscaccianti (2022)).

The first checklist for the Dutch Psychodidae fauna was published in the early 1930s (Barendrecht 1934) listing 34 species for the country. Later, Wagner and Beuk (2002) published an updated checklist with 48 species. In recent years, several new additions to the Dutch fauna have been made (e.g. Boumans (2009a), Boumans (2009b), Cuppen (2009), Boumans (2011), Omelková and Ježek (2017), Ciliberti (2017), Ciliberti et al. (2017), Beuk (2021), Wagner and Beuk (2021)) bringing the total number of species to 61.

During a citizen-science entomological survey conducted in The Netherlands (Amsterdam), a Malaise trap was set in an ecologically-managed portion of one of the city's oldest parks (Vondelpark) (see van Achterberg et al. (2020), Schilthuizen et al. (2021). Derived from this survey, we recorded for the first time two species in The Netherlands bringing the total number of species to 63. Moreover, we re-describe the females of *Panimerus notabilis* (Eaton, 1893) and *P. goetghebueri* (Tonnoir, 1919) and we discuss the morphological characters to distinguish the known females of the genus. Additionally, we make available

for the first time the *COI* Barcodes (sequence of the 5'-end of the cytochrome *c* oxidase subunit I (*COI*) gene) for *Panimerus notabilis*, *P. goetghebueri* and *P. maynei*.

Materials and methods

Terminology

We follow the general terminology proposed by Cumming and Wood (2017).

Collection and preparation of specimens

Specimens of moth flies were collected using a Malaise trap during a citizen-science 'Taxon Expedition' (for the term, see Schilthuizen et al. (2017) and http://www.taxonexpeditions.com). The expedition was conducted in an ecologically-managed part (the 'Koeienweide') in one of the oldest and best-known parks in Amsterdam (Vondelpark, opened in 1865). Specimens were euthanised and preserved in 70% ethanol.

In the material examined section, at the end of each record, the holding institution is given between square brackets ([]). The abbreviations used for collections and their equivalents are given below:

ZFMK: Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels (previously known as Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany.

TXEX: Taxon Expeditions collection, Leiden, Netherlands.

Genetics

A non-destructive methodology for DNA extraction from complete specimens was performed in the facilities of Museum Koenig following the procedure detailed in Jaume-Schinkel and Mengual (in press). Specimen slide preparation was done following the protocol explained by Jaume-Schinkel and Kvifte (2022), modifying it using the whole specimen for DNA extraction. In the examined material section, the GenBank accession numbers for each specimen are given between brackets ().

Furthermore, for the genus *Panimerus*, we downloaded and used all the available sequences from BOLD (www.boldsystems.org) to include in the *COI* tree and we used Geneious Prime ver. 2022.1.1 (Biomatters, Auckland, New Zealand) to perform a distance-based neighbour-joining (NJ) analysis using the Jukes-Cantor model. On the *COI* tree, the name for each specimen contains the following information: name of the species | BOLD accession number | sample ID | GenBank accession number.

Additionally, *COI* barcodes of *Psychoda uniformata* were submitted to the BOLD Identification System (IDS) for animal identification using *COI* sequences and compared with the published sequences by Gibernau and Albre (2022). All sequences can be

accessed in BOLD under the Dataset DS-TEPANI (available: https://doi.org/10.5883/DS-TEPANI).

Taxon treatments

Panimerus albifacies (Tonnoir, 1919)

Nomenclature

Pericoma albifacies Tonnoir, 1919. Tonnoir (1919): 12. TL. Belgium, Brussels.

Telmatoscopus albifacies Tonnoir: Freeman (1950): 86, Jung (1956): 181, Duckhouse (1962): 419, Vaillant (1972): 69, Withers (1989): 32

Telmatoscopus (*Panimerus*) *albifacies* Tonnoir: Tonnoir (1940): 22, Vaillant (1961): 135.

Panimerus albifacies Tonnoir: Krek (1999): 152.

Figures: Fig. 1 A-C

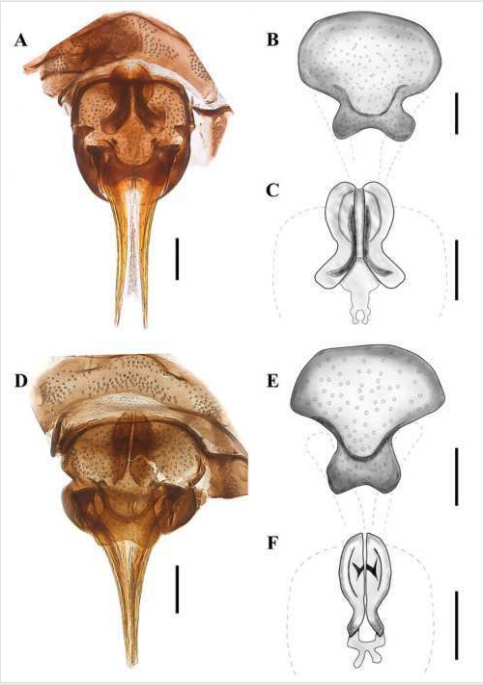

Figure 1. doi

Figure 1. A-C *Panimerus albifacies* female: **A**. Genitalia, **B**. Subgenital plate, **C.** Genital chamber. **D-F** *Panimerus goetghebueri* female: **D.** Genitalia, **E.** Subgenital plate, **F.** Genital chamber. All scale bars equal 0.10 millimetres.

Examined Material: 1 ♀. The Netherlands, Amsterdam, Vondelpark 52.3578°N, 4.8671°E. 19.VII.2019-27.VII.2019. Leg. Taxon Expeditions Team, ZFMK-TIS-2638055 (OR139013) [ZFMK] ; 1 ♂ same data as preceding, except 3.VI.2019-12.VI.2019. Leg. van der Meer, Marrit, ZFMK-TIS-2638076 (OR139014) [ZFMK] ; 2 ♂♂ same data as preceding, except 21.VI.2019-25.VI.2019. ZFMK-TIS-2638086 (OR139004), ZFMK-TIS-2638094 (OR139007) [TXEX].

Diagnosis: Females of *P. albifacies* can be easily differentiated from the known females of the genus by the shape of sternite 8 and the shape of the genital chamber Fig. 1 (A-C). Males can be easily differentiated from other males of the genus by the presence of 9 apical tenacula in the surstyli (8, 9 or more than 20 in other species), the hypandrium setose and by the ejaculatory apodeme angular laterally and rounded anteriorly (as in Vaillant (1972): plate IX).

Female redescription: Sternite 8 (subgenital plate) is wider than its length, with the anterior margin being 2.5 times wider than the posterior margin, it is covered in small setae with a few scattered larger setae on the dorsal surface, two lateral concavities right before the posterior margin, forming two lobes separated by a concavity in the posterior margin. The cerci are longer than sternite 8. The genital chamber is symmetrical as in Fig. 1A-C.

Based on the male description by Tonnoir (1919) and Jung (1956), the female is similar to the male, except the eye bridge is separated by eight facet diameters; the head is without corniculi; the pedicel is symmetrical; the flagellomeres are smaller than those of the male; apical antennal flagellomeres are absent in examined material.

Genetics: four specimens were successfully sequenced: ZFMK-TIS-2638055 (OR139013), ZFMK-TIS-2638076 (OR139014), ZFMK-TIS-2638086 (OR139004) and ZFMK-TIS-2638094 (OR139007). The maximum intraspecific uncorrected pairwise distance for COI sequences was 0.31% or 1 bp.

Distribution

Belgium, Bosnia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Lithuania, Romania, Switzerland, The Netherlands and Turkey (Vaillant 1972, Krek 1972, Salmela and Piirainen 2005, Bernotienė and Rimšaitė 2009, Ježek 2009, Wagner 2013, Wagner et al. 2013, Ježek et al. 2021).

Notes

Tonnoir (1919) stated that the female of *P. albifacies* (Tonnoir, 1919) differs from the female of *P. goetghebueri* by the colouration of the setae on the thorax, head and the base of the antennae (with white spots in *P. albifacies* and mainly black in *P. goetghebueri*). However, Freeman (1950) stated that the female of *P. notabilis* is indistinguishable from those of *P. albifacies* and *P. goetghebueri*. Later, Jung (1956) provided a diagnosis and an illustration of the female of *P. albifacies* (Jung 1956: fig.

238). Based on the published female diagnosis, the drawings provided by Jung (1956) and the re-descriptions and figures herein, the females of *P. albifacies* and *P. goetghebueri* can be differentiated by the shape of sternite 8 (anterior margin being 2.5 times wider than the posterior margin in *P. albifacies* and anterior margin 4 times wider than posterior in *P. goetghebueri*); the shape of the genital chamber (*P. albifacies*, Fig. 1 A-C and *P. goetghebueri*, Fig. 1 D–E). However, there is still a large gap when it comes to the description of females of the genus *Panimerus* and further studies could provide better diagnostic characters to easily differentiate the female specimens. Meanwhile, COI barcodes can be useful when it comes to delimiting undescribed females and associating them with previously described and barcoded male specimens.

Vaillant (1972) female diagnosis refers to the female description of Tonnoir (1919).

Panimerus goetghebueri (Tonnoir, 1919)

Nomenclature

Pericoma goetghebueri Tonnoir, 1919. Tonnoir (1919): 138. TL: Belgium. Gand et Destelberghen

Telmatoscopus goetghebueri Tonnoir: Freeman (1950): 86, Withers (1989): 32.

Panimerus (*Panimerus*) *goetghebueri* Tonnoir: Vaillant (1972): 71.

Panimerus goetghebueri Tonnoir: Jezek (1987): 227.

Figures: Fig. 1 D-F

Examined material: 1 ♀. The Netherlands, Amsterdam, Vondelpark 52.3578°N, 4.8671°E. 19.VII.2019-27.VII.2019. Leg. Taxon Expeditions Team. ZFMK-TIS-2638058 (OR139011) [ZFMK]; 1 ♂ same data as preceding except for 3.VI.2019-12.VI.2019. Leg. Van der Meer, Marrit. ZFMK-TIS-238056 (OR139010) [TXEX].

Diagnosis

Females of *P. goetghebueri* can be easily differentiated from the known females of the genus by the shape of sternite 8 and the shape of the genital chamber (Fig. 1 D and F). Males can be easily differentiated from other males of the genus *Panimerus* by having, at most, eight tenacula on the apex of the surstyli (nine or more in other species) and the ejaculatory apodeme narrowly rod-like (smaller than other species).

Female redescription: Sternite 8 (subgenital plate) is wider than its length, with the anterior margin being four times wider than the posterior margin, covered in small setae with a few scattered larger setae on the dorsal surface, two lateral concavities right before the posterior margin, forming two lobes separated by a concavity in the

posterior margin. Cerci are about the same length as sternite 8. The genital chamber is symmetrical as in Fig. 1D and F.

Based on the male description of Tonnoir (1919) and Vaillant (1972), the female is similar to the male, except the eye bridge is separated by four facet diameters; the head is without corniculi; the pedicel is symmetrical; the flagellomeres are smaller than those of the male. In the examined material, only the first palpal segment is present; apical antennal flagellomeres are absent as well.

Genetics: Two specimens were successfully sequenced: ZFMK-TIS-2638056 (OR139010) and ZFMK-TIS-2638058 (OR139011). The maximum intraspecific uncorrected pairwise distance for COI sequences was 0.45% or 3 bp.

Distribution

Algeria, Czech Republic, Hungary, The Netherlands, Tunisia and the UK (Ciliberti et al. 2017).

Panimerus maynei (Tonnoir, 1920)

Nomenclature

Pericoma maynei Tonnoir, 1920. Tonnoir (1920): 186. TL: Belgium, Ohain, Brabant.

Mormia thienemanni Tonnoir: Vaillant (1954): 91. TL: Algeria, Tala Guilef.

Telmatoscopus maynei Tonnoir: Nielsen (1961): 140.

Panimerus (*Panimerus*) *maynei* Tonnoir: Vaillant (1972): 72.

Examined material: 1 ♂. The Netherlands, Amsterdam, Vondelpark 52.3578°N, 4.867°E. 13.VI.2019-12.VI.2019. Leg. Van der Meer, Marrit. ZFMK-TIS-2638072 (OR139009) [TXEX]. 1 ♂ same data except for 21.VI.2019-25.VI.2019. ZFMK-TIS-2638090 (OR139001) [ZFMK].

Diagnosis: Females of *P. maynei* are unknown. Males can be easily differentiated from other species in *Panimerus* by having more than 30 tenacula on the surstyli (less than 20 in other species), the distribution of the tenacula being scattered in the whole surface of the surstyli (other species in the genus have the tenacula restricted to the apex of the surstyli).

Genetics: Two specimens were successfully sequenced: ZFMK-TIS-2638072 (OR139009) and ZFMK-TIS-2638090 (OR139001). Both obtained sequences are identical.

Distribution

Belgium, Czech Republic, Denmark, France, Germany, Ireland, The Netherlands (this publication, new record) and the UK. (Vaillant 1972, Withers 1989, Vaillant and Withers 1992).

Panimerus notabilis (Eaton, 1893)

Nomenclature

Pericoma notabilis Eaton, 1893. Eaton (1893): 126. TL. Great Britain.

Telmatoscopus notabilis Eaton: Tonnoir (1919): 12; Freeman (1950): 86, Duckhouse (1962): 418, Withers (1989): 32.

Telmatoscopus (*Panimerus*) *notabilis* Eaton: *Tonnoir (1940)*: 28, Jung (1956): 179.

Panimerus (*Panimerus*) *notabilis* Eaton: Vaillant (1972): 68.

Panimerus notabilis Eaton: Krek (1972): 184, Wagner (1979): 42, Ježek (1982): 52, Ježek (1984): 165, Jezek (1987): 237. (See Jezek (1987) for a complete taxonomic history).

Examined material: 1 ♂ The Netherlands, Amsterdam, Vondelpark 52.3578°N, 4.8671°E. 03.VI.2019-12.VI.2019. Leg. Van der Meer, Marrit ZFMK-TIS-2638082 (OR139012) [TXEX]. 1 ♂ same data as preceding, except for 12.VII.2019-19.VII.2019, ZFMK-TIS-2638117 (OR139000) [ZFMK].

Diagnosis: Females of *P. notabilis* are unknown. Males can be easily differentiated from all the males of the genus *Panimerus* by having nine apical tenacula in the surstyli (8, 9 or more than 20 in other species), the ejaculatory apodeme with rounded lateral lobes and is concave anteriorly (Vaillant 1972: plate IX) and by the shape of the aedeagal complex (as in Withers (1989): fig. 96).

Genetics: Two specimens were successfully sequenced: ZFMK-TIS-2638117 (OR139000) and ZFMK-TIS-2638082 (OR139012), these barcodes corresponding to the first barcodes of the species. The maximum intraspecific uncorrected pairwise distance for COI sequences was 0%.

Distribution

Belgium, Croatia, Finland, France, Germany, Hungary, Iran, Ireland, Italy, Poland, Romania, The Netherlands and Turkey (Ježek and Omelková 2012, Ježek et al. 2018, Wagner 2013, Wagner et al. 2013, Kvifte et al. 2013).

Psychoda uniformata Haseman, 1907

Nomenclature

Psychoda uniformata Haseman, 1907. Haseman (1907): 319. TL: USA. Missouri: Columbia.

Psychoda moravica Vaillant, 1966. Vaillant (1966): 225. TL: Czech Republic, Praděd (see Ježek (1990)).

Psychoda uniformata Haseman: Ježek (1990): 67.

Examined material: 1 ♀. The Netherlands, Amsterdam, Vondelpark 52.3578°N, 4.8671°E. 27.V.2019-5.VI.2019. Leg. Taxon Expeditions Team. ZFMK-TIS-2638051 (OR139003) [ZFMK]; 1 ♀ same data, except for 3.VI.2019-12.VI.2019. Leg. Van der Meer, Marrit. ZFMK-TIS-2638081 (OR139015) [TXEX].

Diagnosis

Females of *P. uniformata* can be differentiated from other *Psychoda* species by the shape of sternite 8 (subgenital plate) (as in Ježek (1990) fig. 152) and the shape of the genital chamber (as in Ježek (1990) fig. 145).

Males can be distinguished from other *Psychoda* species on the following combination of characters: the antennae with 13 flagellomeres; the gonostyli apically pointed, the distiphallus is broadly triangular, narrowing towards apex; a single paramere is present, reaching more than four-fifths length of the distiphallus. *Psychoda uniformata* is similar to *Psychoda cultella* Salmela, Kvifte & More, 2012 and *Psychoda obscuripennis* Jezek & van Harten, 2005, but they can be differentiated by the following characters: the antennae with 13 flagellomeres (14 in *P. cultella*, 13 in *P. obscuripennis*); the gonostyli are apically pointed (apically pointed in *P. cultella* and club-shaped in *P. obscuripennis*); the distiphallus broadly triangular (distiphallus parallel-sided in both *P. cultella* and *P. obscuripennis*); the paramere reaching more than four-fifths the length of the distiphallus (paramere subequal in length to the distiphallus in *P. cultella*, paramere reaching roughly two-thirds the length of the distiphallus in *P. obscuripennis*) (Ježek 1990, Ježek and van Harten 2005, Salmela et al. 2012).

Genetics: Two specimens were successfully sequenced: ZFMK-TIS-2638051 (OR139003) and ZFMK-TIS-2638081 (OR139015). The maximum intraspecific uncorrected pairwise distance for COI sequences was 2.12% or 14 bp.

Distribution

Armenia, Austria, Azerbaijan, Czech Republic, Greece, Iran, Israel, Italy, Slovakia, Slovenia, Mongolia, Morocco, The Netherlands (this publication, new record), Poland, Turkey, USA (Ježek et al. 2021, Gibernau and Albre 2022).

Discussion

Citizen-science projects can provide a more accurate picture of the real distribution of species. Previous studies by Maistrello et al. (2016), Alaniz et al. (2018), Dörler et al. (2018), Barahona-Segovia and Barceló (2021), Jaume-Schinkel and Mengual (2022), Kvifte (2023), Jaume-Schinkel et al. (in press) have highlighted the importance of citizen science in capturing species distribution data. In our study, we found two new records for The Netherlands through a citizen-science project, adding valuable information to the existing knowledge base.

These findings demonstrate the power of citizen-science initiatives in uncovering previously unknown distribution patterns and expanding our understanding of species ranges (Barahona-Segovia et al. 2022, Jaume-Schinkel and Mengual 2022, Jaume-Schinkel et al. in press). The integration of citizen-science initiatives has proven to be an invaluable asset in advancing our understanding of species distribution patterns (Barahona-Segovia and Barceló 2021). By engaging and involving the general public in scientific research, citizen-science projects provide a vast network of enthusiastic and motivated individuals who contribute to data collection on a scale that would be otherwise impossible for traditional research teams (Gardiner et al. 2012, Johnson et al. 2020, Mengual and de Soto Molinari 2020, Feldman et al. 2021, Howard et al. 2022).

In addition to their scientific contributions, citizen-science projects foster public engagement and awareness of biodiversity. By involving citizens in scientific research, these projects not only empower individuals, but also enhance their understanding of ecological processes and the importance of conservation efforts. Participants in citizen-science initiatives become ambassadors for the natural world, advocating for the preservation of species and their habitats.

Additionally, our study demonstrates the effectiveness of *COI* barcodes as a valuable tool for species identification within the genus *Panimerus* (Fig. 2). This approach greatly aids taxonomists in associating male and female specimens when there is a lack of distinct morphological features to establish a connection between both sexes. Moreover, DNA barcodes simplify the process of matching specimens from different sexes, especially in cases where only one sex is known. This streamlined matching process contributes to the identification and description of new morphological characters, which are often overlooked when working solely with one sex (such as relying heavily on male genital characters in taxonomy).

Moreover, further investigation into the applications of DNA barcoding, such as the use of other genetic markers or the integration of genomic techniques, could provide even more robust and comprehensive insights into species delimitation and distributions. It would be worthwhile to explore the potential of combining DNA barcoding with other data sources, such as remote sensing or environmental DNA, to gain a more holistic understanding of species distributions and their drivers.

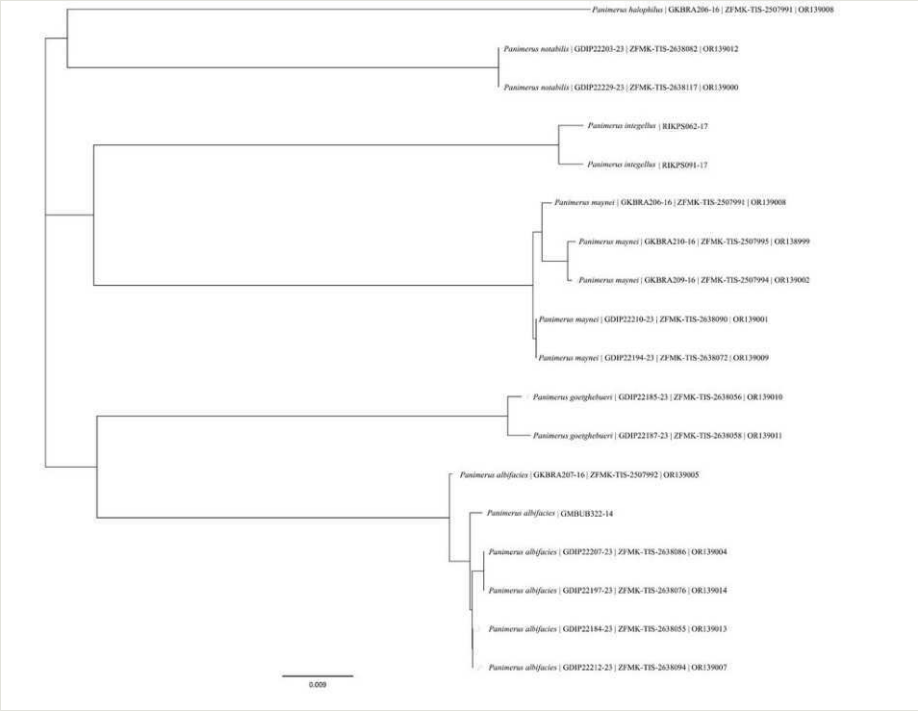

Figure 2. doi

Neighbour-joining (NJ) tree using the Jukes-Cantor model, based on the *COI* sequences of the examined material and publicly-available sequences. NJ tree constructed using Geneious Prime ver. 2022.1.1. The name for each specimen has the following information: name of the species | BOLD accession number | sample ID | GenBank accession number.

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Appendix 6. (Publication chapter 8)

Chapter 8 – Publication

Jaume-Schinkel S, Kvifte GM (in prep) Key to species of the European genus *Periulomyia* Krek, 1999 stat. nov. (Diptera: Psychodidae), with the description of *Periulomyia marijae* sp.nov.

Key to species of the European genus *Periulomyia* Krek, 1999 stat. nov. (Diptera: Psychodidae), with the description of *Periulomyia marijae* sp.nov.

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Abstract

Periulomyia Krek, 1999 was originally described as a subgenus of *Ulomyia* Walker, 1856 and is equivalent to the *Ulomyia cognata* and *Ulomyia hirta* groups described by Vaillant (1983). We diagnose it as distinct from *Ulomyia s.str.* using characters of the adult and larva. We present a catalog and an identification key to the known males of the species of this genus. Furthermore, we redescribe the type species of the genus based on material from Germany and describe *Periulomyia marijae* Jaume-Schinkel & Kvifte **sp.nov.** as a new species from Croatia.

Introduction

The generic name *Ulomyia* Walker, 1856 was proposed as a replacement name for *Saccopteryx* Haliday, 1839 proposed by Walker for the type species *Trichoptera fuliginosa* Walker, 1804 after discovering it was a junior homonym of *Saccopteryx* Illiger, 1811 (Mammalia: Chiroptera) (Kvifte, 2013). It was treated as a separate genus by Enderlein (1935) with an invalid type species designation and later (Enderlein, 1937) synonymized with *Tinearia* Schellenberg, 1803 based on another invalid type species designation. *Tinearia* in this sense was later adopted by Sará (1952). On the other hand, *Ulomyia* in the correct sense (based on *fuliginosa*) was considered a subgenus of *Pericoma* by some authors like Tonnoir (1940), Freeman (1950), and Jung (1956). Later, Vaillant (1981, 1983) discussed the morphological characters of both larval and adult stages establishing it as a separate genus and differentiating it from related pericomoid taxa such as *Bazarella* Vaillant, 1961*, Pericoma* Haliday, 1856*, Pneumia* Enderlein, 1935*,* and *Thornburghiella* Vaillant, 1982.

Moreover, Vaillant (1983) proposed different species groups within *Ulomyia,* namely, *Ulomyia fuliginosa group* [*Ulomyia fuliginosa* (Meigen, 1804); *Ulomyia basaltica* Vaillant, 1983]*, Ulomyia*

undulata group [*Ulomyia undulata* (Tonnoir, 1919); *Ulomyia hispanica* (Sara, 1954); *Ulomyia umbripennis* Vaillant, 1983; *Ulomyia scurina* (Vaillant, 1958); *Ulomyia incerta* (Wagner, 1978); *Ulomyia annulata* (Tonnoir, 1919); *Ulomyia maculosa* (Wagner, 1979)], *Ulomyia cognata group* [*Ulomyia cognata* (Eaton, 1893); *Ulomyia rostrata* Vaillant, 1983; *Ulomyia montium* Vaillant, 1983] and *Ulomyia hirta group* [*Ulomyia hirta* (Szabó, 1960); *Ulomyia ophicornis* Vaillant, 1983; *Ulomyia szaboi* Vaillant, 1983; *Ulomyia szaboi* ssp. *meridionalis* Vaillant, 1983; *Ulomyia miriabilis* (Sara, 1952); *Ulomyia spinosa* Krek, 1972]

Krek (1999) revised the genus *Ulomyia* for the Balkan Peninsula and proposed three subgenera, namely *Ulomyia* (*Ulomyia*) [*U. (U) fuliginosa* (Meigen); *U. (U) spinifera* Krek, 1990], *Ulomyia* (*Sijarićia)* [*U. (S) erinacea* Krek, 1970], and *Ulomyia (Periulomyia)* [*Ulomyia (P) cognata* (Eaton) and *Ulomyia (P) bulgarica* Wagner]. The subgenera were diagnosed with the following characters: *Ulmoyia (Ulomyia)*: Wing with central pouch, eye bridge with 7 rows of facets; *Ulomyia* (*Sijarićia)* Wing without a pouch, first three flagellomeres fused into a single segment, and, eye bridge with 5 rows of facets; *Ulomyia (Periulomyia)* wing without a central pouch, first flagellomeres clearly separated from each other, and eye bridge with six rows of facets. *Sijaricia* was later placed in synonymy with *Thornburghiella* by Ježek et al. (2022).

In this study, we revisited the subgeneric classification proposed by Krek (1999), and the species groups proposed by Vaillant (1983). In this context, the genus *Periulomyia* Krek **stat. nov.**, corresponds to the *Ulomyia cognata* and *Ulomyia hirta* species groups proposed by Vaillant (1983). We diagnose it as a distinct genus from *Ulomyia s. str.,* using morphological characters of larvae and adults, thus transferring and creating new combinations for *Periulomyia cognata* (Eaton, 1893) **comb. nov**., *P. hirta* (Szabó, 1960) **comb. nov.**, *P. meridionalis* (Vaillant, 1983) **comb. nov.**, *P. mirabilis* (Sarà, 1952) **comb. nov**., *P. montium* (Vaillant, 1983) **comb. nov.**, *P. ophicornis* (Vaillant, 1983) **comb. nov**., *P. rostrata* (Vaillant, 1983) **comb. nov.**, *P. szaboi* (Vaillant, 1983) **comb. nov.**, *P. vaseki* (Ježek, 2002) **comb. nov**., and describing a new species from specimens collected in Croatia, *P. marijae* Jaume-Schinkel & Kvifte **sp. nov**.

Material and Methods

The examined material is deposited in different entomological collections referred to by their abbreviations in the text. The abbreviations used for collections and their equivalents are given below:

NHM: Natural History Museum, London, United Kingdom.

ZMBN: University Museum of Bergen, Bergen, Norway.

ZFMK: Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels (previously known as Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany.

Terminology. We follow the general terminology proposed by Cumming & Wood (2017) and Kvifte & Wagner (2017a). Additionally, we use the term 'hypopods' to refer to the caudal appendages, which are also referred to as cercopods, epandrial appendages, or surstyli (see the discussion in Kvifte & Wagner, 2017b).

Results

Taxonomic account

Class Insecta Linnaeus, 1758

Order Diptera Linnaeus, 1758

Suborder Psychodomorpha Hennig, 1968

Family Psychodidae Newman, 1834

Subfamily Psychodinae Newman, 1834

Genus *Periulomyia* Krek, 1999 stat. nov.

Ulomyia subgen. *Periulomyia* Krek, 1999: 279. Type species: *Periulomyia cognata* (Eaton, 1893).

Differential diagnosis. Eyebridge six facets wide at its widest point (except 7 in *P. szaboi* Vaillant), first and second flagellomeres (third and fourth antennal segments) carrying stiff bristles in aseriate clusters; allurement organs originating from proepisternum; 4th instar larvae with accessory setae not deciduous, usually present on preserved specimens. In *Ulomyia* Walker, 1856, the eye bridge is seven facets wide at its widest point, stiff bristles are present only on the first flagellomere (third antennal segment), or absent; allurement organs originate from anepisternum and 4th instar larvae have accessory setae deciduous, usually missing from preserved specimens.

Species: *Periulomyia cognata* (Eaton, 1893) **comb. nov.**, *P. hirta* (Szabó, 1960) **comb. nov.**, *P. marijae* **sp. nov.,** *P. meridionalis* (Vaillant, 1983) **comb. nov.**, *P. mirabilis* (Sarà, 1952) **comb. nov.**, *P. montium* (Vaillant, 1983) **comb. nov.**, *P. ophicornis* (Vaillant, 1983) **comb. nov.**, *P. rostrata* (Vaillant, 1983) **comb. nov.**, *P. szaboi* (Vaillant, 1983) **comb. nov.**, *P. vaseki* (Ježek, 2002) **comb. nov.**

***Periulomyia cognata* (Eaton, 1893) comb. nov.**

(Fig. 1A)

Pericoma cognata Eaton, 1893: 121. Type locality: not given, probably the UK (Great Britain).

Ulomyia cognata (Eaton): Vaillant, 1983: 319

Ulomyia (Periulomyia) cognata (Eaton): Krek, 1999: 288

Diagnosis. Eye bridge separated by 5-6 facet diameters; interocular suture V-shaped; first flagellomeres (3rd and 4th antennal segments) clearly separated; the number of spines on flagellomeres 1, flagellomere 2; Gonostyli with bulbous base, gonostyli base broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli shorter than the bulbous base; hypopods with 7-8 tenacula.

Examined Material. m#, slide mounted, "Germany // Rheinland-Pfalz, Niederzissen //MF7B, Bausenberg, Wiese // 50.46541667, 7.2259 // 16-29.03.2013 // Leg. Rulik et al. // Malaise Trap" "ZFMK-DIP-00081613" "Psychodidae // Periulomyia // cognata // Det. Jaume-Schinkel, S." [ZFMK].

Syntypes 2m#, pinned United Kingdom, Somerset, Pen Selwood, Near Wincanton, 51.083541, -2.353065. Leg. Eaton. 12. Sep. 1891. BMNH(E)235384; BMNH(E)235385 [NHM]. f#, pinned United Kingdom; Somerset, Cockload Wood, Pen Selwood. Leg. Eaton, 08 Sep. 1892. BMNH(E)235383 [NHM]. m#, pinned, United Kingdom, Somerset, Godminster Wood, Near Bruton, Leg. Eaton, 05 Sep. 1891. BMNH(E)235382 [NHM].

Redescription.

Measurements in mm (n=1). Wing length 2.50, width 1.10; head length 0.52, width 0.51; antennal segments, scape: 0.10, pedicel: 0.06; flagellomeres 1-11: 0.05, flagellomeres 12-14: 0.03; palpomeres 1: 0.12, 2: 0.18, 3: 0.18, 4: 0.21.

Male. Head around the same length as width, eyes separated by approximately 3 facet diameters at the narrowest point, separated by 5 facet diameters at the widest point; eye bridge with rows of 4,6,6,6,6 facets; interocular suture curved, as a wide-open u. Fontal patch with anterior margin extending towards interocular suture, posterior margin rounded, not divided. Antenna with scape about 1.6 times longer than its width, about 2 times the length of the pedicel, cylindrical; pedicel spherical; first two flagellomeres separated, symmetrical, barrel-shaped, first flagellomere with five-six strong bristles, the second flagellomere with five strong bristles, remaining flagellomeres symmetrical and barrel-shaped without strong bristles, apical three flagellomeres shorter than previous 11, apical flagellomere with a digitiform apical projection, shorter than the length of the engrossed portion of flagellomere. Palpal proportions, 1.0:1.5:1.5:1.7; palpomeres cylindrical with apical palpomere corrugated.

Wing 2.2 times longer than wide, hyaline; Sc ending in the wing membrane, ending before the base of R_{2+3}; R_4 ending before the wing apex, R_5 ending after wing apex; forks of R_{2+3} and M_{1+2} weakly sclerotized, both forks at the same level on the wing membrane; CuA reaching wing margin.

Terminalia. Hypandrium narrow, plate-like; gonocoxites shorter than gonostylus, cylindrical, longer than wide; gonostylus with a basal broad base, broader than gonocoxites, with an apical

abruptly narrowing projection, apical projection length is equal to about half the wide of the gonosyli; gonocoxal apodemes plate-like, partially fused; aedeagal sheath with apical margin concave at mid, covering basal half of the edeagal complex, reaching the junction between ejaculatory apodeme and aedeagus; aedeagus symmetrical; ejaculatory apodeme dorsoventrally flattened, narrow in its entire length; epandrium wider than long; basal margin concave around entire length, apical margin almost straight; surstylus about 1.5 times the length of gonocoxites, base broad slightly narrowing towards the apex, apical margin rounded with 7-8 apical tanacula on each; tenacula apex rounded; epiproct tongue-shaped with rounded margin, covered in small setulae.

Distribution. Austria, Belgium, Czech Republic, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Poland, Slovakia, Slovenia, Ukraine, Turkey, United Kingdom (Vaillant, 1983; Wagner, 2004; Ježek and Omelková 2012; Salmela et al. 2014; Ježek et al. 2017; Jezek, 2002; Jezek et al 2018a)

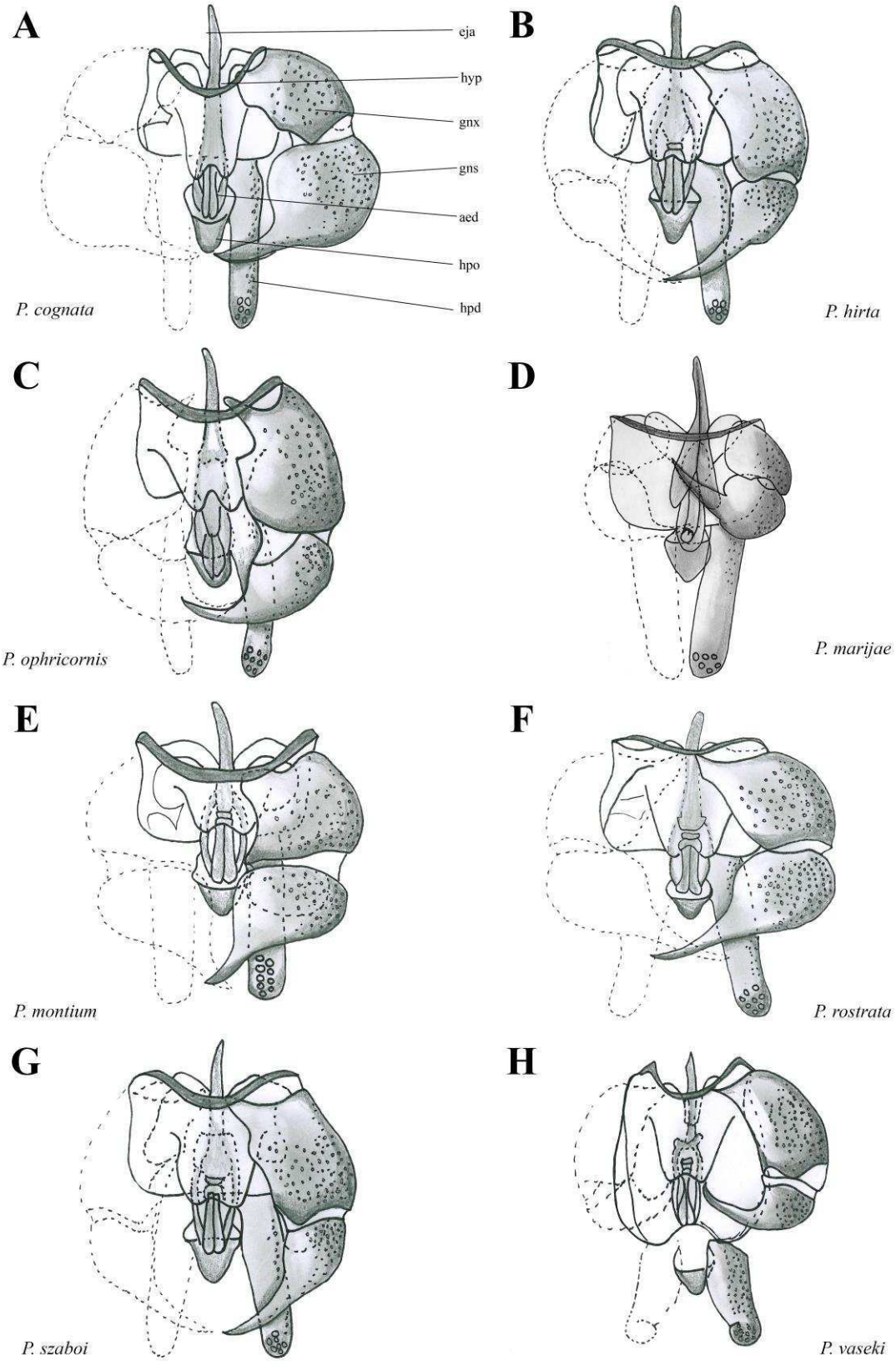
A
eja
hyp
gnx
gns
aed
hpo
hpd
P. cognata
B
P. hirta
C
P. ophricornis
D
P. marijae
E
P. montium
F
P. rostrata
G
P. szaboi
H
P. vaseki

Figure 1 Male genitalia of *Periulomyia* species. **A.** *P. cognata* (Eaton, 1893) **B.** *P. hirta* (Szabò, 1960); **C.** *P. ophicornis* (Vaillant, 1983); **D.** *P. marijae* sp. nov. Jaume-Schinkel & Kvifte; **E.** *P. montium* (Vaillant, 1983); **F.** *P. rostrate* (Vaillant, 1983) **G.** *P. szaboi* (Vaillant, 1983); **H.** *P. vaseki* (Ježek, 2002).

***Periulomyia hirta* (Szabò, 1960) comb. nov.**

(Fig. 1B)

Pericoma hirta Szabò, 1960: 169. Type locality: Romania, Mehadia.

Ulomyia hirta (Szabò): Kvifte, 2012: 63. (for a discussion of the nomenclature of this species see Kvifte (2012)).

Diagnosis: Eye bridge separated by 2 facet diameters; interocular suture Y-shaped; first flagellomeres (3rd and 4th antennal segments) clearly separated; the number of spines on flagellomeres 1: 6, flagellomere 2: 3, flagellomere 3: 1; Gonostyli without a bulbous base, gonostyli base not broader than gonocoxites, gonostyli gradually tapering towards apex; hypopods with 6 tenacula.

Distribution. Austria, France, Germany, Hungary (Vaillant, 1983; Wagner, 2004)

***Periulomyia ophicornis* (Vaillant, 1983) comb. nov.**

(Fig. 1C)

Ulomyia ophicornis Vaillant, 1983: 322. Type locality: Romania, Carpathian Mountains.

Diagnosis: Eye bridge separated by 4-5 facet diameters; interocular suture U-shaped; first flagellomeres (3rd and 4th antennal segments) clearly fused; the number of spines on flagellomeres 1+2: 3; Gonostyli with bulbous base, gonostyli base not broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli shorter than bulbous base, incurved; hypopods with 6-7 tenacula.

Distribution. Romania (Vaillant, 1983)

Periulomyia marijae **Jaume-Schinkel & Kvifte, sp.nov.**

Figures (1D, 2)

Ulomyia sp. nov. - Ivković et al. 2015: 50-51, 53.

Examined material. *Holotype*, m#, slide mounted, "*CROATIA: Pritrice Jezera // izbor Bijele Rijeke p4 // IX.2009 // Emergence trap P4 // M. Ivkovié // XIIPS1038" "Ulomyia marijae sp.nov." "B9205"* [ZMBN].

Differential Diagnosis.

Periulomyia marijae **sp. nov**. can be differentiated from other species in *Periulomyia* by the following characters: the first two flagellomeres (3rd and 4th antennal segments) fused; 3 to 5 spines on flagellomere 1+2 (3rd and 4th antennal segments); the eye bridge is separated by 4 facet diameters; the bulbous base of gonostyli not broader than the gonocoxites. This species is closely related to *Periuolmyia ophicornis*, but they can be differentiated by the following characters: *P. marijae* **sp. nov**. with the eye bridge separated by 4 facet diameters (4-5 facet diameters in *P. ophicornis)*; interocular suture slightly curved, almost straight in *P. marijae* **sp. nov.** (interocular suture U-shaped in *P. ophicornis*); *P. marijae* **sp. nov.** with flagellomere 1+2 (3rd and 4th antennal segments) with 5 spines (flagellomere 1+2 (3rd and 4th antennal segments) with 3 spines in *P. ophicornis*) in *P. marijae* **sp. nov**. the narrowed part of gonostyli longer than bulbouse base of gonostyli (narrowed part of gonostyli shorter than bulbous base in P. ophicornis).

Description.

Measurements in mm (n=2). Wing length 2.25, width 1.07; head length 0.52, width 0.50; antennal segments, scape: 0.10, pedicel: 0.06; palpomeres 1: 0.10, 2: 0.18, 3: 0.17, 4: 0.22.

Male. Holotype. Head around the same length as width, eyes separated by approximately 4 facet diameters; eye bridge with rows of 4,5,6,6,6 facets; interocular suture slightly curved, almost straight. Fontal patch with anterior margin extending towards interocular suture, posterior margin rounded, not divided. Antenna with scape about 1.3 times longer than its width, about 2 times the length of the pedicel, cylindrical; pedicel spherical; first two flagellomeres fused, symmetrical, barrel-shaped, first flagellomere with a row of 3 bristles, the second flagellomere with a row of 2 bristles, remaining flagellomeres not fused, symmetrical, barrel-shaped. The total number of

flagellomeres is unknown as apical flagellomeres are missing in the examined material, the maximum number of flagellomeres = 11. Palpal proportions, 1.0:1.8:1.7:2.2.

Wing 2 times longer than wide, hyaline; Sc ending in the wing membrane, extending towards the base of R2+3_5; R_4 ending before the wing apex, R5 ending after wing apex; CuA reaching wing margin.

Terminalia. Hypandrium narrow, plate-like; gonocoxites shorter than gonostylus, reniform, wider than long; gonostylus with a basal broad base, as broad as gonocoxites, and apical part narrow; gonocoxal apodemes plate-like, partially fused; aedeagal sheath deeply divided at mid, covering basal half of the edeagal complex; aedeagus symmetrical; ejaculatory apodeme dorsoventrally flattened, rounded at the anterior margin and tapering towards aedeagus, paramere curved towards coverglass, with a sclerotized apex, horse-shoe-shaped, phallomeres curved, joined at middle; epandrium wider than long; basal margin concave around entire length, apical margin almost straight; surstylus about 1.75 times the length of gonocoxites, base broad getting narrow towards the apex, apical margin rounded with 6-7 apical tanacula on each; tenacula apex rounded, concave; proctiger not visible on the slide.

Female. unknown.

Etymology. We take great pleasure in naming the new species after Marija Ivkovic, collector of the holotype, excellent freshwater biologist and dipterist, and not least a great friend.

Biology. The new species was collected in emergence traps from a karstic spring stream, and some details of its biology are given by Ivkovic et al (2015).

Distribution. only known from the type locality in Croatia.

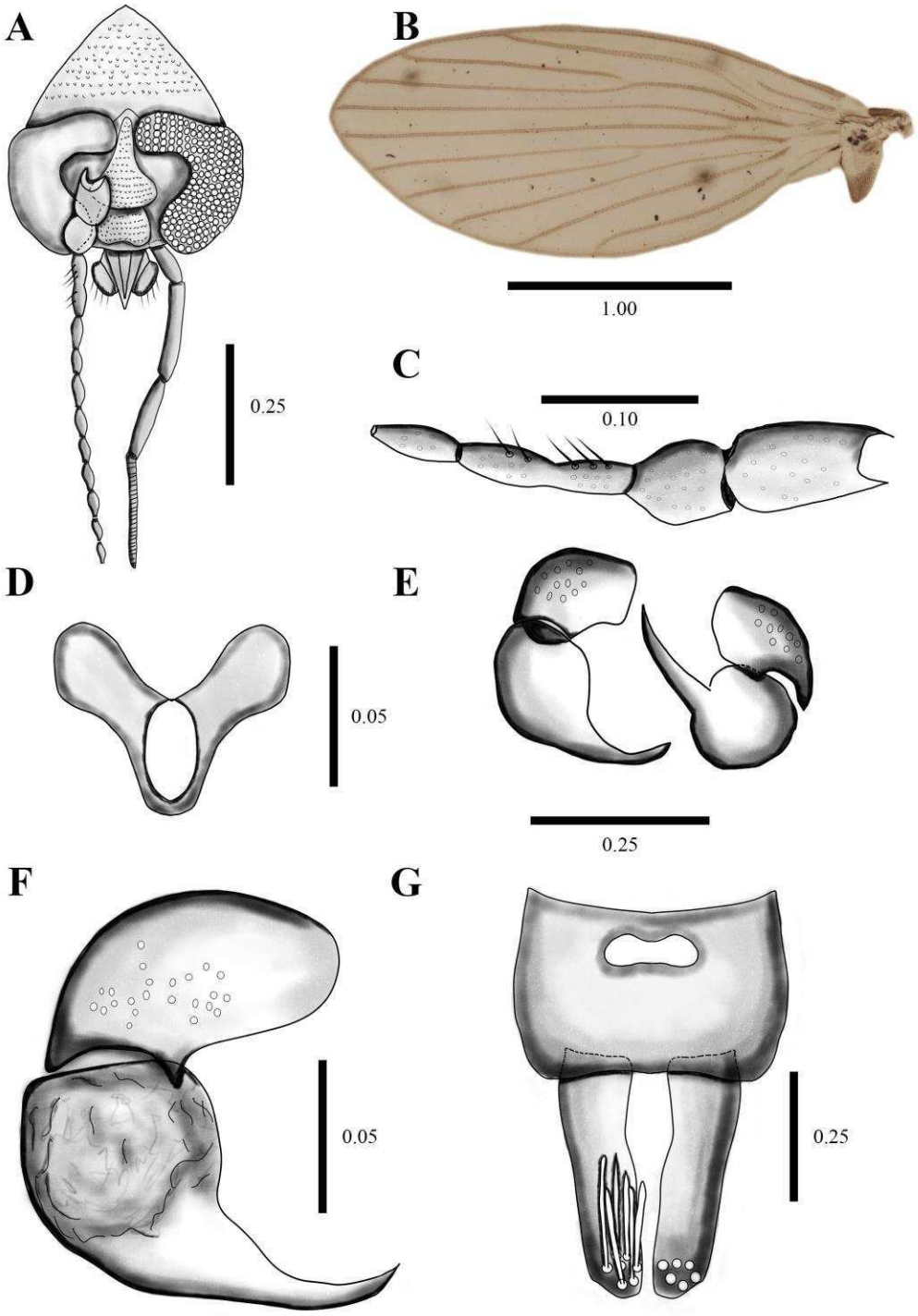

Figure 2 *Periuolomyia marijae* sp. nov. Jaume-Schinkel & Kvifte. Male holotype. **A.** head; **B.** wing; **C.** first antennal segments; **D.** gonocoxal apodemes; **E.** gonocoxites and gonostyli; **F.** gonocoxite and gonostylus; **G.** Epandrium and hypopods. All scales in milimiters (mm)

***Periulomyia mirabilis* (Sarà, 1952)**

Tinearia mirabilis Sarà, 1952: 2. Type locality: Italy, Campagnia (Forli), Lugilo.

Ulomyia mirabilis (Sarà): Vaillant, 1983: 323.

Diagnosis: Eye bridge separated by 1 facet diameters; interocular suture V-shaped; first flagellomeres (3rd and 4th antennal segments) clearly separated; the number of spines on flagellomeres 1: 7, flagellomere 2: Gonostyli with bulbous base, gonostyli base broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli, straight and very narrow, shorter than the bulbous base; hypopods with 8-10 tenacula.

Distribution: Italy (Sara, 1952; Vaillant, 1983)

***Periulomyia montium* (Vaillant, 1983) comb. Nov.**

(Fig. 1E)

Ulomyia montium Vaillant, 1983: 320. Type locality: France.

Diagnosis: Eye bridge separated by 4 facet diameters; interocular suture wide U-shaped; first flagellomeres (3rd and 4th antennal segments) fused; the number of spines on flagellomeres 1+2: 2, flagellomere 3: 5; Gonostyli with bulbous base, gonostyli base broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli shorter than the bulbous base; hypopods with 6 tenacula.

Distributio.: France, Germany (Vaillant, 1983)

***Periulomyia rostrata* (Vaillant, 1983) comb. nov.**

(Fig. 1F)

Ulomyia rostrata Vaillant, 1983: 320. Type locality: Romania.

Diagnosis: Eye bridge separated by 4 facet diameters; interocular wide open U-shaped; first flagellomeres (3rd and 4th antennal segments) clearly separated; the number of spines on flagellomeres 1: 6, flagellomere 2: 6; Gonostyli with bulbous base, gonostyli base broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli longer than the

bulbous base; hypopods with 6-8 tenacula.

Distribution: Romania (Vaillant, 1983)

***Periulomyia szaboi* (Vaillant, 1983) comb. nov.**

(Fig. 1G)

Ulomyia szaboi Vaillant, 1983: 322. Type locality: France.

Ulomyia szaboi meridionalis Vaillant, 1983: 322. **syn. nov.**

Diagnosis: Eye bridge separated by 1.5 facet diameters; interocular suture closed U-shaped; first flagellomeres (3rd and 4th antennal segments) fused; the number of spines on flagellomeres 1+2: 7, flagellomere 3: 4; Gonostyli with bulbous base, gonostyli base not broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli longer than the bulbous base; hypopods with 5-6 tenacula.

Distribution: France, Germany (Vaillant, 1983; Wagner, 2004)

Remarks. Vaillant (1983) found no morphological differences in the larval stages between *Ulomyia szaboi* and *Ulomyia szaboi ssp. meridionalis*, and furthermore mentioned that the differences between the adults are subtle, however he did not state which differences can be seen between the adults. We found not enough evidence in the descriptions from Vaillant (1983) to consider them as separate species, therefore, we consider them as synonyms until further evidence is presented.

***Periulomyia vaseki* (Ježek, 2002) comb. nov.**

(Fig. 1H)

Ulomyia vaseki Ježek, 2002: 94. Type locality: Czech Republic.

Diagnosis: Eye bridge separated by 3 facet diameters; interocular suture short U-shaped; first flagellomeres (3rd and 4th antennal segments) clearly fused; the number of spines on flagellomeres 1+2: 5, flagellomere 3: 6; Gonostyli with bulbous base, gonostyli base not broader than gonocoxites, gonostyli abruptly tapering towards apex, narrowed part of gonostyli longer than the

bulbous base; hypopods with 6-9 tenacula.

Distribution: Czech Republic, Slovenia, Slovakia (Jezek, 2002; Wagner, 2004)

Key to adult males of *Periulomyia* Krek, 1999

1. The first two flagellomeres (3rd and 4th antennal segments) separated..... **2**

-. The first two flagellomeres (3rd and 4th antennal segments) fused (as in Fig. 2C)… **5**

2. Interocular suture Y-shaped; Gonostyli without bulbous base, gradually tapering towards apex (Fig. 1B)… ***P. hirta***

-. Interocular suture variable in shape, but not Y-shaped; Gonostyli with bulbous base, abruptly narrowing towards apex (Fig. 1A, C, E)… **3**

3. Eye bridge separated by 4-6 facet diameters…4

-. Eye bridge separated by 1 facet diameters; interocular suture V-shaped; narrowed part of gonostyli, straight and very narrow, shorter than the bulbous base… ***P. mirabilis***

4. Eye bridge separated by 5-6 facet diameters; interocular suture V-shaped; Narrowed part of the gonostyli is noticeably shorter than the bulbous base (Fig. 1A)… ***P. cognata***

-. Eye bridge separated by 4 facet diameters; interocular suture wide open U-shaped; Narrowed part of the gonostyli as long or longer than bulbous base (Fig. 1 F)… ***P. rostrata***

5. eye bridge separated by less than 2 facet diameters… ***P. szaboi***

-. eye bridge separated by 3 or more facet diameters … **6**

6. Bulbous base of gonostyli broader than gonocoxite (Fig. 1E) … ***P. montium***

-. bulbous base of gonostyli not broader than gonocoxite (Fig. 1H) … **7**

7. eye bridge separated by 3 facet diameters; flagellomere 1+2 (3rd and 4th antennal segments) with 5 spines … ***P. vaseki***

-. eye bridge separated by 4 or more facet diameters; flagellomere 1+2 (3rd and 4th antennal

segments) with 3 or 5 spines… **8**

8. eye bridge separated by 4-5 facet diameters; flagellomere 1+2 (3rd and 4th antennal segments) with three spines; narrowed part of gonostyli shorter than bulbous base (Fig. 1C)… ***P. ophicornis***

-. eye bridge separated by 4 facet diameters (Fig. 2 A); flagellomere 1+2 (3rd and 4th antennal segments) with 5 spines (Fig. 2C); narrowed part of gonostyli longer than bulbous base (Fig. 1D)… ***P. marijae*** **sp. nov.**

Discussion.

Periulomyia was defined by Krek (1999) as a subgenus of *Ulomyia* to include the species *Ulomyia (P) cognata (Eaton)* and *Ulomyia (P) bulgarica Wagner*, diagnosing it with the following morphological characters: wing without a central pouch, first flagellomeres clearly separated from each other, and eye bridge with six rows of facets. We dispute the placement of *Ulomyia bulgarica* Wagner & Joost, 1988 as a species of *Periulomyia*. In the original description by Wagner & Joost (1988) it is clearly mentioned that the wing of *Ulomyia bulgarica* has the typical undulate (sinuous) shape of the *Ulomyia undulata* species group proposed by Vaillant (1983). Moreover, Wagner & Joost (1988) discussed *Ulomyia bulgarica* as probably closely related to *Ulomyia opaca* (Tonnoir, 1922) and the possibility that this species group may be closer to *Pneumia* due to similarities with *Pneumia trivialis* (Eaton, 1893). Based on the presented evidence in the original description by Wagner & Joost (1988) we do not consider this species to belong to *Periulomyia*, contrary to the placement in Krek (1999).

The original drawings provided by Sara (1952), later reproduced by Vaillant (1981), for *Periulomyia mirabilis* **comb. nov.** (described as *Tinearia mirabilis*) do not show the aedeagal structure, nor the complete genitalia, making it hard to compare with others. Nonetheless, the combination of characters provided in the description is sufficient to separate it from other species in *Periuolomyia*. It is worth mentioning that we did not examine the holotype and it would be desirable to produce new illustrations of the male genitalia including the missing characters in the original description.

To date, the genus *Periulomyia* has nine extant species, namely, *P. cognata, P. hirta, P. marijae* **sp. nov.**, *P. meridionalis, P. mirabilis, P. montium, P. ophicornis, P. szaboi,* and, *P. vaseki*. All of them are restricted to the West Palearctic region.

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Appendix 7. (Publication chapter 9)

Chapter 9 – Publication

Jaume-Schinkel, S. (in prep) A key to the Moth flies (Diptera: Psychodidae) of Europe, including subfamilies and genera.

Disclaimer: This unpublished work is an ongoing effort to update the taxonomical key of the subfamilies and genera of Psychodidae present in Europe. It is intended to be submitted to a journal. Author list is not final and is likely to change.

A key to the Moth flies (Diptera: Psychodidae) of Europe, including subfamilies and genera.

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Abstract

The most comprehensive identification keys for the family in the Palearctic Region were published over 50 years ago and numerous nomenclatural changes have been published since then. Thus, the need arises for a new key with updated nomenclature. In this manuscript, we comprehensively discuss all the subfamilies present in Europe, offering an identification key with illustrations to distinguish each subfamily. Additionally, we introduce each subfamily with a concise summary of historical classification and known biology of larvae and adults, along with comments on the known number of genera and species. For subfamilies with multiple genera, we provide a key for genus identification. Each couplet in the identification key is illustrated, highlighting the characters necessary to follow the couplet.

Introduction

The family Psychodidae has nearly 3,400 described species distributed worldwide, including two introduced species in the Antarctic Region (Curler & Courtney, 2009; Frenot et al., 2005; Galati & Rodrigues, 2023; Wagner & Ibáñez-Bernal, 2009). Several different intrafamilial classifications have been proposed throughout history and the family subdivision is still under debate (Curler & Courtney, 2009), but six subfamilies are generally recognized, namely Bruchomyiinae, Horaiellinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyiinae. Nevertheless, the monophyly of the family is still debatable (Henning, 1972; Quate & Vockeroth, 1981; Bertone et al., 2008; Wiegemann et al., 2011). On the contrary, some authors have recognized Phlebotominae as a separate family (Azar et al. 1999; Williams, 1993) although this recognition would require the elevation of all subfamilies to family and since the phylogenetic relationships between subfamilies remain unresolved this system is not worldwide accepted (Curler & Moulton, 2012; Kvifte & Wagner, 2017). There are several studies aiming to resolve the suprageneric Psychodidae classification, especially within the subfamily Psychodinae (e.g., Vaillant, 1971; Ježek, 1983; Duckhouse, 1987; Rispail & Leger, 1998,

Kvifte, 2018). Recent studies based on nuclear and mitochondrial DNA sequence data (E.g. Curler & Moulton, 2012; Espindolá *et al*., 2012; Kvifte, 2018) revealed that the tribal classification proposed by Vaillant (1971) and Ježek (1984) comprises non-monophyletic groups. Until now, systematic classification systems based on different character datasets do not agree with the subdivision of the family (Kvifte, 2018), especially at the tribe level, thus, no tribal classification is followed in the present work.

Adult psychodids are small nematoceran flies (less than 5mm) easily recognizable by their setose vestiture and their distinctive wing shape and venation. Many of them hold their wings at rest horizontally over the abdomen, giving them the appearance of small moths; hence the common name "moth flies" (Curler & Courtney, 2009; Wager & Ibáñez-Bernal, 2009). Adults have somewhat erratic and weak flights, but despite that, they have managed to live in a wide variety of habits and niches from high montane streams to very contaminated wastewater and drains. Their larvae can be found in several aquatic, semi-aquatic, and even in not very humid habitats such as rotting wood, carrion, fungi, dung, and soil (Wagner, 1977; Wager & Ibáñez-Bernal, 2009; Bravo et al., 2014). The majority of the known moth fly larvae are considered detritivores as they require decaying organic matter as a substrate or food source, but the larval biology of a vast number of species is still unknown (Wagner, 1977; Wager & Ibáñez-Bernal, 2009; Bravo et al., 2014; Kvifte & Wagner, 2017).

Some species of moth flies are vectors of zoonotic diseases, especially human leishmaniasis (Munstermann, 2004), and some others are considered possible indicators of habitat quality (Alexander et al., 2001; Barrett et al., 1996). But their very small size and very often scarce numbers in samples make them a neglected and understudied group among the Diptera.

Europe has the fauna of Psychodidae most thoroughly studied, where more than 500 described species are recorded (Wagner, 2004; Kvifte, 2015). The taxonomic knowledge for moth flies in Europe is uneven, though, with a few countries having high numbers of studies and records (e.g., Bulgaria, France, Germany, Czech Republic, Slovakia, and Yugoslavia) but many others lack systematic collection of specimens. This current situation results in many unknown species' distributions and poor taxonomic treatment in Europe (Kvifte & Andersen, 2012; Kvifte *et al*., 2016; Wagner, 2001). In addition, the most complete identification keys for the family Psychodidae in Europe were published 50 years ago by Vaillant (1971) and they are only available in German. Several major taxonomic changes took place in the last five decades, which justify the urgent need for an updated identification key for the European Psychodidae

fauna. The major aim of this study is, therefore, to provide an identification key for the five subfamilies and all genera present in Europe, together with a brief introduction to each subfamily.

Material and Methods.

Terminology.

We follow the terminology used by Galati et al. (2017) and Kvifte & Wagner (2017).

Definition of Europe.

We follow the proposed geographic boundaries by Jong *et al*. (2014) which comprehends: East: Ural (E 60°), West: Atlantic Ocean (W 30°), South: Mediterranean (N 35°), North: Atlantic Islands (N 82°). As stated in the database Fauna Europea (Jong *et al* 2014) some species are included with their distribution stated as "European Turkey", some species included here are distributed in "not European Turkey", but both are included as Turkey (Figure 1).

We follow the classification of the subfamilies that have been generally accepted in Curler & Moulton (2012), Kvifte & Wagner (2017), Wagner (2009), and Wagner & Ibáñez-Bernal (2009). Therefore, we recognize six subfamilies: Bruchomyiinae, Horaiellinae, Phlebotominae, Psychodinae, Sycoracinae, and Trichomyiinae. All with the exception of Horaiellinae are present in the European Fauna.

Results

From the previous database Fauna Europea (Wagner, 2004) approximately 500 species were recorded for Europe, with the update of the records and descriptions of new species we now report 546 species for Europe, 49 more species in the last two decades for the previously reported database. The following subfamilies are present in Europe: Bruchomyiinae, 1 genus; Phlebotominae, 2 genera; Psychodinae, 40 genera; Sycoracinae, 1 genus; Trichomyinae, 1 genus.

Additionally, we present a brief discussion for each subfamily trying to address the taxonomical problematics through the years and notes on the known biology for each subfamily. Moreover, we present a key to the subfamilies present in Europe and a Key to the genera of Each subfamily, except Bruchomyiinae, Sycoracinae, and Trichomyinae since only one genus each is present in Europe.

Finally, we update the nomenclatural changes that have occurred in the last two decades for all the species present in Europe (Based on the nomenclature followed by Wagner, 2004), in addition, we update the geographical distribution and the records for each country in Europe.

Key to the subfamilies of Psychodidae in Europe

The identification key is based on the works of Wagner (1997), Wagner & Ibáñez-Bernal (2009), and Kvifte & Wagner (2017). The present key is valid for adult flies.

1	Eyes with a dorsal extension towards the midline (Eye Bridge) (Fig. 1 A); IF absent, THEN often a dorsal suture between eyes. Male terminalia with spatulate or feather-like tenacula	***Psychodinae***
-	Eyes without dorsal extension towards midline (Fig. 1 B); dorsal suture between eyes never present. Male Terminalia with or without tenacula	**2**

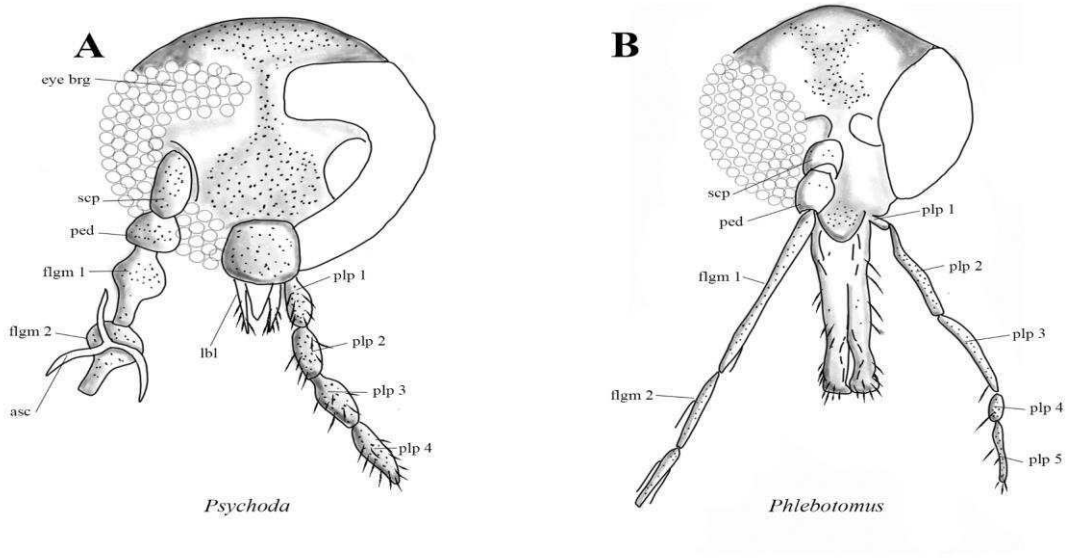

Fig. 1. A. Head of *Psychoda sp.* **B.** Head of *Phlebotomus sp*. Abbreviations: asc – ascoid, eye brg – eye bridge, flgm – flagellomere, lbl – labella, ped – pedicel, plp – palpal segment, scp – scape. No scale.

2	Wing vein R with five branches, with two longitudinal veins between radial and medial forks (Fig. 2 A, C)	**4**
-	Wing vein R with four branches, with one longitudinal vein between radial and medial fork (Fig. 2 B, D)	**3**

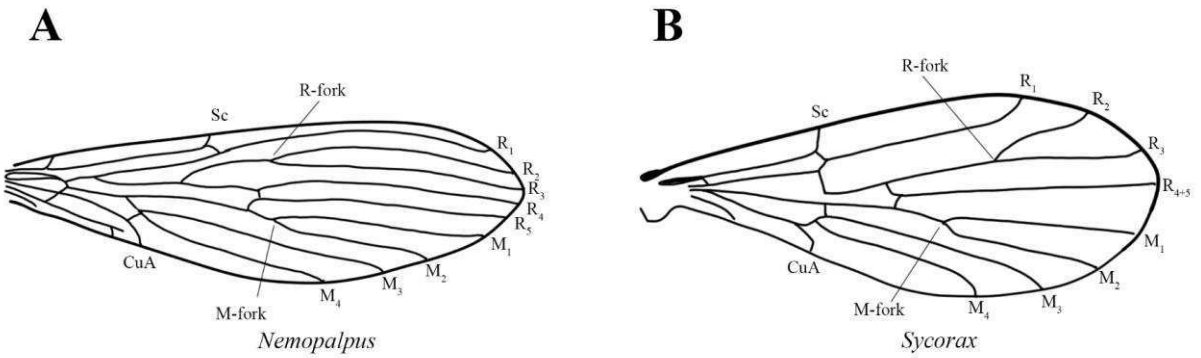

Fig. 2. A. Wing of *Nemopalpus sp.* **B.** Wing of *Sycorax sp.* Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

3	Wing vein CuA long, extending beyond the level of the medial fork (Fig. 2 D); only the first basal cell developed. Female mouthparts non-functional (Fig. 1 A, C, D) . Male genitalia inverted	***Trichomyiinae***
-	Wing vein CuA short, ending at about the level of the origin of the medial fork (Fig 2 B); basal cells developed. Females with functional mouthparts (Fig. 1 B). Male genitalia in European species not inverted	***Sycoracinae***

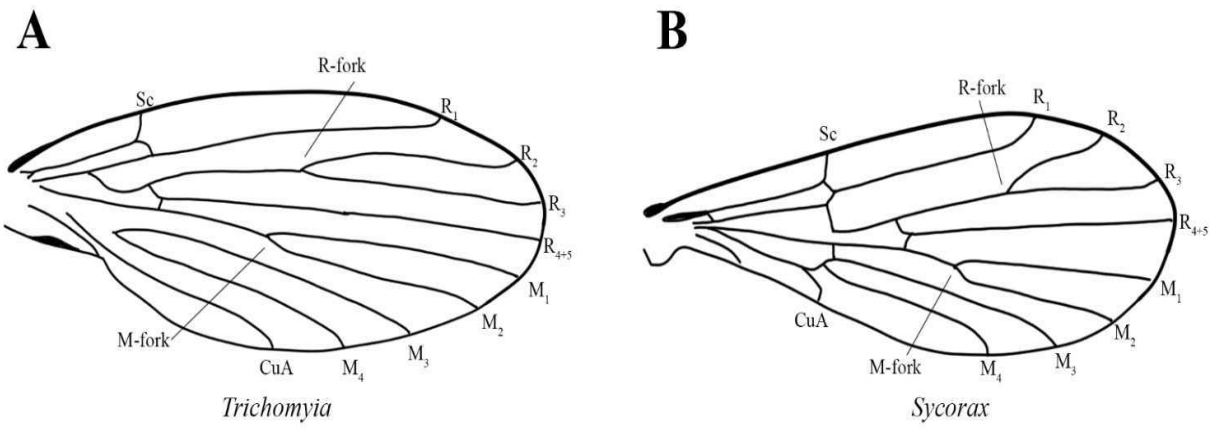

Fig. 3. A. Wing of *Trichomyia sp.* **B.** Wing of *Sycorax sp.* Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

4	Both sexes: Antennae with 14 flagellomeres. Females: with functional mouthparts (Fig. 1 B) and two spermathecas. Male terminalia: epandrium with fixed appendages; gonostylus with at least one, usually several, spiniform setae	***Phlebotominae***
-	Both sexes: Antennae with 14 or more flagellomeres. Females: without functional mouthparts (Fig 1 A), with only one spermatheca. Male terminalia: epandrium without appendages; gonostylus usually without spiniform setae	***Bruchomyiinae***

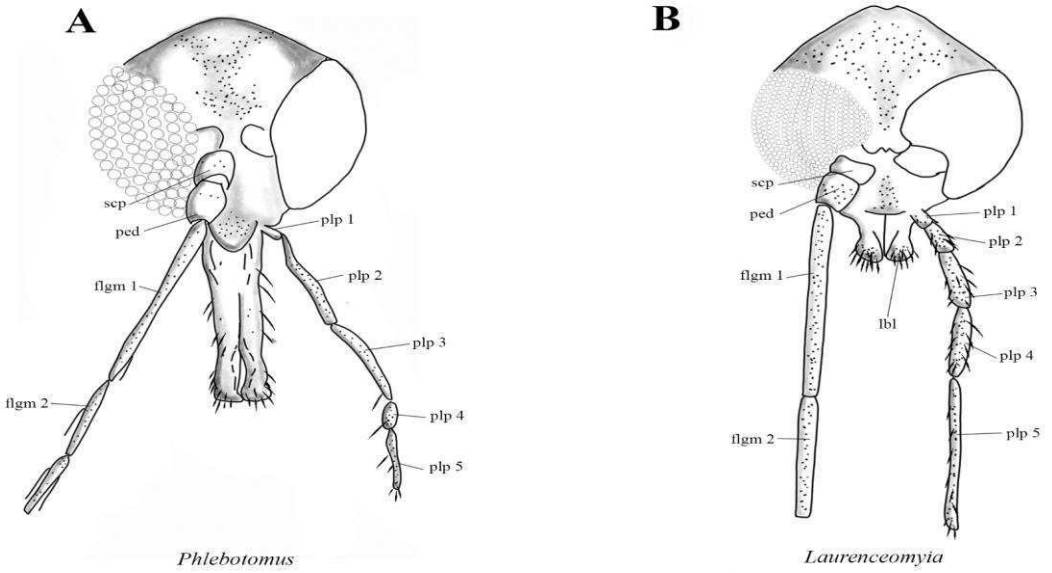

Fig. 4. A. Head of *Phlebotomus sp.* **B.** Head of *Laurenceomyia sp*. Abbreviations: flgm – flagellomere, lbl – labella, ped – pedicel, plp – palpal segment, scp – scape. No scale.

Subfamily Bruchomyiinae Alexander, 1921

The subfamily Bruchomyiinae is a small, widely distributed subfamily with seven described genera that currently include 53 extant and seven fossil species (Curler & Jacobson, 2012; Wagner & Stuckenberg, 2016; Ježek *et al*., 2018). This subfamily recently received attention, particularly in the Neotropical Region, with several new species described (Quate et al. 2000; Quate & Alexander 2000; Santos et al. 2009, 2013; Bravo & Barata 2012; Wagner & Stuckenberg 2012; Ježek *et al*., 2018). Formerly only three genera were recognized in this subfamily, namely *Bruchomyia* Alexander, 1921, *Eutonnoiria* Alexander, 1940 and *Nemopalpus* Macquart, 1838. However, Wagner & Stuckenberg (2016) provided a cladistic analysis based on morphological characters for the subfamily and resolved it into two major clades: one clade with the Old-World genera *Nemopalpus* and *Eutonnoiria*, and a second clade with the New World genus *Bruchomyia* and three new genera, i.e., *Boreofairchildia* Wagner & Stuckenberg, 2016, *Laurenceomyia* Wagner & Stuckenberg, 2016, and *Notofairchildia* Wagner & Stuckenberg. 2016. More recently, a new genus, *Alexanderia* Wagner & Kvifte, 2019, was described to include the species from the Oriental Region earlier placed within *Nemopalpus* (Polseela et al. 2019). The two Australian species of *Nemopalpus* are regarded as unplaced within the subfamily due to their uncertain affinities (Polseela et al. 2019).

Only the genus *Nemopalpus* is present in Europe, represented by a single Palearctic species, *Nemopalpus flavus* Macquart, 1838. Wagner & Suckenberg (2016) made a comprehensive analysis of the subfamily, including synonyms and detailed information on its taxonomic history.

Biology. It is not known if adults of Bruchomyiinae feed (Kvifte & Wagner, 2017). Larval development has been reported together with larvae of Phlebotominae feeding on decaying organic matter (Kvifte & Wagner, 2017). A single species from New Zealand was reported to develop in rotting wood (Duckhouse, 1980). Williams (2003) reported an unidentified species in the Neotropical region as myrmecophilous.

Genus *Nemopalpus* Macquart, 1838

Nemopalpus Macquart, 1838b: 219 (1838). Type species: *Nemopalpus flavus* Macquart, 1838, by monotypy. Detailed synonyms list in Wagner & Stuckenberg (2016).

Remarks: A single species is known in Europe.

Subfamily Phlebotominae Rondani, 1840

Sandflies (subfamily Phlebotominae) comprise around 1,000 species distributed worldwide (Akhoundi *et al*., 2016; Galati, 2018; Galati & Rodrigues, 2023; Seccombe, *et al.*, 1993), with around 25 species occurring in Europe (Wagner, 2004; Cazan *et al*., 2019). Historically they have been treated as a separate family (Phlebotomidae) (Léger & Depaquit, 2002) or even as a tribe inside Psychodidae (Wagner, 1997b). Akhoundi *et al*. (2016), Galati (2018), and, Galati & Rodrigues (2023) gave detailed information about the historical classification of this subfamily and its different arrangements. Several systematic treatments have been raised over time, especially for the Old World Phlebotominae where some authors recognize *Demeillonius* Davidson 1980, *Grassomyia* Theodor 1958, *Parvidens* Theodor & Mesghali 1964, *Spelaeomyia* Theodor 1948 and *Spelaeophlebotomus* Theodor 1948 as separate valid genera (Artemiev, 1991; Rispail & Léger, 1998; Depaquit *et al*., 2008; Kvifte & Wagner, 2017), but, on the contrary, those same genera have been considered as subgenera of *Sergentomyia* França & Parrot, 1920 or *Phlebotomus* Rondani & Berté, 1840 (Abonnenc, 1972; Abonnenc *et al*., 1965; Lewis, 1982; Leng, 1987). Recently, Akhoundi *et al*. (2016) decided based on practical criteria, but not justified, to classify the Old World (Mainly the Palearctic region) sand flies into three genera, i.e., *Phlebotomus* Rondani & Berté, 1840 (with 13 subgenera), *Sergentomyia* França & Parrot, 1920 (with 10 subgenera), and *Chinius* Leng, 1987, however, according to Randrianambinintsoa *et al*. (2014) some subgenera are based on symplesiomorphies, with not enough material to compare from different localities and, in addition, some species are not even classified at the subgeneric level.

We follow Blavier *et al*. (2019), Rispail & Léger (1998), and Kvifte & Wagner (2017), and recognize the following genera as part of the Palearctic fauna: *Grassomyia* Theodor. *Parvidens* Theodor & Mesghali 1964, *Phlebotomus* Rondani & Berté 1840, *Sergentomyia* França & Parrot 1920. But only *Phlebotomus* and *Sergentomyia* are present in Europe, therefore, the key only covers these two genera. Despite some subgenera being recognized and partially accepted worldwide, we avoid the inclusion of subgenera in the key until further information is available.

Biology. Most of the larval stages develop on the soil, some species are associated with small mammal burrows (Quate & Vockeroth, 1987; Wagner, 1997b) Adult females have functional mouthparts and feed on vertebrate blood (Kvifte & Wagner, 2017). Phlebotominae species are well-known vectors of *Leishmania* and some microfilarial worms (Kvifte &

Wagner, 2017). This capability of transmitting *Leishmania* makes this subfamily a medically important group for humans.

Key of the genera of Phlebotominae (adults)

(Following Lane, 1993, Wagner, 1997b; Theodor, 1958; Kvifte & Wagner, 2017)

1	Both sexes: abdominal terguites 2-6 with reclined (recumbed) setae (Fig. 1 A), the alveoli much smaller than those on terguites 1 (Fig. 1 A). Males: antenna with first flagellomeres with ascoids; terminalia with gonostylus with 3-5 spiniform setae. Females: cibarium with or without teeth. female antenna with paired ascoids on flagellomeres 1-13; anepisternum without patches of setae; female spermatheca without rows of spicules	***Sergentomyia***
-	Both sexes: abdominal terguites 2-6 with erect setae (Fig. 1 B), the alveoli of similar size to those of terguite 1 (Fig. 1 B). Males: terminalia with gonostylus with 1-6 spiniform setae, terminalia with paramere bilobate or not, if bilobate, then lower lobe not dorsoventrally compressed, without a row of stout spines. Females: cibarium with teeth; thorax with anepisternum with 1 patch of setae; spermathecal wall ornamented	***Phlebotomu***

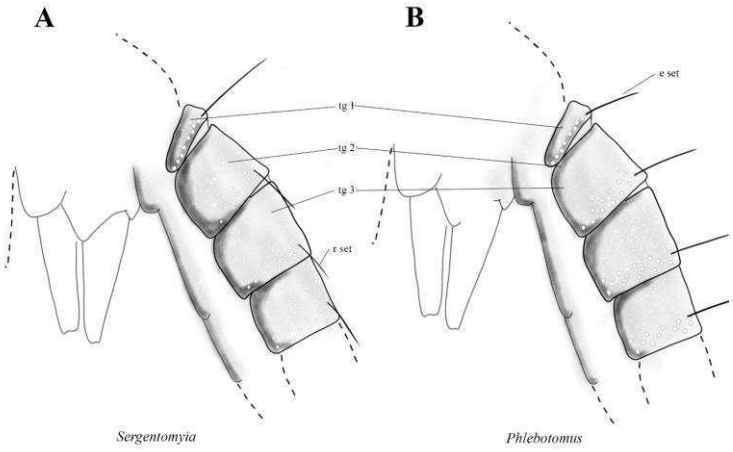

Fig. 5. A. Abdominal terguites of *Sergentomyia sp*. **B.** Abdominal terguites of *Phlebotomus sp.* Abbreviations: Abbreviations: e set – erect setae, r set – reclined setae, tg – abdominal terguite.

Genus *Phlebotomus* Rondani & Berté, 1840

Flebotomus Rondani & Berté in Rondani, 1840: 12 [invalid original spelling of *Phlebotomus*].

Phlebotomus Rondani & Berté in Rondani, 1840: 12 (as *Flebotomus*). Spelling corrected by ICZN 1954: 201 (Opinion 256). Type species: *Bibio papatasi* Scopoli, 1786. **Remarks:** 19 species present in Europe

Genus *Sergentomyia* França & Parrot, 1920

Newsteadia França, 1919: 148 (as subgenus of *Phlebotomus*). Type species: *Hebotomus* [sic] *minutus* Rondani, 1843 by designation of França (1920: 234) [junior homonym of *Newsteadia* Green, 1902].

Sergentomyia França & Parrot, 1920: 699 [replacement name for *Newsteadia* França]. **Remarks:** 4 species in Europe

Subfamily Psychodinae Rondani, 1856

The subfamily Psychodinae is considered the most diverse inside Psychodidae. With high variation in morphology and ecology, allowing the species to be present almost worldwide (Curler & Moulton, 2012; Espíndola *et al*., 2012; Kvifte & Wagner, 2017, Wagner, 1997b). There are more than 100 genera and 2,000 species described worldwide, including some Antarctic islands (Curler & Moulton, 2012; Kvifte, 2018), however, the history of this subfamily is complex. In the last 50 years 14 tribal or subtribal classifications have been proposed (e.g. Duckhouse, 1987; Vaillant, 1971; Ježek, 1983; Kvifte, 2015, 2018; Quate & Brown, 2004) but none of them has been adopted among all the specialists (Curler & Moulton, 2012; Kvifte & Wagner, 2017). A comparison of the classifications proposed by Vaillant (1971) and Ježek, (1983) based on mitochondrial DNA by Espíndola *et al*. (2012) found that some of the tribes are nonmonophyletic. Each tribal classification system has been based on non-compatible characters, making it very difficult to compare among them and with the results of molecular analyses (Kvifte, 2018). Therefore, researchers have emphatically discussed the relationships among the tribes inside Psychodinae without reaching a consensus, as most of them are not supported by robust phylogenetic analyses (Kvifte & Wagner, 2017). The most recent and tentative classification for the tribes inside Psychodinae, including a good overview of the classification through the years, is given by Kvifte (2018). However, this lack of consensus, and until more robust phylogenetic analyses are available, no tribal classification was followed in this key.

Likewise, the subgeneric classification has been treated in different ways through the

years, several taxa have been treated as subgenera or as a full genus category, one example of this is the subgenus *Tinearia* Schellenberg, 1803 which has been treated as a genus (Ježek, 1977, Wagner, 2004) or as a subgenus (Kvifte & Andersen, 2012, Kvifte *et al*, 2016), the inclusion of *Tinearia* as a subgenus of *Psychoda* allows monophyletic units, and recognizing it as a genus leaves *Psychoda* as a paraphyletic unit (see remarks under *Psychoda alternata* Say, 1824 in Kvifte *et al* 2016). The same problematic is present in genera like *Mormia* Enderlein, 1935, *Pericoma* Haliday in Curtis, 1856 and the different subgenera closely related, so until further analysis are conducted we avoid the usage of subgeneric classification in the present work, and we treat all species only at genus level , recognizing the following genera for Europe: *Atrichobrunettia* Satchell, 1953, *Bazarella* Vaillant, 1961, *Berdeniella* Vaillant, 1976, *Clogmia* Enderlein, 1935, *Clytocerus* Eaton, 1904, *Feuerborniella* Vaillant, 1971, *Ježekiella* Wagner & Kvifte 2015, *Jungiella* Vaillant, 1972, *Lepiseodina* Enderlein, 1930, *Lobulosa* Szabo, 1960, *Mormia* Enderlein, 1935, *Mystropsychoda* Duckhouse, 1975, *Neoarisemus* Botoseneanu & Vaillant, 1970, *Nielseniella* Vaillant, 1972, *Panimerus* Eaton, 1913, *Parabazarella* Vaillant, 1983, *Parajungiella* Vaillant, 1972, *Paramormia* Enderlein, 1935, *Pericoma* Walker, 1856, *Peripsychoda* Enderlein, 1935, *Periulomyia* Krek, 1999, *Philosepedon* Eaton, 1904, *Phyllotelmatoscopus* Vaillant, 1982, *Pneumia* Enderlein, 1935, *Promormia* Ježek, 1984, *Psychoda* Latreille, 1796, *Saraiella* Vaillant, 1981, *Seoda* Enderlein, 1935, *Szaboiella* Vaillant, 1979, *Telmatoscopus* Eaton, 1904, *Thornburghiella* Vaillant, 1982, *Threticus* Eaton, 1904, *Tonnoiriella* Vaillant, 1982, *Trichopsychoda* Tonnoir, 1933, *Trichosepedon* Krek, 1999, *Ulomyia* Haliday in Walker, 1856, *Vagmania* Krek, 1972, *Vaillantodes* Wagner, 2001.

Biology. Most information on the biology of the larvae of this subfamily is based on the Palearctic and Nearctic taxa (Kvifte & Wagner, 2017; Wagner & Ibáñez-Bernal, 2009). The species present a wide range of suitable habitats with the majority of species depending on water or moist substrates and requiring decomposing organic matter to develop (Kvifte & Wagner, 2017; Wagner & Ibáñez-Bernal, 2009). Some species have been associated with decaying wood, carrion, and vertebrate feces, compost, and fungal bodies (Kvifte & Wagner, 2017). A few cases of opportunistic myiasis are confirmed, but rare (Taylan-Ozkan et al. 2004; Tu et al. 2007).

Notes. The genus *Ježekiella* Wagner & Kvifte 2015 was described from the Mediterranean region; however, the characters of the genus are ambiguous making it difficult to place near other genera inside the subfamily, therefore this genus is not present in the key. The genus *Vagmania* Krek, 1972 was mainly described on larval characters, placing it near the genus

Duckhousiella, however, it is still not clear how this genus groups with other genera and this genus is out of the key.

Key to genera of Psychodinae (adults)

(Based on the keys by Wagner, 1997b; Wagner & Ibáñez-Bernal, 2009; Withers, 1989; Kvifte & Wagner, 2017)

1	Antennae with all flagellomeres cylindrical or fusiform (Fig. 6 A)	**23**
-	Antennae with at least flagellomeres 2-10 divided into a basal bulb and a distal neck (Fig. 6 B)	***2***

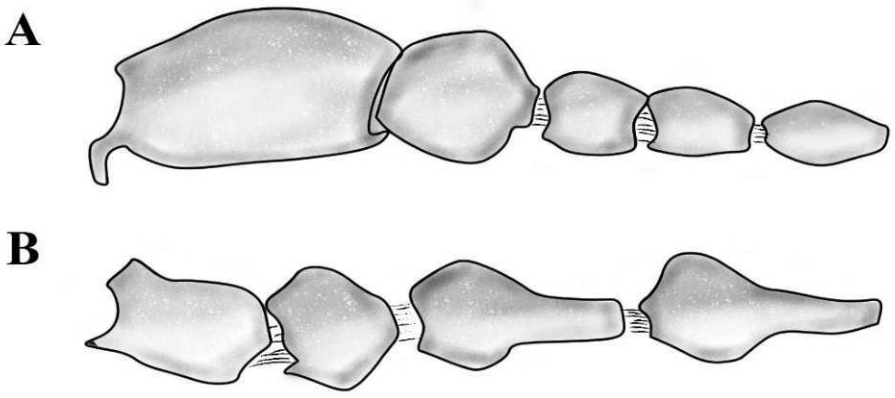

Fig. 6. A. First antennal segments showing cylindrical/fusiform flagellomeres. **B.** First antennal segments showing flagellomeres with basal bulb an distal neck

2	Eye bridge comprising 3 rows of facets (Fig. 7 A); wing vein R5 ending below wing apex (Fig. 7 B); wing costal vein (C) with a node at base less than 2X width of the rest of the costa; aedeagus symmetrical	***Promormia***
-	Eye bridge comprising 3, 4, or more rows of facets (Fig. 7 A); wing vein R5 ending at or below wing apex (Fig. 7 B); wing costal vein (C) without a node at base; aedeagus symmetrical or asymmetrical	**3**

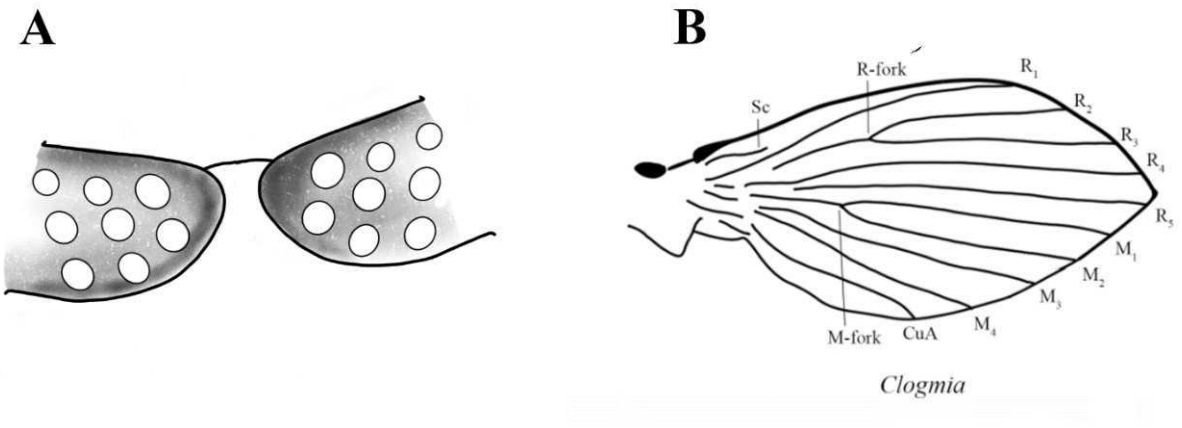

Fig. 7. A. Eye bridge with 3 rows of facets and interocular suture. **B.** Wing showing vein R5 ending below wing apex. Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

3	Eye bridge comprising 4 rows of facets, without interocular suture (Fig. 8 A); wing vein R5 ending at wing apex, radial fork basal of the median fork; male terminalia with aedeagus asymmetrical; feather-like tenacula	***Neoarisemus***
-	Eye bridge with variable rows of facets, interocular suture present (Fig. 8 B); other characters variable	**4**

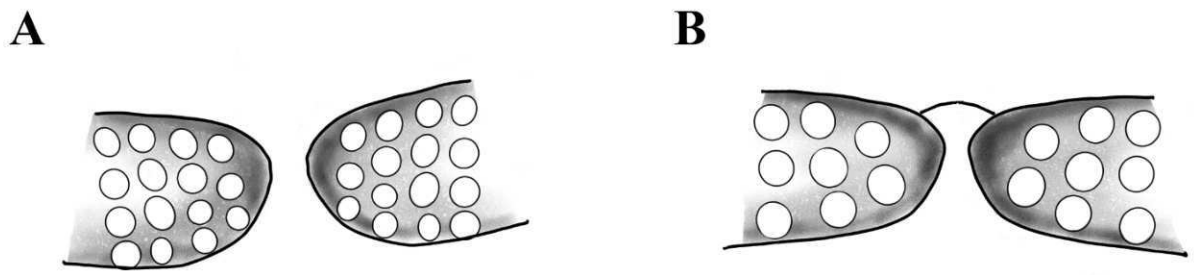

Fig. 8. A. Eye bridge with four facet rows, wihout interocular suture. **B.** Eye bridge with three facet rows, with interocular suture

4	Flagellomeres of antennae carrying ascoids with a single, curved branch or with only anterior branches (Fig. 9 A)	**11**

-	Flagellomeres of antennae carrying ascoids with both anterior and posterior branches (Fig. 9 B)	**5**

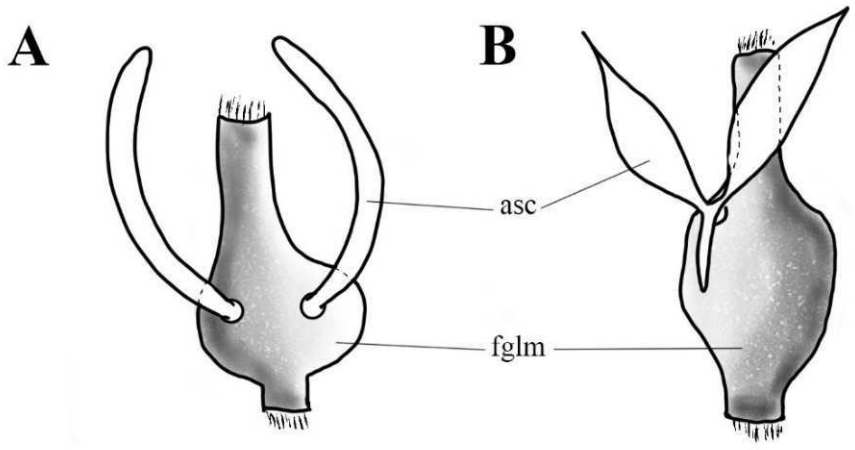

Fig. 9. A. Fagellomere carrying ascoids with only anterior branches. **B.** Flagellomere carrying ascoid with both anterior and posterior branches

5	Surstylus of male terminalia with more than one tenaculum (Fig. 10 A)	**7**
-	Surstylus of male terminalia with single tenaculum (Fig. 10 B)	**6**

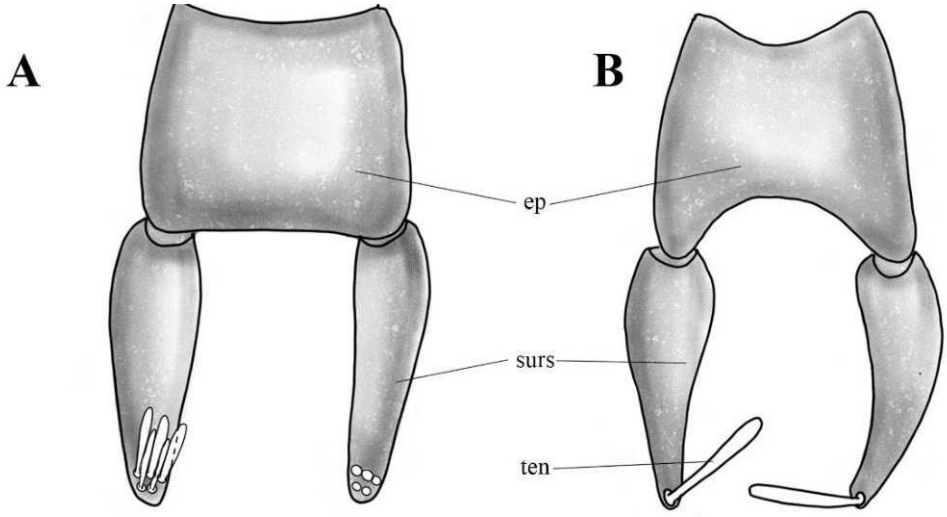

Fig. 10. A. Male genitalia showing one tenaculum in the surstylus**. B.** Male genitalia showing more than one tenaculum in the surstylus. Abbreviations: ep – epandrium, surs – surstylus, ten – tenacula

6	Labellum of palp round, without rod-like setae. Interocular suture present. The aedeagus is symmetrical with paired parameres (Fig. 11 A)	***Feuerborniella***

Labellum of palp flat, with apical rod-like setae. Interocular suture

- absent. The aedeagus is asymmetrical, or symmetrical without parameres ***Psychoda*** (Fig. 11 B)

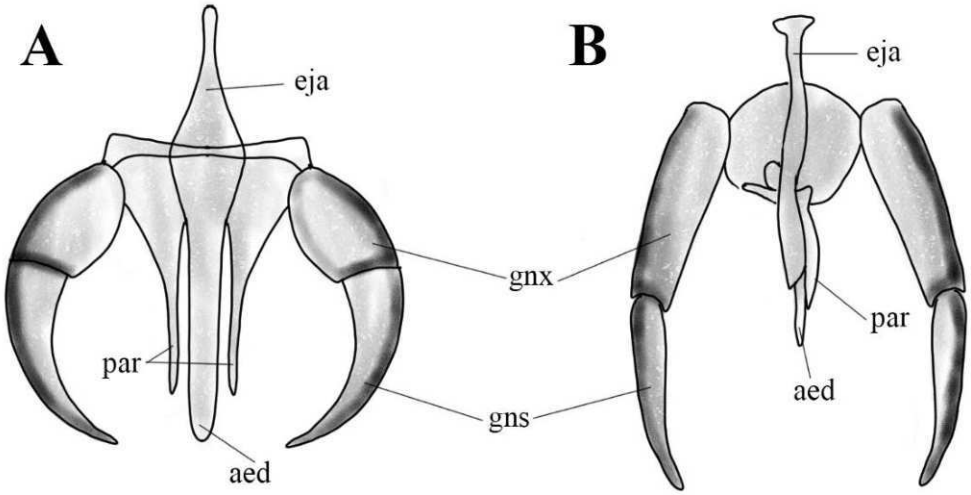

Fig. 11. A. Male genitalia of Feuerborniella sp. **B.** Male genitalia of Psychoda sp. Abbreviations: aed – aedeagus, eja – ejaculatory apodeme, gns – gonostyli, gnx – gonocoxites, par – paramere(s)

7 Wing membrane without setae (Fig. 12 A) **10**

- Wing membrane with setae (Fig. 12 B) **8**

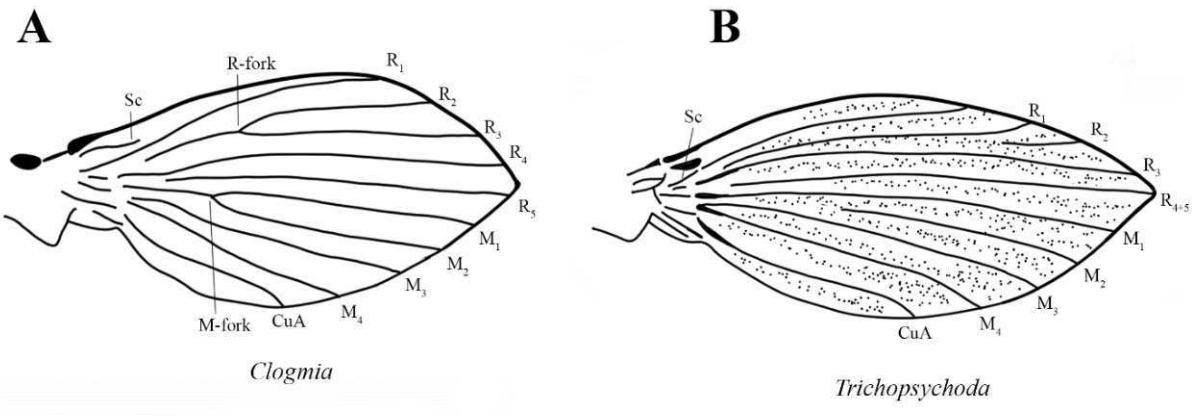

Fig. 12 A. Wing of *Clogmia sp.* showing bare membrane. **B.** Wing of Trichopsychoda sp. showing setose membrane. Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

8	Wing forks incomplete (Fig. 13 A). Aedeagus asymmetrical, without parameres. Surstylus with distal row of more than 2 rod-like tenacula and subapical field of setiform tenacula with complex bell-shaped apices	***Trichopsychoda***
-	Wing forks complete (Fig. 13 B). The aedeagus is symmetrical or asymmetrical, with parameres. Surstylus with 1–2 rod-like tenacula (in North European species with 2 tenacula), lacking setiform tenacula	**9**

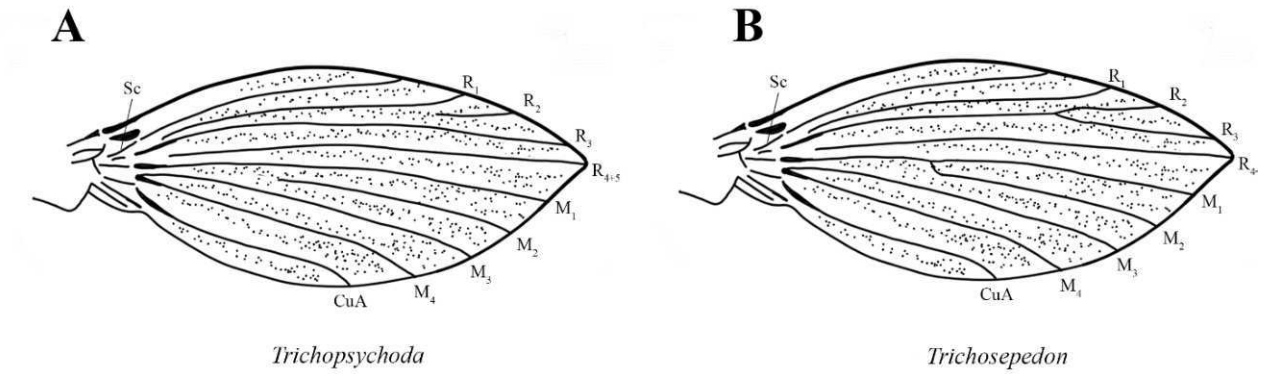

Fig. 13. A. Wing of *Trichopsychoda sp.* showing forks R and M incomplete. **B.** Wing of *Trichosepedon sp.* showing forks R and M complete. Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

9	Ascoids with a single, leaf-shaped anterior branch (Fig. 14 A) or with Z-shaped digitiform ascoids or with Y-shaped digitiform ascoids (fig.) Setae on wing membrane only present in stripes parallel to veins. The aedeagus is asymmetrical	***Nielseniella***
-	Ascoids with only two digitiform anterior branches (Fig. 14 B). Setae on wing membrane evenly distributed. The aedeagus is symmetrical	***Trichosepedon***

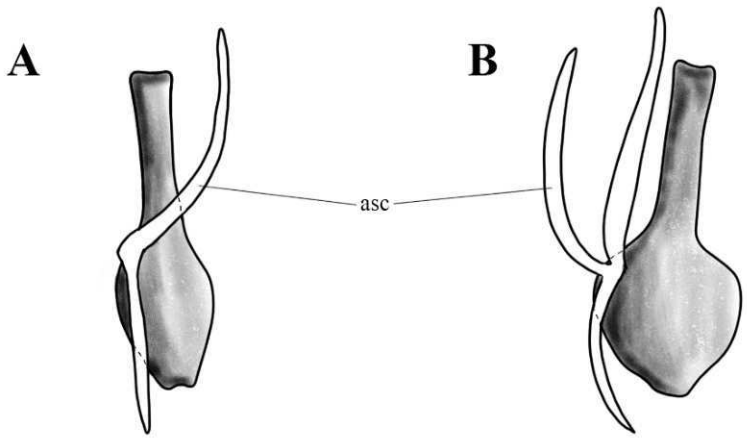

Fig. 14. A. Falgellomere showing ascoid with a single anterior branch. **B.** Flagellomere showing ascoid with two anterior brances. Abbreviations: asc – ascoid

10	Ascoids with a single anterior branch (Fig. 15 B). Ejaculatory apodeme expanded laterally, blade-shaped; distal part of aedeagus projecting beyond parameral sheath. Parameres are obviously asymmetrical. Surstylus with 3 tenacula (Fig. 15 A)	***Threticus***
-	Ascoids with two digitiform anterior branches (Fig. 15 D). Facets of the eye bridge are narrower than facets of the eye. Ejaculatory apodeme narrow except at extreme base where it may be T-shaped; distal part of aedeagus not projecting beyond parameral sheath. Parameres are symmetrical or diffusely asymmetrical. Surstylus with 2 tenacula (Fig. 15 C)	***Philosepedon***

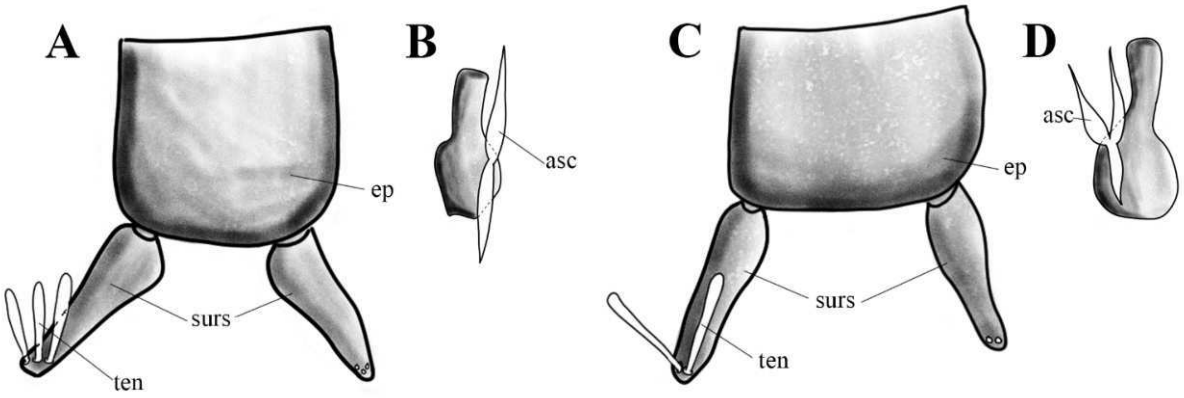

Fig. 15. A. Male genitalia showing three tenacula. **B.** Antennal flagellomere showing ascoid with single anterior branch. **C.** Male genitalia showing two tenacula. **C.** Antennal flagellomere showing ascoid with two anterior branches. Abbreviations: asc – ascoid, ep – epandrium, surs – surstyli, ten – tenaculum

11	Origin of R2+3 not connected to R4 and usually at the same level as the origin of R5 (Fig. 16 A)	**13**
-	Origin of R2+3 connected to R4 and distal to the origin of R5 (Fig. 16 B)	**12**

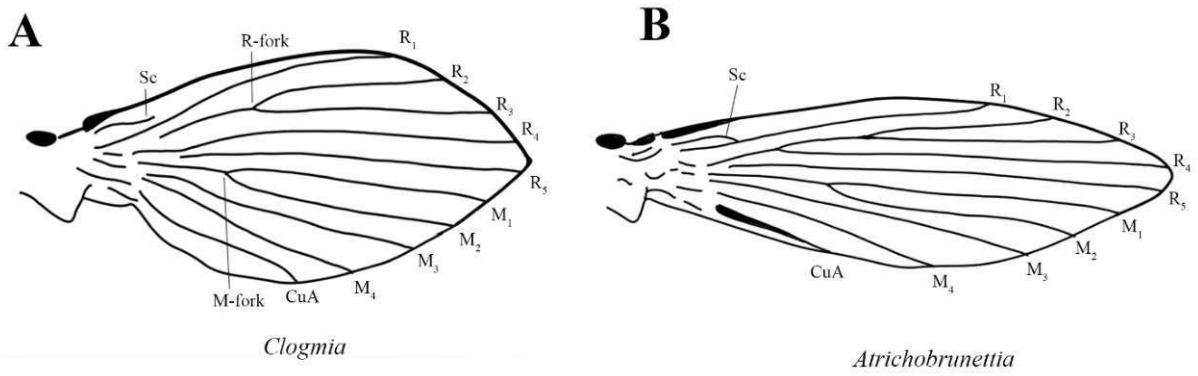

Fig. 16. A. Wing of *Clogmia sp.* **B.** Wing of *Atrichobrunettia sp.* Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

12	Eyes separated (Fig. 17 B). Wing is very narrow, 3.5 times as long as wide. Surstylus conical, tapering towards apex, length is less than three times the width of surstyli base, with tenacula shorter than half length of surstylus	***Atrichobrunettia***
-	Eyes contiguous (some species with eyes separated by less than 2 facet diameters) (Fig. 17 B). Wing not as narrow, at most 3 times as long as wide. Surstylus of even width, not tapering towards apex, length is more than 3 times the width of surstyli base, with tenacula usually as long as surstylus	***Mormia***

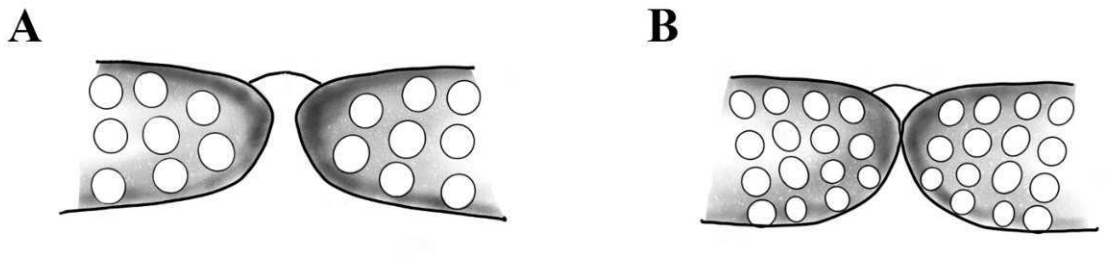

Fig. 17. A. Eye bridge showing eyes separated **B.** Eye bridge showing eyes contiguous.

13	R5 terminating below wing apex (Fig. 18 A)	**16**
-	R5 terminating in wing apex (Fig. 18 B)	**14**

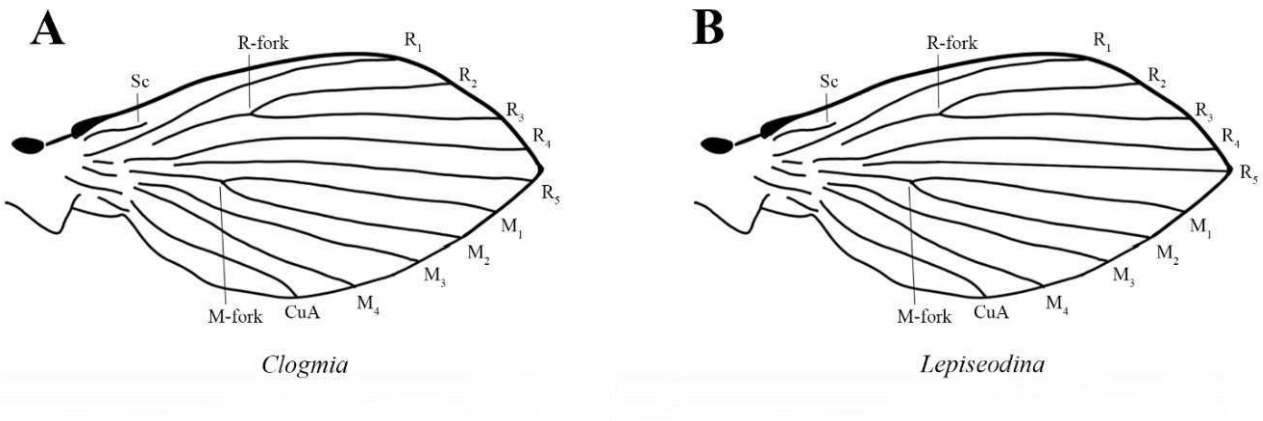

Fig. 18. A. Wing of *Clogmia sp.* **B.** Wing of *Lepiseodina sp.* Abbreviations: CuA – anterior branch of cubital vein, M – medial vein, M-fork – medial fork, R – radial vein, R-fork – radial fork, Sc – subcostal vein.

14	Ascoids bifurcate (Fig 19 A)). Tenacula distally knife-shaped, as short as basal width of surstylus	***Clogmia***
-	Ascoids leaf-shaped (Fig. 19 B) or digitiform (Fig. 19 C). Tenacula distally feathery, longer than twice basal width of surstylus	**15**

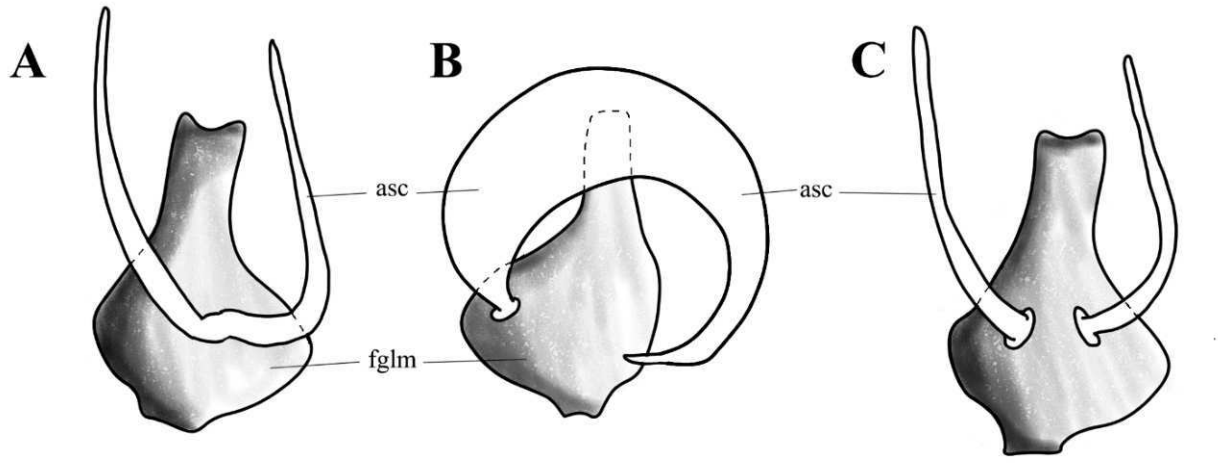

Fig. 19. A. Flagellomere showing bifurcate ascoids. **B.** Flagellomere showing leaf-shaped ascoid. **C.** Fagellomere showing digitiform ascoids. Abbreviations: asc – ascoid, fglm – flagellomere

15	Ascoids leaf-shaped or digitate coiled. Aedeagus symmetrical, Y-shaped, with ejaculatory apodeme narrower than distal elements (Fig. 20 A). Gonostylus longer than the length of epandrium	***Telmatoscopus***
-	Ascoids digitate S-shaped, never leaf-shaped. Aedeagus asymmetrical, not Y-shaped, with ejaculatory apodeme broader than distal element (Fig. 20 B). Gonostylus as long as, or shorter than the length of epandrium	***Lepiseodina***

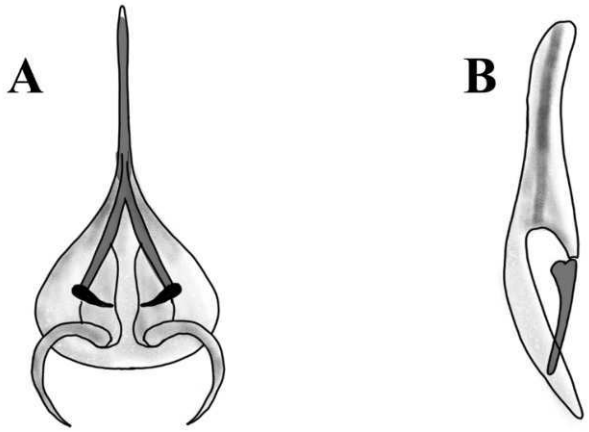

Fig. 20. A. Aedeagus symmetrical and Y-shaped. **B.** Aedeagus asymmetrical, not Y-shaped.

16	Ascoids paired (Fig. 21 A)	**18**
-	Ascoids encircling flagellomeres (Fig. 21 B)	**17**

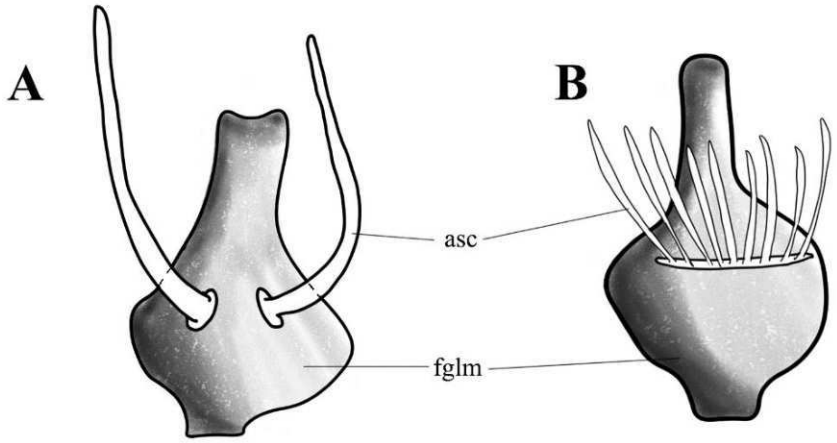

Fig. 21. A. Flagellomere showing paired ascoids. **B.** Flagellomere showing ascoids encircling flagellomere. Abbreviations: asc – ascoids, fglm – flagellomere

17	Ascoids in one row. Femora 1 with two ventral longitudinal rows of thorns (Fig. 22 A). Aedeagus distally with two separate phallomeres	***Paramormia***
-	Ascoids in two rows. Femora 1 without ventral thorns (Fig. 22 A). Aedeagus with phallomeres distally fused	***Phyllotelmatosc opus***

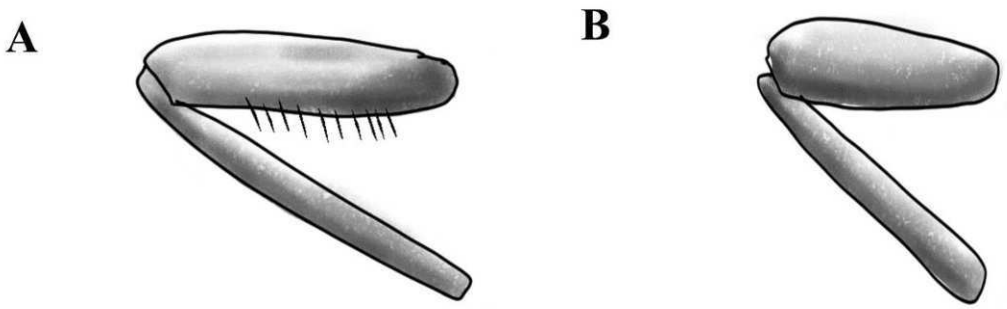

Fig. 22. A. Femora 1 with ventral row of thorns. **B.** Femora 1 without ventral row of thorns

18	Vertex without corniculi (Fig. 23 A)	**20**
-	Vertex with corniculi (Fig. 23 B)	**19**

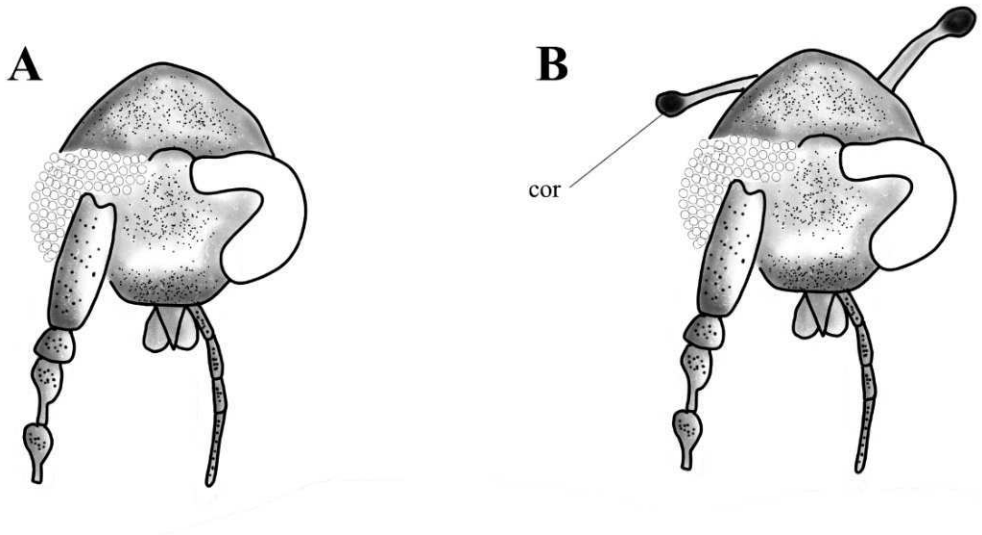

Fig. 23. A. Head without corniculi. **B.** Head with corniculi. Abbreviations: cor – corniculi

19	Corniculi stalked (Fig. 24 A). Pedicel with stiff spine-like bristles. Aedeagus with curved, sickle-shaped lateral elements	***Panimerus***
-	Corniculi ball-shaped (Fig. 24 B). Pedicel globular shape, without spines. Aedeagus with lateral elements not curved and sickle-shaped	***Jungiella***

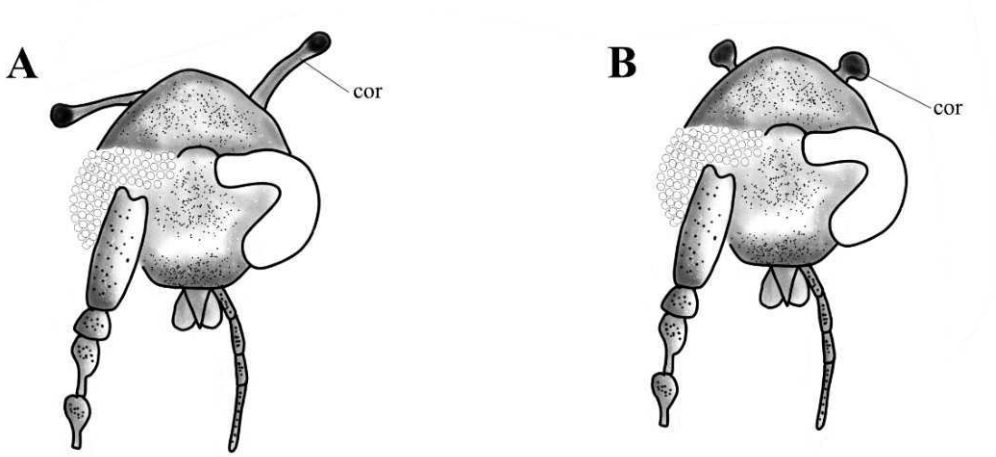

Fig. 24. A. Head with stalked corniculi. **B.** Head with ball-shaped corniculi. Abbreviations: cor – corniculi

20	Wing less than 2.2 times as long as wide. Ascoids palmate (Fig. 25 A)	***Peripsychoda***
-	Wing more than 2.4 times as long as wide. Ascoids digitate (Fig. 25 B)	**21**

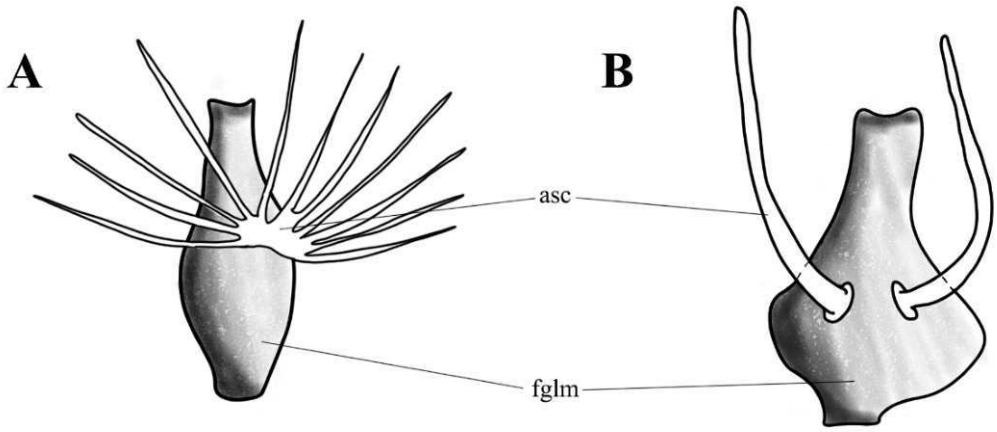

Fig. 25. A. Flagellomere showing palmate ascoids**. B.** Flagellomere showing digitate ascoids. Abbreviations: asc – ascoid, fglm – flagellomere

21	Ejaculatory apodeme without median keel. Parameres are usually broadly fused to form triangular or bullet-shaped median elements, or reduced (Fig. 26 A)	***Seoda***
-	Ejaculatory apodeme with Y-shaped median keel. Parameres fused to form V-shaped furca (Fig. 26 B)	**22**

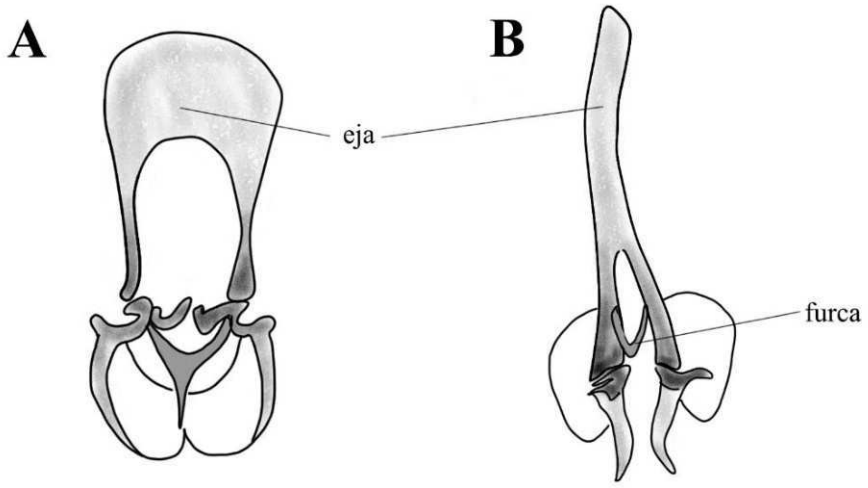

Fig. 26. A. Ejaculatory apodeme of *Seoda sp*. **B.** Ejaculatory apodeme showing V-shaped furca. Abbreviation: eja – ejaculatory apodeme

22	Scape more than 3.4 times the length of the pedicel. Ascoids as long as flagellomeres, simply S-curved (Fig. 27 A). Aedeagus not covered by dorsal hood	***Parajungiella***

-	Scape less than 2 times the length of the pedicel. Ascoids more than twice the length of flagellomeres, curved in a spiral (Fig. 27 B). Aedeagus covered by dorsal hood	***Vaillantodes***

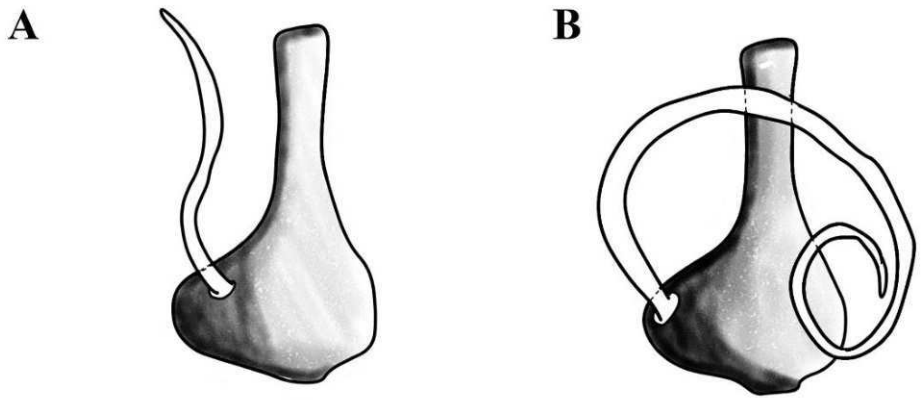

Fig. 27. A. Flagellomere showing ascoid S-curved. **B.** Flagellomere showing ascoid longer than flagellomere and coiled

23	Aedeagus symmetrical, and with ejaculatory apodeme not laterally expanded (Fig. 28 A)	**25**
-	Aedeagus asymmetrical, and with ejaculatory apodeme laterally expanded (Fig. 28 B)	**24**

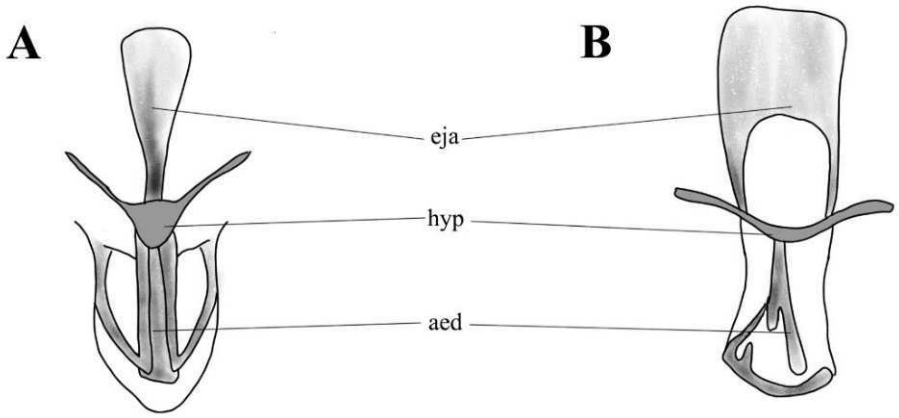

Fig. 28. A. Aedeagus symmetrical, with ejaculatory apodeme not laterally expanded. **B.** Aedeagus asymmetrical with ejaculatory apodeme laterally expanded. Abbreviations: aed – aedeagus, eja – ejaculatory apodeme, hyp – hypandrium

24	Surstylus with just a single tenaculum (Fig. 29 A)	***Lobulosa***

-	Surstylus with multiple tenacula (Fig. 29 B)	***Tonnoiriella***

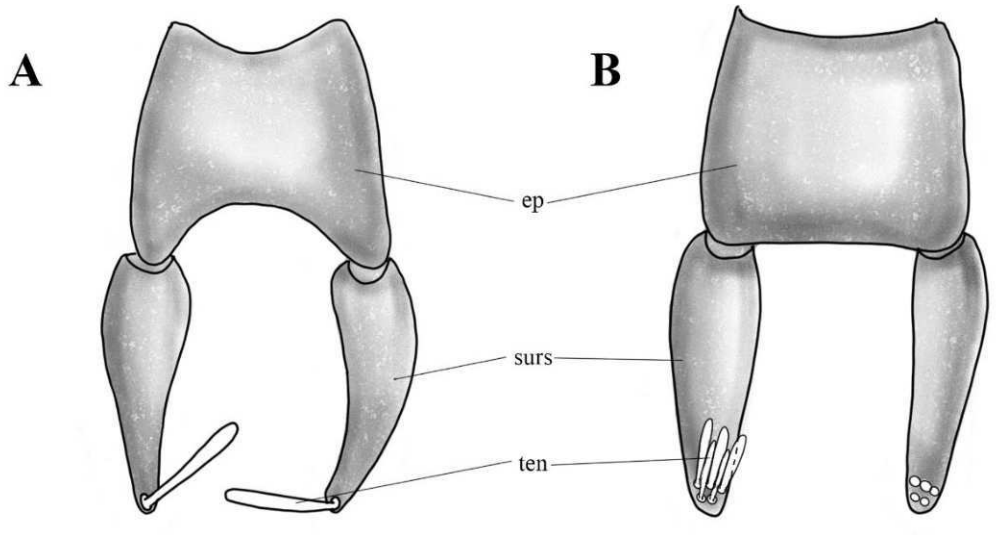

Fig. 29. A. Male genitalia showing one tenaculum in the surstylus. **B.** Male genitalia showing more than one tenaculum in the surstylus. Abbreviations: ep – epandrium, surs – surstylus, ten – tenacula

25	Corniculi are usually present. The antennal scape is more than 3 times the length of the pedicel, flagellomere 1 with a distal wavy brush of setae (Fig. 30 A)	***Clytocerus***
-	Corniculi are always absent. The antennal scape is less than 3 times the length of the pedicel, flagellomere 1 without a wavy brush of setae (Fig. 30 B)	**26**

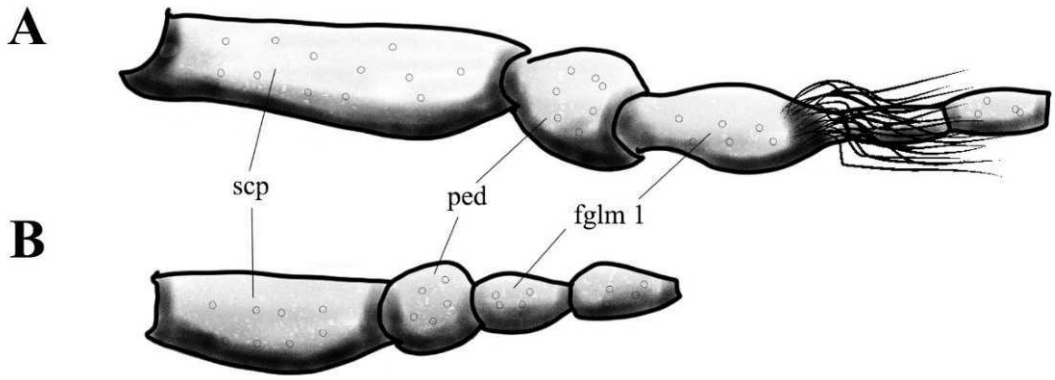

Fig. 30. A. First antennal segments of Clytocerus sp. showing length of scape and wavy bursh of setae. **B.** First antennal segments showing length of scape. Abbreviations: fglm 1 – flagellomere 1, ped – pedicel, scp – scape

26	First flagellomere without strong bristles or spines (Fig. 31 A)	**31**
-	First flagellomere with strong bristles or spines (absent in some species of *Ulomyia* Haliday in Walker, 1856) (Fig. 31 B)	**27**

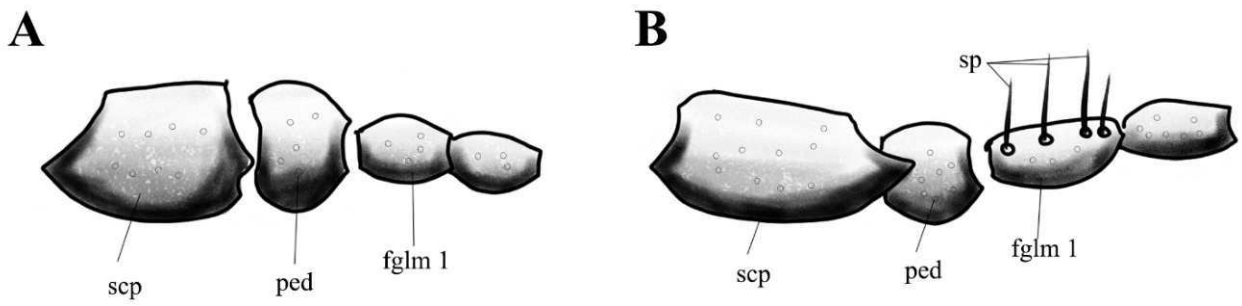

Fig. 31. A. First antennal segments, flagellomere 1 without spines. **B.** First antennal segments, flagellomere 1 with spines. Abbreviations> fglm 1 – flagellomere 1, ped – pedicel, scp – scape, sp – spines

27	First flagellomere with three or four spines in a row. Gonostylus attached to the ventral side of gonocoxite (Fig. 32 A). Surstyli distally pointed	***Parabazarella***
-	First flagellomere with seven to twelve spines. Gonostylus attached to the distal end of gonocoxite (Fig. 32 B). Surstyli distally rounded	**28**

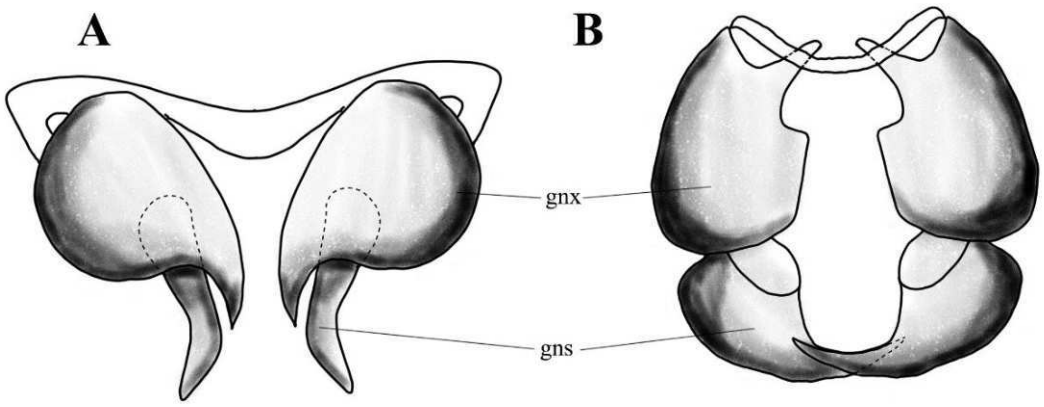

Fig. 32. A. Gonocoxites and gonostyli of *Parabazarella sp*. **B.** Gonocoxites and gonostyli of *Periulomyia sp*. Abbreviations: gns – gonostyli, gnx – gonocoxites

28	First and second flagellomere with stiff bristles in seriate clusters (some species with stiff bristles in the third flagellomere) (Fig. 33 A); eye bridge with six facets at widest point. Sensory organs bifurcate, originating from proepisternum. Wing without large central pouch	***Periulomyia***
-	Only the first flagellomere with spines in a seriate row (Fog. 33 B); eye bridge with five or seven facets at the widest point. Wing with or without central pouch	**29**

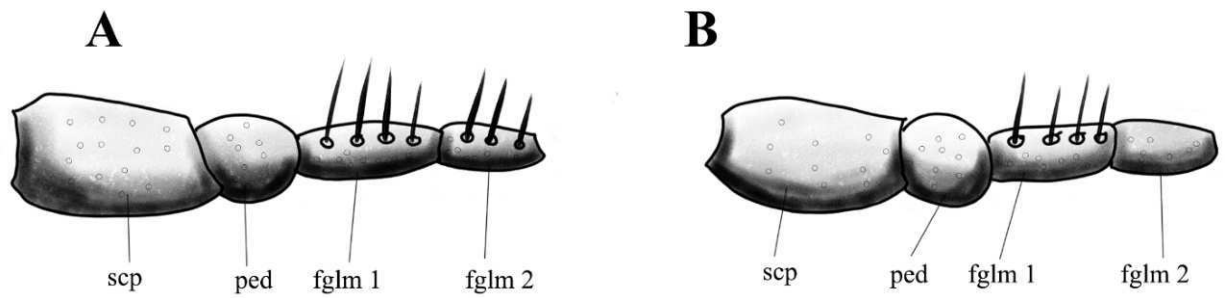

Fig. 33. A. First antennal segments, flagellomeres 1 & 2 with spines. **B.** First antennal segments, only flagelommere 1 with spines. Abbreviations: fglm – flagellomere, ped – pedicel, scp – scape

29	Eyebridge with rows of seven facets wide at widest point (Fig. 34 A); wing in some species with large central pouch	***Ulomyia***
-	Eyebridge with rows of 5 facets (Fig. 34 B); wings always without pouch	**30**

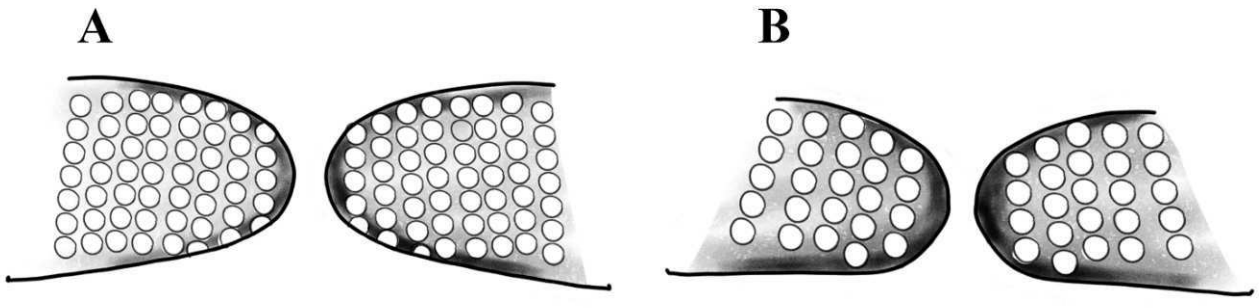

Fig. 34. A. Eye bridge showing seven facets rows. **B.** Eye bridge showing five facet rows

30	Gonocoxites globular, close to each other, often fused (Fig. 35 A)	***Bazarella***
-	Gonocoxites elongate, never fused (Fig. 35 B)	***Thornburghiella***

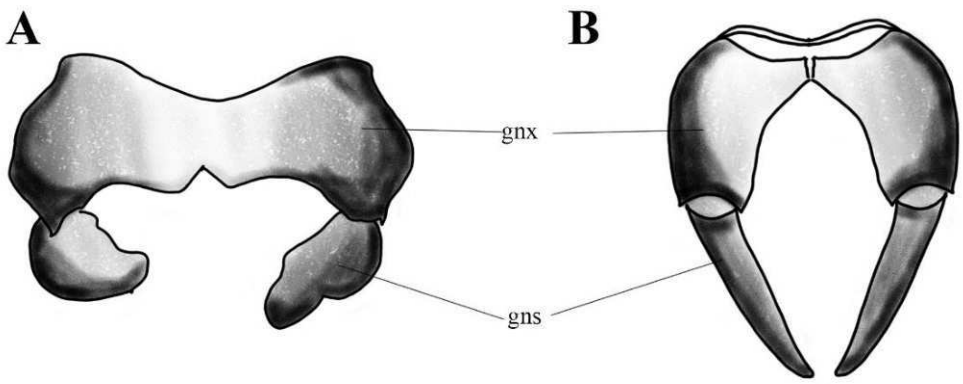

Fig. 35. A. Male terminalia showing globular fused gonocoxites. **B.** Male terminalia showing elongate gonocoxites. Abbreviations: gns – gonostyli, gnx – gonocoxites

31	Surstyli with four or more tenacula (Fig. 36 A)	**33**
-	Surstyli with three or fewer tenacula (Fig. 36 B)	**32**

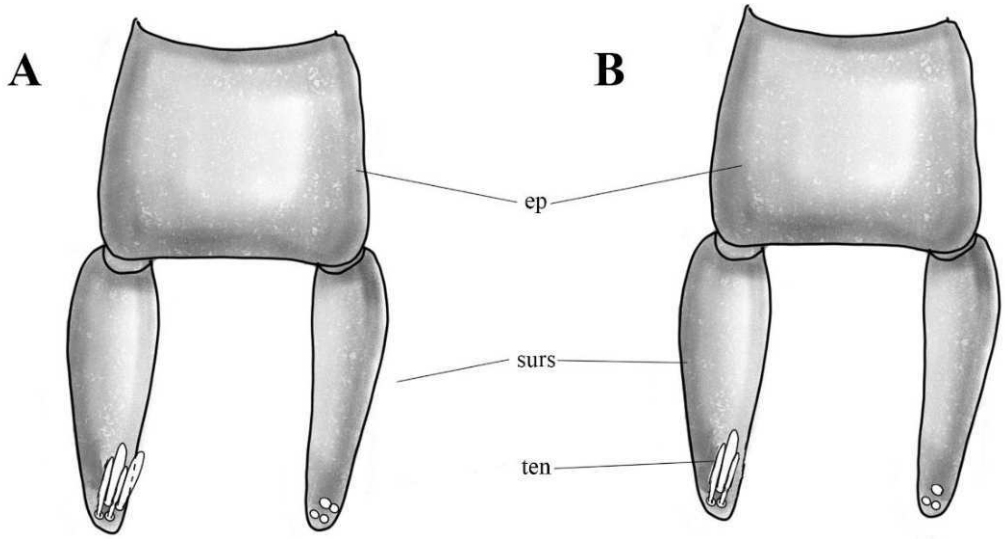

Fig. 36. A. Male terminalia showing surstyli with four tenacula. **B.** Male terminalia showing surstyli with three tenacula. Abbreviations: ep – epandrium, surs – surstyli, ten – tenacula

32	Surstylus with three tenacula arranged in a triangle; apical tenaculum less than half the length of subapical two tenacula (Fig. 37 A)	***Szaboiella***

-	Surstylus in North European species with one tenaculum only, some Mediterannean species with two tenacula of equal length (Fig. 37 B)	***Berdeniella***

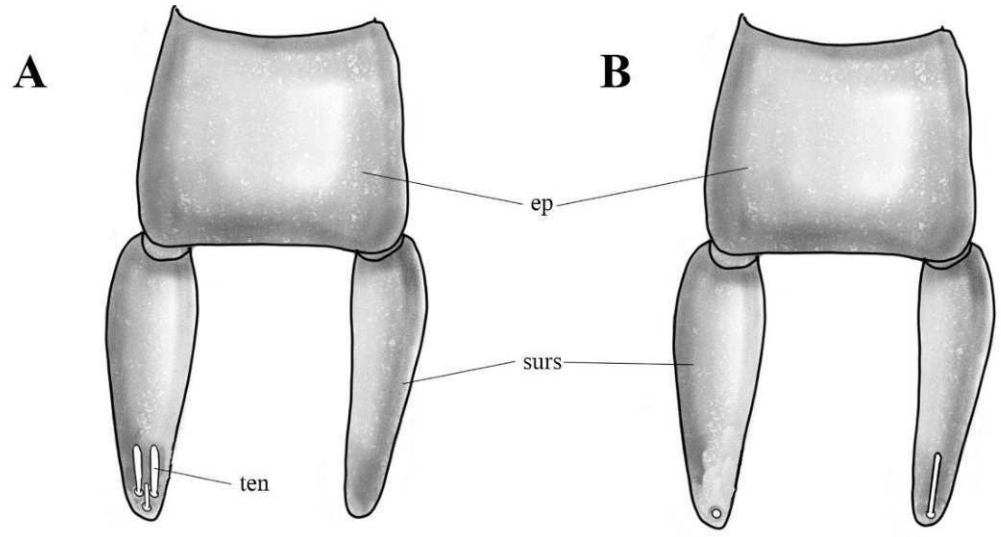

Fig. 37. A. Male terminalia showing surstyli with three tenacula. **B.** Male terminalia showing surstyli with one tenaculum. Abbreviations: ep – epandrium, surs – surstyli, ten – tenacula

33	Terminal flagellomere without apical neck (Fig. 38 A). Surstyli with tenacula bases separated by approximately one tenacula base diameter	***Saraiella***
-	Terminal flagellomere with an apical neck (apiculus) (Fig. 38 B). Surstyli with tenacula bases adjacent	**34**

Fig. 38. A. Terminal flagellomeres, last flagellomere without apical neck. **B.** Terminal flagellomeres, last flagellomere with apical neck

34	Eyebridge of five or six rows of facets (Fig. 39 A). Antennae with terminal flagellomere with apiculus as long as segment	***Pneumia***

- Eyebridge of four rows of facets (Fig. 39 B). Antennae with terminal flagellomere with apiculus at most a third of segment length — ***Pericoma***

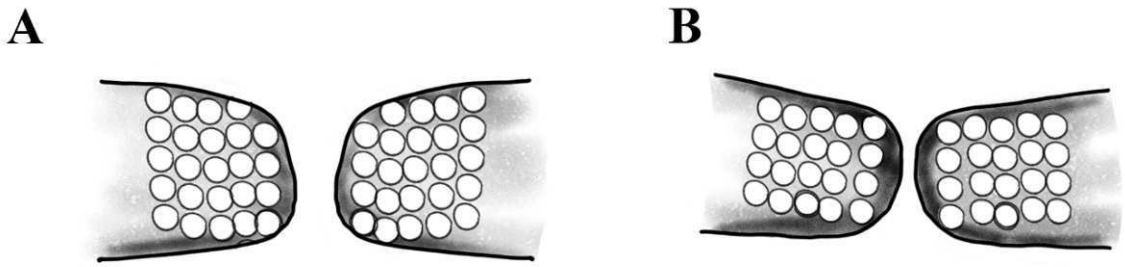

Fig. 39. A. Eye bridge showing six rows of facets. **B.** Eye bridge showing four rows of facets

Genus *Atrichobrunettia* Satchell, 1953

Atrichobrunettia Satchell, 1953: 413. Type species: *A. alternata* Satchell, 1953. **Remarks.** 5 species known in Europe

Genus *Bazarella* Vaillant, 1961

Bazarella Vaillant, 1961: 140. Type species: *Pericoma atra* Vaillant, 1955. **Remarks.** 2 species known in Europe

Genus *Berdeniella* Vaillant, 1976

Berdeniella Vaillant, 1976: 183. Type species: *Pericoma helvetica* Sarà, 1957. **Remarks.** 60 species known in Europe

Genus *Clogmia* Enderlein, 1935

Clogmia Enderlein, 1935: 87. Type species: *Psychoda albipennis* Williston = albipunctata Williston. **Remarks.** 2 species known in Europe

Genus *Clytocerus* Eaton, 1904

Clytocerus Eaton, 1904: 59. Type species: *Pericoma (Clytocerus) africanus* Tonnoir, 1920. **Remarks.** 16 species known in Europe

Genus *Feuerborniella* Vaillant, 1971

Fuerborniella Vaillant, 1971: 37. Type species: *Psychoda obscura* Tonnoir, 1919. **Remarks.** 1 species known in Europe

Genus *Jungiella* Vaillant, 1972

Jungiella Vaillant, 1972: 81. Type species: *Pericoma soleata* Walker, 1856. **Remarks.** 40 species known in Europe

Genus *Lepiseodina* Enderlein, 1930

Lepiseodina Enderlein, 1930: 93. Type species: *Psychoda tristis* Meigen, 1830. **Remarks.** 2 species known in Europe

Genus *Lobulosa* Szabó, 1960

Lobulosa Szabó, 1960:163. Type species: Pericoma transsylvanica Szabó, 1960. **Remarks.** 2 species known in Europe

Genus *Mormia* Enderlein, 1935

Mormia Enderlein, 1935: 248. Type species: *Pericoma revisenda* Eaton, 1893. **Remarks.** 41 species known in Europe

Genus *Neoarisemus* Botoseneanu & Vaillant, 1970

Neoarisemus Botoseneanu & Vaillant, 1970: 178. Type species: *Psychoda nigra* Banks, 1894. **Remarks.** 2 species known in Europe

Genus *Nielseniella* Vaillant, 1972

Nielseniella Vaillant, 1972: 105. Type species: *Trichopsychoda maderensis* Satchell, 1955. **Remarks.** 2 species known in Europe

Genus *Panimerus* Eaton, 1913

Panimerus Eaton, 1913: 425. Type species: *Pericoma notabilis* Eaton, 1893. **Remarks.** 14 species known in Europe

Genus *Parabazarella* Vaillant, 1983

Parabazarella Vaillant, 1983: 341. Type species: *Pericoma subneglecta* Tonnoir, 1922. **Remarks.** 1 species known in Europe

Genus *Parajungiella* Vaillant, 1972

Parajungiella Vaillant, 1972: 83. Type species: *Pericoma longicornis* Tonnoir, 1919. **Remarks.** 7 species known in Europe

Genus *Paramormia* Enderlein, 1935

Paramormia Enderlein, 1935: 248. Type species: *Pericoma fratercula* Eaton, 1893.. **Remarks.** 9 species known in Europe

Genus *Pericoma* Haliday in Walker, 1856

Pericoma Haliday in Walker, 1856:256 Type species: *Trichoptera trifasciata* Meigen, 1804. **Remarks.** 56 species known in Europe

Genus *Peripsychoda* Enderlein, 1935

Peripsychoda Enderlein, 1935: 249. Type species: *Psychoda fusca* Macquart, 1826. **Remarks.** 4 species known in Europe

Genus *Periulomyia* Krek, 1999

Periulomyia Krek, 1999: Type species: Periulomyia cognata (Eaton, 1893). **Remarks.** 9 species known in Europe

Genus *Philosepedon* Eaton, 1904

Philosepedon Eaton, 1904: 57. Type species: *Psychoda humeralis* Meigen, 1818. **Remarks.** 18 species known in Europe

Genus *Phyllotelmatoscopus* Vaillant, 1982

Phyllotelmatoscopus Vaillant, 1982: 295. Type species: *Pericoma decipiens* Eaton, 1893. **Remarks.** 3 species known in Europe

Genus *Pneumia* Enderlein, 1935

Pneumia Enderlein, 1935: 247. Type species: *Psychoda palustris* Meigen, 1818. **Remarks.** 48

species known in Europe

Genus *Promormia* Ježek, 1984

Promormia Ježek, 1983: 191. Type species: *Telmatoscopus eatoni*, Tonnoir, 1940. **Remarks.** 1 species known in Europe

Genus *Psychoda* Latreille, 1796

Psychoda Latreille, 1796: 152. Type species:*Tipula phalaenoides* Linneaus. **Remarks.** 46 species known in Europe

Genus *Saraiella* Vaillant, 1981

Saraiella Vaillant, 1981: 283. Type species: *Pericoma crypta* Vaillant, 1955. **Remarks.** 22 species known in Europe

Genus *Seoda* Enderlein, 1935

Seoda Enderlein, 1935: 248. Type species: *Pericoma labeculosa* Eaton, 1893. **Remarks.** 23 species known in Europe

Genus *Szaboiella* Vaillant, 1979

Szaboiella Vaillant, 1979: 242. Type species: *Pericoma hibernica* Tonnoir, 1940. **Remarks.** 5 species known in Europe

Genus *Telmatoscopus* Eaton, 1904

Telmatoscopus Eaton, 1904: 58. Type species: *Pericoma advena* Eaton, 1893 (See Quate, 1965: 93). **Remarks.** 5 species known in Europe

Genus *Thornburghiella* Vaillant, 1982

Thornburghiella Vaillant, 1982: 299. Type species: *Psychoda albitarsis* Banks, 1895. **Remarks.** 2 species known in Europe

Genus *Threticus* Eaton, 1904

Threticus Eaton, 1904: 57. Type species: *Pericoma lucifaga* Walker, 1856. **Remarks.** 9 species known in Europe

Genus *Tonnoiriella* Vaillant, 1982

Tonnoiriella Vaillant, 1982: 213. Type species: *Pericoma pulchra* Eaton, 1893. **Remarks.** 14 species known in Europe

Genus *Trichopsychoda* Tonnoir, 1933

Trichopsychoda Tonnoir, 1933: 59. Type species: *Psychoda hirtella* Tonnoir, 1919. **Remarks.** 1 species known in Europe

Genus *Trichosepedon* Krek, 1999

Trichosepedon Krek, 1999: Type species: *Philosepedon balcanicus* Krek, 1971. **Remarks.** 2 species known in Europe

Genus *Ulomyia* Haliday in Walker, 1856

Ulomyia Halidy in Walker, 1856: 261. Type species: *Psychoda fuliginosa* Meigen, 1804. **Remarks.** 19 species known in Europe

Genus *Vaillantodes* Wagner, 2001

Vaillantodes Wagner, 2001: 87. Type species: Vaillantia. **Remarks.** 6 species known in Europe

Genus *Vagmania* Krek, 1972

Vagmania Krek, 1972: 437. Type species: *Vagmania ramulosa* Krek, 1972. **Remarks.** 1 species known in Europe

Subfamily Sycoracinae Jung, 1954

The subfamily Sycoracinae is a rather small moth fly subfamily with 45 extant species distributed worldwide (Ježek, 1999; Barretto, 1956; Bejarano *et al,* 2008; Bravo, 2003, 2007; Bravo & Salazar-Valenzuela, 2009; Bravo *et al.,* 2010; Curler & Jacobson, 2012; Santos & Bravo, 2009; Santos *et al*, 2013). Sycoracinae was first classified within the subfamily Trichomyiinae (Duckhouse, 1972), but it is currently considered a separate subfamily (Duckhouse, 1972; Vaillant, 1978; Wagner, 1997a). Most specialists recognize three genera for this subfamily (Duckhouse, 1972; Santos *et al*., 2009), namely *Aposycorax* Duckhouse, 1972, *Parasycorax* Duckhouse, 1972, and *Sycorax* Haliday in Curtis, 1839, but others classify all the species under a single genus, *Sycorax* i.e. Ježek (1999). Some species have functional mouthparts and feed on blood, with a similar feeding behavior as the members of the subfamily Phlebotominae (Azar *et al*., 2007). Sycoracinae is represented in Europe by the genus *Sycorax*

and only 12 species occur in the Palearctic Region, of those 10 occur in Europe (Curler & Jacobson, 2012).

Biology: All known larvae for the Sycoracinae are only described from the Palearctic Region, they are found on aquatic mosses, leaf litter, or habitats with a high content of lime (Duckhouse, 1972; Kvifte & Wagner, 2017; Wagner, 1997a). Adults have functional mouthparts and females are known to feed on vertebrate blood (Kvifte & Wagner, 2017). The European species *Syzcorax silacea* Haliday in Curtis (1839) can transmit microfilarial worms between frogs (Desportes, 1941)

Genus *Sycorax* Haliday in Curtis, 1839

Sycorax Haliday in Curtis, 1839: 745. Type species: *Sycorax silacea* Haliday in Curtis, 1839, by monotypy.

Microdixa Müller, 1927: 535. Type species: *Microdixa scutig*era Müller, 1927, by original designation. **Remarks.** 10 species are present in Europe .

Subfamily Trychomyiinae Tonnoir, 1922

The subfamily Trychomyiinae has a worldwide distribution with five described genera, four out of those include only fossil species, i.e., *Axenotrichomyia* Azar, Huang, Cai & Nel, 2015, *Eatonisca* Meunier, 1905, *Eotrichomyia* Nel, Meunier & De Plöeg, 2002, and *Xenotrichomyia* Azar, Mouawad & Salame, 2015 (Meunier, 1905; Nel et al., 2002; Azar et al., 2015a, b). The fifth genus, *Trichomyia* Haliday in Curtis, 1839, comprises 198 extant species (Araujo & Bravo, 2018). The genus *Trichomyia* has the highest richness in the Neotropical Region with 127 species (Araújo & Bravo, 2016, 2018; Araújo *et al*. 2017a, b). Two groups and several subgenera have been proposed; however, the subgeneric classification is rather unclear and lacks strong phylogenetic support (Duckhouse, 1978, 1985; Bravo, 1999, 2001; Omelková & Ježek, 2012). Therefore, no subgeneric classification is followed here. Only eight species are recorded for Europe in a single genus *Trichomyia* Haliday in Curtis, 1839 (Omelková & Ježek, 2012).

Biology: It is not known if adult Trychomyiinae feed (Kvifte & Wagner, 2017). All known larvae of this subfamily have been found developing inside decaying wood (Kvifte & Wagner, 2017). Adult collections around the globe suggest that species of this subfamily are associated with natural forests (Omelková & Ježek, 2012; Kvifte & Wagner, 2017).

Genus *Trichomyia* Haliday in Curtis, 1839

Trichomyia Haliday, 1839 in Curtis (1839): 745. Type species: Trichomyia urbica Haliday, 1839, by monotypy. Detailed Synonyms in: Omelková & Ježek (2012). **Remarks.** 8 species are present in Europe .

Discussion

Five out of the six recognized subfamilies of Psychodidae are present in Europe. The subfamily Bruchomyiinae, represented by a single species *Nemapalpus flavus* is only recorded from the Canary Islands therefore this subfamily is not present in continental Europe, but it is still included in the European Fauna. The subfamily Phlebotominae is represented by 23 species in two genera (*Phlebotomus* and *Sergentomyia*); Spain has the richest Phlebotominae fauna with 11 species . The subfamily Psychodinae is the richest of all subfamilies with 504 species and 38 genera distributed all over Europe; France, Germany, and Slovakia present the greater number of species (159, 113, and 114 respectively) . The subfamily Sycoracinae is represented by a single genus (*Sycorax*) with 10 species; the Czech Republic has the highest number of species followed by Yugoslavia (6 and 5 species respectively) . Finally, Trichomyiinae is represented by 8 species in a single genus (*Trichomyia*); being Germany, the UK and the Czech Republic the species rich countries with 3 species each .

Overall, The Czech Republic (171), France (163), Germany (161), Italy (106), Slovakia (118), and Yugoslavia (113) are the countries with the most records of species, on the contrary, Andorra (2), Belarus (2), Iceland (2), Latvia (1) Lithuania (1) and Lichtenstein (0) present the lowest number of species recorded . *Psychoda alternata* (Say, 1826) has the widest distribution, being recorded for 30 countries, followed by *Psychodae cinerea* (Banks, 1894) in 28 countries, *Psychoda phalenoides* Linneaus, 1758, in 27 countries, *Pericoma blandula* Eaton, 1893, and *Pneumia nubila* (Meigen, 1818) in 26 countries each . On the contrary, 485 out of the 540 species currently reported in Europe are present in 10 or fewer countries.

Historically, the Psychodidae fauna in Europe has been studied since the late 1890s by Eaton (Svensson, 2009). Followed by Tonnoir studies almost two decades later (1919-1922), until his work culminated in the 1940s (Tonnoir, 1940; Svensson, 2009). Other authors such as Feuerborn (1922a, 1922b) and Satchell (1947a, 1947b, 1948) made significant contributions to the studies of Psychodidae in Europe. Later, in the 50s, Jung (1956) contributed significantly to the European knowledge of the family. From the 19070s to the 80s Vaillant contributed with a very comprehensive review included in Lindner's: Die Fliegen der Palaearktischen Region

(1971-1983), summarizing all the previous knowledge and providing a general overview of the fauna in Europe (Svensson, 2009).

In more recent years, the work on European/Palearctic Psychodidae has improved with the works of Krek (1999), Wagner (e.g. 1973, 1979, 1980, 1990, 1997, 2000), Whiters (e.g. 1988, 1989), and Ježek (e.g. 1981, 1983, 1984, 1990, 1998) (Svensson, 2009), Kvifte (e.g. 2019, 2023), Kvifte & Andersen (2012), Kvifte et al (2019), Oboňa et al. (e.g. 2021, 2023), Salmela (e.g. 2005, 2008), and Jaume-Schinkel et al. (e.g. Jaume-Schinkel, et al. 2022, 2023)). Nonetheless, since the beginning of the studies in Europe, most of the works have been conducted locally, focusing on specific regions or countries, or focusing on specific genera/species, resulting in some countries having more records/species (as mentioned above). All of the work so far has contributed to the general knowledge of the fauna in the region, nonetheless, almost none of the previous work has taken a broader approach, meaning, nobody has taken the task to target the group as a whole, considering other regions, genera or species for a more comprehensive study. Ultimately, studies will have to gather information scattered across multiple publications in different years and future studies should aim to approach and tackle the information having a broader view.

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Appendix 8. (Publication chapter 10)

Chapter 10 – Publication

Jaume-Schinkel S, Kvite GM (2022) *Platyplastinx ibanezbernali* sp. nov., a new species of moth fly (Diptera: Psychodidae) from Ecuador. Acta Entomologica Musei Nationalis Pragae, 62(2), 383–389. https://doi.org/10.37520/aemnp.2022.020

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RESEARCH PAPER

Platyplastinx ibanezbernali sp. nov., a new species of moth fly (Diptera: Psychodidae) from Ecuador

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Abstract. A new species of *Platyplastinx* Enderlein, 1937, *Platyplastinx ibanezbernali* sp. nov., is described from Ecuador based on morphological characters as well as DNA barcodes from male and female specimens. We provide the first brief description of an egg for the genus. Furthermore, this species is included in the key to world species of the genus, and we provide a key to adult males of *Platyplastinx* from Ecuador.

Key words. Lower Diptera, Psychodinae, new species, taxonomy, Ecuador, Neotropical Region

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:86625BEC-601B-443C-8AED-78D89109D1E7

Introduction

Platyplastinx Enderlein, 1937 was described based on a single broken female specimen of *P. solox* Enderlein, 1937. It remained as such until Quate (1963) redescribed this species, but the genus was first reliably defined when Quate (1999) transferred the male of *P. moragai* (Quate, 1996) (previously described as *Tonnoira moragai*) to this group and provided a diagnosis of the genus based on male characters. According to Lopes & Bravo (2015), there are 13 described species in this genus (not including the one described here). A single species, *P. sycophantos* Quate, 1955, represents the northernmost record of the genus collected in a subtropical part of the Nearctic Region. The majority of other *Platyplastinx* species occur in the Neotropical Region: *P. amazonensis* Lopes & Bravo, 2015, *P. apodastos* Quate & Brown, 2004, *P. crossomiscos* Quate & Brown, 2004, *P. culmosus* Quate & Brown, 2004, *P. duckhousei* Lopes & Bravo, 2015, *P. exiguus* Lopes & Bravo, 2015, *P. hirsutus* Lopes & Bravo, 2015, *P. moragai* (Quate, 1996), *P. obscurus* (Bravo, Lago & Castro, 2004), *P. plumaris* (Quate, 1996), *P. solox* Enderlein, 1937, *P. sycophantos* (Quate, 1955), *P. tango* Quate & Brown, 2004, with São Paulo state in Brazil being the southernmost record for the genus. Distribution of known species is shown in Table 1. In the present paper, we describe a new species of *Platyplastinx* based on male and female specimens from Ecuador, increasing the total number of species of this genus recorded from the country to 3 and the total species worldwide to 14. Additionally, we provide DNA barcodes (5′-end of the cytochrome *c* oxidase subunit 1 or COI) for the new species. Finally, we include this species in the Key to World Species of *Platyplastinx* (Lopes & Bravo 2015) and we provide a key to the adult males recorded in Ecuador.

Material and methods

Study area. Pichincha province is located in the northern part of Ecuador. Canton Pedro Vicente Maldonado (0°10′00″N 79°00′00″W) includes an area of 620 km^2 with average altitude of 600 m a.s.l. The climate in this area is warm-humid, with annual precipitation of 4,341 mm and average annual temperature of 24.5 °C. The main biotope is a very humid pre-mountain rainforest (HPPC 2015).
Collection and preparation of specimens. Specimens were collected using a Malaise trap set for three days (25–28 January, 2020), with 96% ethanol used as preservative. Psychodidae were sorted from the trap sample and stored at -20 °C. The preparation of permanent slides follows the procedure outlined by Ibáñez-Bernal (2005) with the following modifications: before the clearing process, the head, left-wing and abdomen were dissected. The thorax, right-wing, and legs (if present) were used for DNA extraction and barcoding. The pieces of the specimens (head, wing, and abdomen) were not placed in water with dish soap; instead, they were rinsed in bi-distilled water for 5 minutes, and then cleared in a solution of 10% NaOH for

24 hours at room temperature. Then, they were rinsed in bi-distilled water and dehydrated in 70%, 96%, and 100% ethanol for 10 minutes each, and finally placed in clove oil for 10 more minutes. Euparal was used as a mounting medium. Specimen dissections were performed with the aid of a Leica M205C stereomicroscope; once permanently mounted, specimens were examined using a Nikon E600 microscope equipped with a Nikon Y-IDT drawing arm. Measurements were taken using an ocular micrometer. Drawings were completed with a mixed technique of charcoal and ink, and then digitally processed in Adobe Photoshop CS6.

Terminology. We follow the general terminology proposed by Cumming & Wood (2017) and for the male genital terminology we follow the term of hypopods instead of cerci or surstyli as these caudal appendages seem to have origins in both the proctiger and epandrium (see discussion of Kvifte & Wagner 2017). Egg poles follow the definition of Dutra et al. (2011), the anterior pole is the end of the egg that bears the pedicel or a slight projection, while the posterior pole is usually smooth and rounded with no external structures or openings.

Measurements. Head width was taken in the widest part, approximately above the insertion of antennal scape, whereas the length was taken from the vertex to the lower margin of clypeus; wing length was measured from the base of the wing at the start of the costal node to the apex of the wing tip, while the width was taken approximately at an imaginary vertical line crossing the radial and medial forks; palp segment proportions are given considering the length of the first palp segment as a unit (1.0).

In the material examined section, at the end of each record and between square brackets ([]), the holding institution and the unique identifier or number are given. The abbreviations used for collections and their equivalents are given below:

INABIO Instituto Nacional de Biodiversidad, Quito, Ecuador;

IEXA Coleccion Enomologica IEXA, Instituto de Ecologia (INECOL) A.C., Xalapa, Veracruz;

ZFMK Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

In the description of the type labels, the contents of each label are enclosed in double quotation marks (“ ”), and the individual lines of data are separated by a double forward-slash (//).

Taxonomic account

Platyplastinx Enderlein, 1937

Platyplastinx Enderlein, 1937: 107, type species: *Platyplastinx solox* Enderlein. 1937.

Platyplastinx: Quate (1963) (diagnosis); Quate (1999) (revised description); Quate & Brown (2004) (revised description); Lopes & Bravo (2015) (diagnosis, world species list).

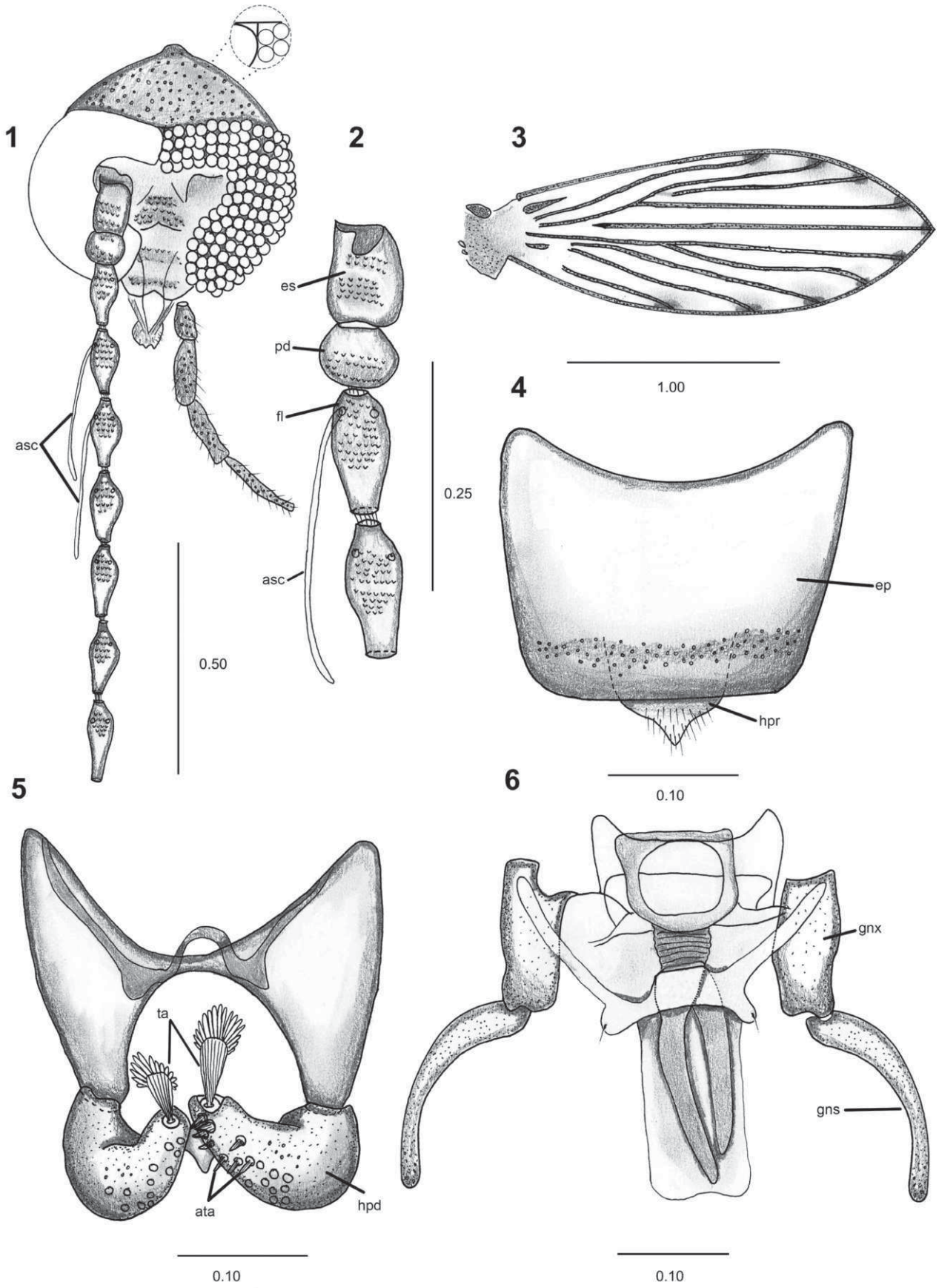
Diagnosis. Modified from Lopes & Bravo (2015) and Quate & Brown (2004).

Male. Interocular suture present; frontal patch of alveoli undivided; antennae with 14 flagellomeres, flagellomere 14 with cylindrical apiculus; ascoids simple, digitate and paired, approximately length of two antennal flagellomeres. Thorax without allurement organs; wings with infuscate patterns, usually with dark spots on apices of longitudinal veins; wing forks R_{2+3} and M_{1+2} basal to wing center, R_5 ending at wing apex. Genitalia with asymmetrical aedeagal complex (except in *P. exiguous* where it is symmetrical); epandrium with single foramen; hypopods with two types of tenacula; often with single principal elongated apical tenaculum (some species with 3, 12 or 15) with fimbriate, feathered or clavate apices; another group of 2–20 small, spine-like accessory tenacula; principal elongate apical tenaculum 2–8 times longer than accessory tenacula.

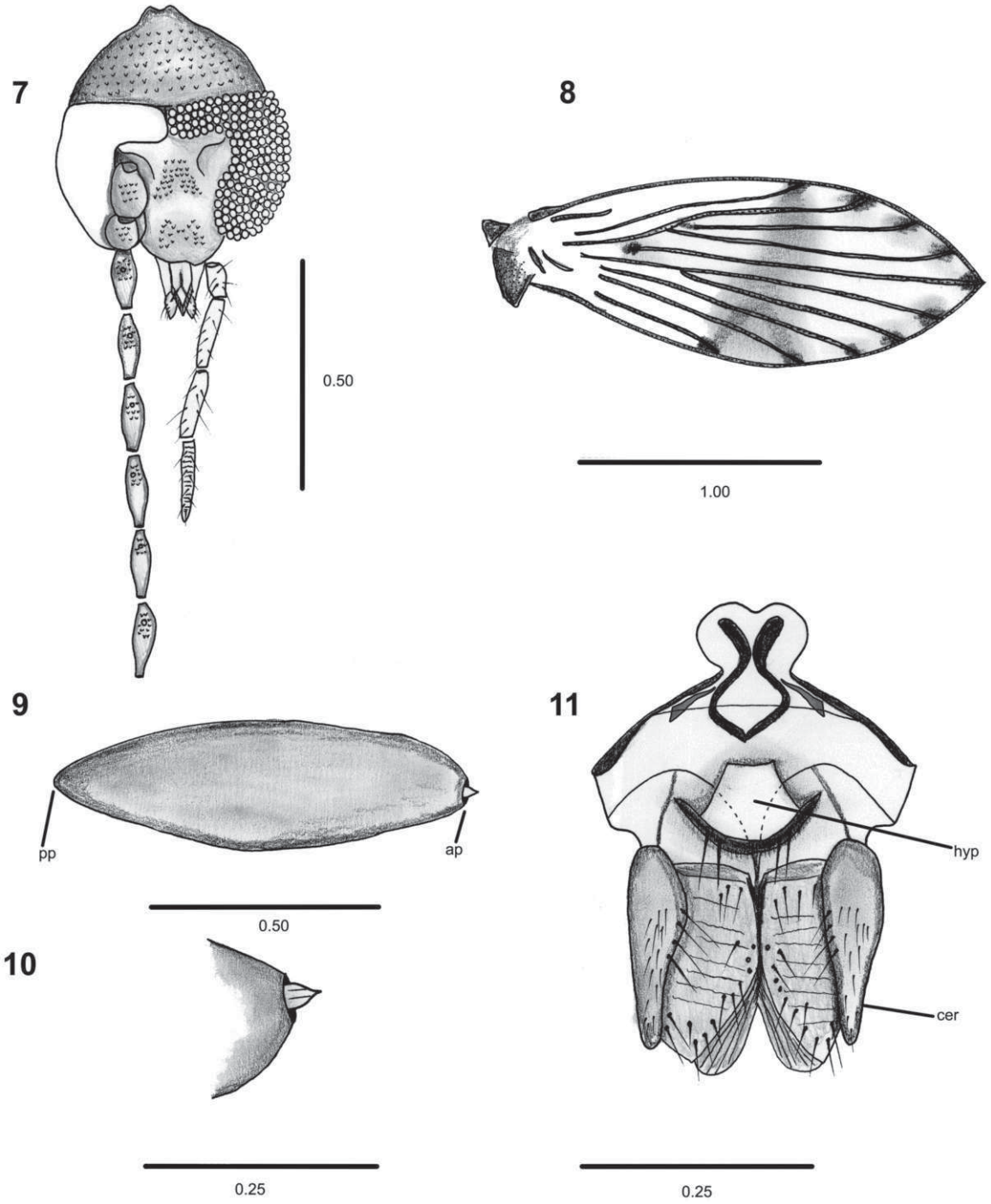
Female. Subgenital plate bilobed and ending with rounded apices; genital ducts comma-shaped and small, not hemispherical; lateral strut present; cerci short, broad and longer than wide.

Table 1. Known distribution of *Platyplastinx* species.

Species	Author	Distribution	Reference
P. amazonensis	Lopes & Bravo, 2015	Brazil	Lopes & Bravo (2015)
P. apodastos	Quate & Brown, 2004	Brazil	Lopes & Bravo (2015); Quate & Brown (2004)
P. crossomiscos	Quate & Brown, 2004	Brazil	Lopes & Bravo (2015); Quate & Brown (2004)
P. culmosus	Quate & Brown, 2004	Ecuador	Lopes & Bravo (2015); Quate & Brown (2004)
P. duckhousei	Lopes & Bravo, 2015	Brazil	Lopes & Bravo (2015)
P. exiguus	Lopes & Bravo, 2015	Brazil	Lopes & Bravo (2015)
P. hirsutus	Lopes & Bravo, 2015	Brazil	Lopes & Bravo (2015)
P. ibanezbernali	Jaume-Schinkel & Kvifte, 2022	Ecuador	Present manuscript
P. moragai	(Quate, 1996)	Brazil, Costa Rica, Panama	Lopes & Bravo (2015); Quate (1996, 1999)
P. obscurus	(Bravo, Lago & Castro, 2004)	Brazil	Bravo et al (2004); Lopes & Bravo (2015)
P. plumaris	(Quate, 1996)	Costa Rica	Lopes & Bravo (2015); Quate (1996)
P. solox	Enderlein, 1937	Costa Rica	Lopes & Bravo (2015); Quate (1996)
P. sycophantos	(Quate, 1955)	United States of America	Lopes & Bravo (2015)
P. tango	Quate & Brown, 2004	Costa Rica	Lopes & Bravo (2015); Quate & Brown (2004)



Figs 1–6. *Platyplastinx ibanezbernali* sp. nov., male holotype. 1 – head; 2 – antennal scape, pedicel and first two flagellomeres; 3 – wing; 4 – epandrium and hypandrium; 5–6 – male genitalia. Abbreviations: asc – ascoids, ata – accessory tenacula, ep – epandrium, es – scape, fl – flagellomere, gns – gonostyli, gnx – gonocoxites, hpd – hypopods, hpr – hypandrium, pd – pedicel, ta – tenaculum. Scale in millimeters.



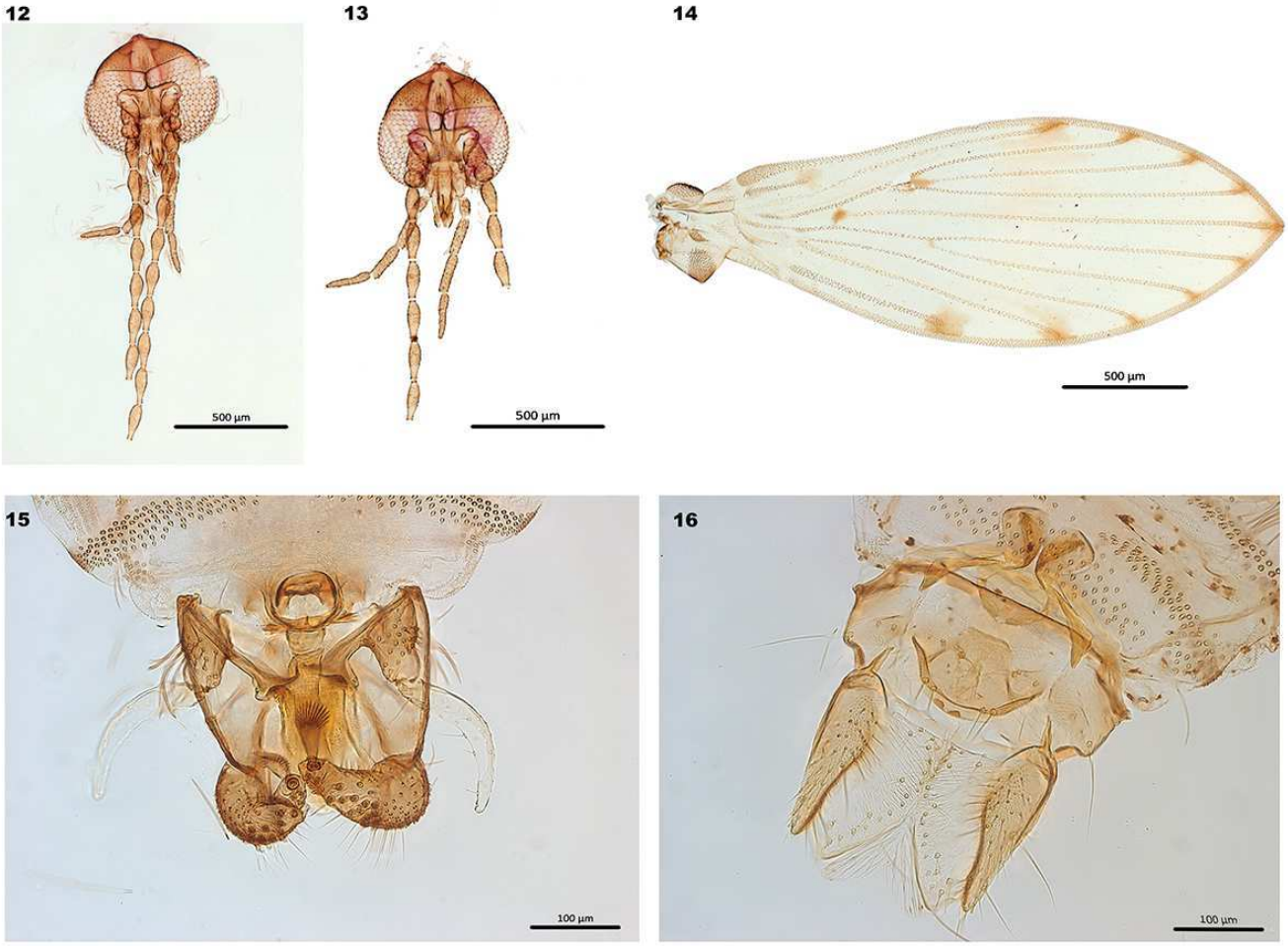
Figs 7–11. *Platyplastinx ibanezbernali* sp. nov., female paratype. 7 – head; 8 – wing; 9–10 – egg; 11 – terminalia. Abbreviations: ap – anterior pole, cer – cercus, hyp – hypogynium, pp – posterior pole. Scales in millimeters.

Platyplastinx ibanezbernali sp. nov.

(Figs 1–8)

Type material. Holotype: ♂, slide mounted (on the slide: head, left wing, terminal segments of abdomen and genitalia; thorax, right wing and legs removed for DNA barcoding). Deposited at INABIO with the following labels: "Ecuador, Pichincha Prov., // Parroquia Pedro Vicente // Maldonado 0.118626, - // 78.958022400000004 // 779 m. 25-January-28- // January-2020. // Kilian, Isabel" "PSYCHODIDAE ♂ // Platyplastinx // ibanezbernali // Jaume-Schinkel, S. & Kvifte, G. M // det. Jaume-Schinkel, S. 2021" "HOLOTYPE" [red] "ZFMK-DIP-00082164" [barcode]. Paratypes: 2 ♂♂, slide mounted as holotype, same label information [deposited at INABIO] [ZFMK-DIP-00081679, ZFMK-DIP-00081672]; 1 ♂, 1 ♀, slide mounted as holotype, same label information [deposited at ZFMK] [ZFMK-DIP-00082161, ZFMK-DIP-00082180]; 1 ♂, slide mounted as holotype, same label information [deposited at IEXA] [ZFMK-DIP-00082168]; 1 ♂ complete specimen stored in 96% ethanol, same label information [deposited at ZFMK] [ZFMK, ZFMK-DIP-000-81982].

Diagnosis. ***Adult male.*** Males of this species can be differentiated from other species of *Platyplastinx* by the following characters: hypopods with only 1 apical elongated tenaculum; hypopods pyriform, tapering towards apex; eye bridge with 4 facet rows; 16–18 short spiniform accessory tenacula; eyes contiguous with interocular suture T-shaped; wing membrane not darkened except for dark spots on apices of longitudinal veins.



Figs 12–16. *Platyplastinx ibanezbernali* sp. nov., photographies. 12 – head, male holotype; 13 – head, female paratype; 14 – wing, male paratype; 15 – genitalia, male holotype; 16 – genitalia, female paratype. Scale in µm.

It can be separated from *P. sycophantos*, *P. cromossomiscos* and *P. culmosus* by the presence of only one apical elongated tenaculum in the hypopods. It differs from *P. hirsutus*, *P. tango*, *P. exiguus*, *P. moragai*, and *P. plumaris* by the shape of the hypopods (pyriform and tapering towards the apex in *Platyplastinx ibanezbernali* sp. nov.). Finally, it can be differentiated from the most similar species, *Platyplastinx duckhousei* Lopes & Bravo 2015, by the following characters: in *Platyplastinx ibanezbernali* sp. nov. the interocular suture is T-shaped and eyes contiguous with 4 facet rows, wings are not darkened in the apical half and bear dark spots at apices of wing veins, gonostyli are incurved; in *P. duckhousei* the interocular suture is inverted U-shaped and eyes separated by less than 1 facet diameter, wings are darkened in the apical half except for clear spots between wing veins, gonostyli are straight.

Description. ***Adult male*** (Figs 1–6, 12, 14–15). Measurements (averages, n = 6): Head width 0.58 mm, length 0.53 mm; wing width 0.93 mm, length 2.34 mm.

Head round in frontal view; surface regularly covered with setae alveoli. Eyes contiguous; Interocular suture T-shaped; eye bridge with 4 facet rows. Frontal alveoli patch bell-shaped, with lower margin bilobed. Clypeus with alveoli patch square, very sparse at median. Antenna with scape cylindrical, about twice length of pedicel; pedicel spherical; flagellomeres broadly fusiform, about same length as scape, terminal flagellomeres absent in material examined, maximum number of flagellomeres present in one specimen: 11, ascoids paired and long, digitiform, about length of 2.1 flagellomeres. Palpus short, not extending beyond flagellomere 6, all palp segments cylindrical; palp segment 4 striated; proportions of palp segments 1 : 2 : 2 : 2.4. Labellum fleshy, longer than broad, with 3–5 setae and no spiniform or tooth-like sensilla.

Wing length 2.5 times its maximum width, oblanceolate with acute apex, general coloration of membrane yellowish with brown spots on apex of all longitudinal veins and at origin of R_5 and fork R_{2+3}; costa with one basal node; Sc ending freely at about start of M_3; Rs pectinate, radial fork slightly basal to medial fork, junction of $R_3 + R_2$ very faint (Figs 3, 14), R_5 ending at wing apex.

Terminalia. Hypandrium bilobed and joining gonocoxites at basal margin, lobes with single bristle near apex (Fig. 6), it appears membranous, giving appearance of "cleft" near median line; gonostyli longer than gonocoxites, curved inward, with apices at same level as that of aedeagus; ejaculatory apodeme almost square with anterior margin little wider than posterior margin; aedeagal-paramere complex with two finger-like appendages; epandrium approximately as wide as long, with anterior margin concave, with bristles distributed at apical margin; hypopods curved pyriform, with one apical feathered

tenaculum and additional 16–18 short spiniform accessory tenacula distributed in distal half of ventral surface.

Female (Figs 7–10, 13, 16). Measurements (n = 1) head width 0.48 mm, length 0.53 mm; wing width 0.76 mm, length 1.92 mm. Same as male except for proportion of palp segments 1 : 2 : 2 : 2.3 (Fig. 7). Terminal flagellomeres missing in material examined; maximum number of flagellomeres present in one specimen: six. Ascoids missing in material examined. Wing length 2.5 times its maximum width, general coloration as in male wing, with faint darkening in middle section but not extending towards apex (Fig. 8).

Terminalia. Hypogynium apical margin rounded (hypogynium rounded and not bilobed is a characteristic of the genus, see diagnosis in Quate 1999; Quate & Brown 2004 [as subgenital plate]) trapezoid-shaped with broadly sclerotized U-shaped margin carrying small setulae and 4 strong bristles as in Figs 11, 16. Hypogynium short, about 0.45 length of cerci. Cerci length is about 1.5 its maximum width. Genital chamber simple, shaped like inverted S on each side, as in Figs 11, 16.

Egg. The single female specimen contained eggs inside the abdomen, the overall shape is ovoid (Fig. 9). All eggs present micropyle as conical-shaped structure on anterior pole as in Figs 9–10. Microsculpture is not preserved well enough to allow diagnosis.

Etymology. The specific epithet is in honor of a great mentor, entomologist and Psychodidae specialist, Dr. Sergio Ibáñez-Bernal.

Genetics. The GenBank accession numbers for the DNA barcodes (5′-COI) for this species are: ON002471 [ZFMK-DIP-00082164], ON002470 [ZFMK-DIP-00082168], ON002472 [ZFMK-DIP-00082180].

Distribution. Currently known only from the type locality.

Differential diagnosis. This species can be included in the key to males of world *Platyplastinx* presented by Lopes & Bravo (2015), modified as follows:

6 Hypopods (cercus in Lopes & Bravo 2015) with accessory tenacula numbering 16–18. 7
– Hypopods (cercus in Lopes & Bravo 2015) with accessory tenacula numbering 6 to 9. 8
7 Eyes separated by less than 1 facet diameter; interocular suture inverted U-shaped; Wing membrane darkened in apical half with exception of small apical areas between wing veins; genitalia as in Lopes & Bravo (2015: fig. 4). .... ***Platyplastinx duckhousei*** Lopes & Bravo, 2015
– Eyes contiguous; interocular suture T-shaped (Fig. 1); wing membrane not darkened in apical half, with darkened apices of wing veins; genitalia as in Figs 5–6. ... . ***Platyplastinx ibanezbernali*** **sp. nov.**

Key to adult males of *Platyplastinx* in Ecuador

1 Hypopods with only 1 elongated tenaculum (Fig. 5); eyes contiguous (Fig. 1), genitalia as in Figs 5–6, 15. ..... ***Platyplastinx ibanezbernali*** **sp. nov.**
– Hypopods with more than 1 elongated tenaculum; eyes separated. 2
2 Eyes separated by 1.5 facet diameters, interocular suture as inverted Y; accessory tenacula 20, genitalia as in Quate & Brown (2004: figs 171–172). .... ***P. crossomiscos*** Quate & Brown, 2004
– Eyes separated by less than 1 facet diameter; interocular suture curved, not Y-shaped; accessory tenacula 10, genitalia as in Quate & Brown (2004: figs 164–166). ..... ***P. culmosus*** Quate & Brown, 2004

Discussion

In the most recent paper focused on *Platyplastinx* (Lopes & Bravo 2015) as well as in the revised descriptions of Quate (1999) and Quate & Brown (2004), females of only two species were known: *P. solox*, for which only the female is known, and *P. moragai*. With the inclusion of the description of a female *Platyplastinx ibanezbernali* sp. nov. three females are now known for the genus, which leads us to emphasize the importance of biological inventories and that there is still a lot of diversity waiting to be discovered in the Neotropical Region.

Eggshell structures of Psychodidae species are considered to be species-specific and have been used as a character for species identification (Rocha et al. 2011), although it is recommended to base such descriptions on electron microscopy; however, biological material for new species is sometimes hard to obtain. Unfortunately, the eggs of the single female specimen are still inside the abdomen, and no microsculpture can be differentiated through the microscope. Nonetheless, the description and drawings of the conical structure of the eggs of *Platyplastinx ibanezbernali* sp. nov. represent the first known description of the egg in the genus *Platyplastinx* which establishes a base for additional studies in the near future.

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ERRATUM

JAUME-SCHINKEL S. & KVIFTE G. M. 2022: *Platyplastinx ibanezbernali* sp. nov., a new species of moth fly (Diptera: Psychodidae) from Ecuador. *Acta Entomologica Musei Nationalis Pragae* 62 (2): 383–389. https://doi.org/10.37520/aemnp.2022.020

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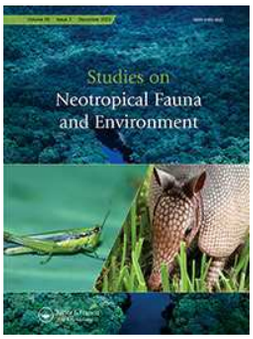
Appendix 9. (Publication chapter 11)

Chapter 11 – Publication

Jaume-Schinkel S (2022) Description of *Tonnoira conistylus* sp. nov. from Costa Rica and a new record of Tonnoira distincta Bravo et al. 2008 from Ecuador, *Studies on Neotropical Fauna and Environment*, 1–8, https://doi.org/10.1080/01650521.2022.2081466



Studies on Neotropical Fauna and Environment

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Description of *Tonnoira conistylus* sp. nov. from Costa Rica and a new record of *Tonnoira distincta* Bravo et al. 2008 from Ecuador

Santiago Jaume-Schinkel

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ORIGINAL ARTICLE

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Description of *Tonnoira conistylus* sp. nov. from Costa Rica and a new record of *Tonnoira distincta* Bravo et al. 2008 from Ecuador

Santiago Jaume-Schinkel

Zoologisches Forschungsmuseum Alexander Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels, Bonn, Germany

ABSTRACT

Tonnoira conistylus **sp. nov**. is described based on a male collected in 1921 from Costa Rica. Illustrations and photographs of the new species are provided. This species is included in the world key of males of the genus *Tonnoira* Enderlein and an updated distributional map for the species in the genus is provided, as well as a new record of *Tonnoira distincta* from Ecuador.

http://www.zoobank.org/urn:lsid:zoobank.org:pub:FC930575-34FB-4CEE-A456-773F0083B7C5

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Received 25 March 2022
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KEYWORDS
Distribution; moth flies; new taxa; species list; taxonomy

Introduction

Tonnoira; Enderlein, 1937 is a Neotropical genus of moth flies (Diptera: Psychodidae) with 26 extant and one fossil species described so far (Azar & Maksoud 2020; Bravo et al. 2020). The genus was first described by Enderlein (1937) based on a single female specimen of *T. pelliticornis* Enderlein, 1937 from Peru (Santos & Curler 2014), but currently, the extant *Tonnoira* species have been recorded in ten countries ranging from Nicaragua to Brazil, excluding the Caribbean Islands (Bravo et al. 2008, 2020; Santos & Curler 2014).

Brazil is the country with the highest number of *Tonnoira* species, with records from 18 out of the 26 described species so far, followed by Suriname (5 species), French Guiana (3 species), Costa Rica, Panama, and Venezuela (2 species), and Bolivia, Ecuador, Nicaragua, and Peru (1 species) (Bravo et al. 2020).

In the present study, a new species is described based on a single male collected in Costa Rica in 1921. Additionally, a new geographical record for *Tonnoira distincta* Bravo, Alves & Chagas, 2008 from Ecuador is provided, a checklist and an updated distributional map for the species of the genus is provided.

Material and methods

The material examined for this study is deposited at The National History Museum (NHM) in London, UK. Collector, collection method, date, and locality are specified under the material examined after each specimen. In the material examined section, at the end of each record and between square brackets ([]), the holding institution is indicated. In the description of type labels, the contents of each label are enclosed in double quotation marks (‘ ’), italics denote handwriting, and the individual lines of data are separated by a double forward-slash (//).

The specimens were diaphanized, dissected, and mounted on permanent slides. Slides are thought to be prepared by Laurence W. Quate (G.R. Curler, pers. comm. 2021) based on the type of preparation and notes on slides.

Terminology

General terminology follows Cumming and Wood (2017) and Kvifte and Wagner (2017).

Measurements

Head width was taken at the widest part, approximately above the insertion of the antennal scape, whereas the length was taken from the vertex to the lower margin of the clypeus; wing length was measured from the base of the wing at the start of the costal node to the apex of the wingtip, while the width was taken approximately at an imaginary vertical line crossing the radial and medial forks; palpal proportions are given considering the length of the first palpal segment as a unit (1.0).

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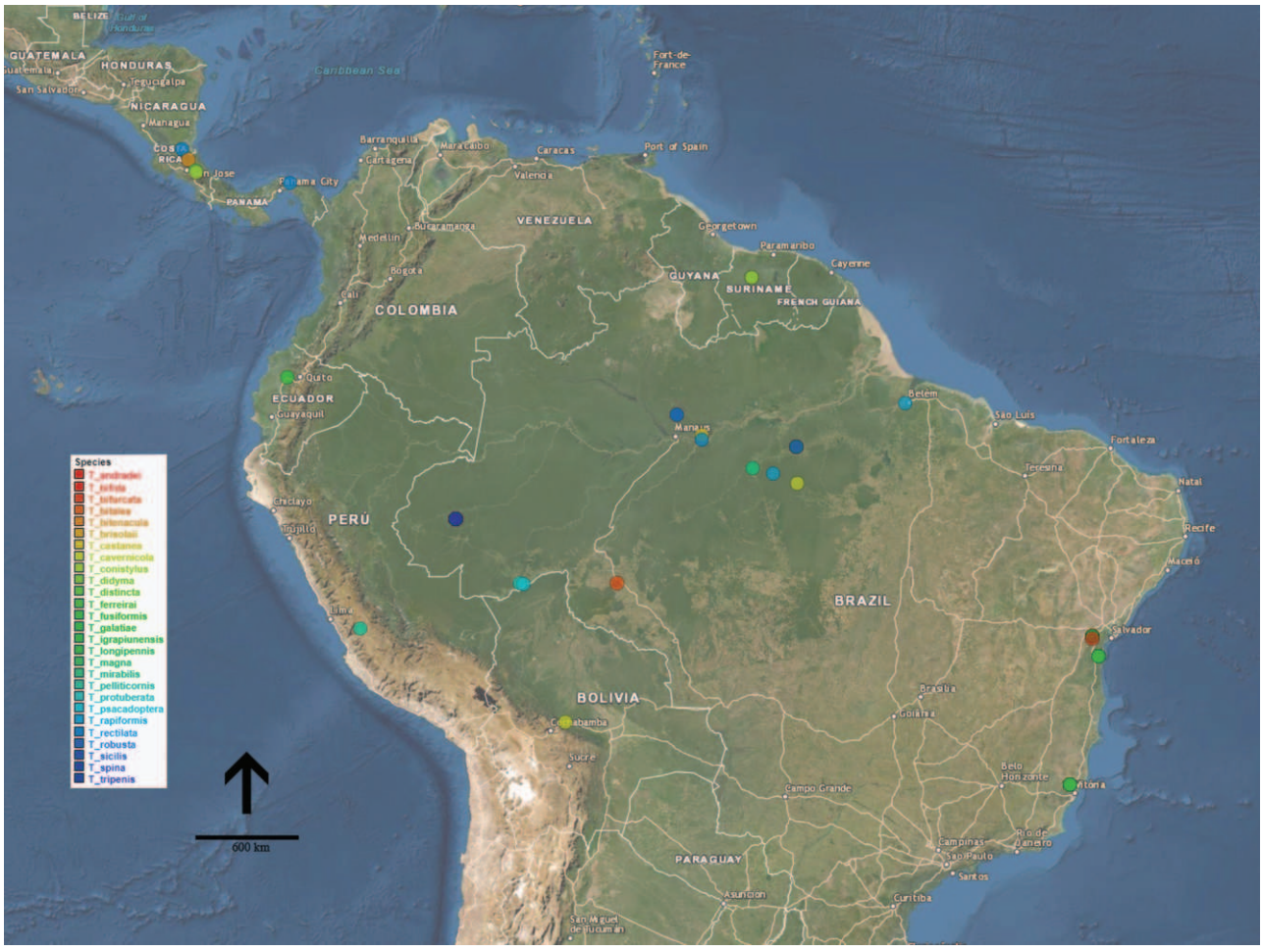

Figure 1. Updated distribution map of known *Tonnoira* species.

Results

Genus Tonnoira *Enderlein, 1937*

Type species: *Tonnoira pelliticornis*, 1937: 106. Type locality: PERU, Callanga. Enderlein 1937:106 (original description); Quate 1963:189 (diagnosis); Quate 1996: 33 (revised description); Quate & Brown 2004: 25 (revised description); Santos & Curler 2014: 464 (diagnosis); Bravo et al. 2020: 4 (species list). The distribution of all species can be seen in Figure 1 and Table 1.

Species included

T. andradei Santos & Curler, 2014; *T. bifida* Bravo & Chagas, 2004; *T. bifurcata* Quate & Brown, 2004; *T. bitalea* Quate, 1999; *T. bitenacula* Quate, 1996; *T. brisolaii* Santos & Curler, 2014; *T. castanea* Quate & Brown, 2004; *T. cavernicola* Quate & Brown, 2004; *T. conistylus* **sp. nov.**; *T. didyma* Quate & Brown, 2004; *T. distincta* Bravo, Alves & Chagas, 2008; *T. ferreirai* Santos in Santos & Curler, 2014; *T. fusiformis* Quate & Brown, 2004; *T. galatiae* Santos & Curler, 2014; *T. igrapiunensis* Bravo, Vilarinho & Araújo, 2020; *T. longipennis* Bravo & Chagas, 2004; *T. magna* Bravo & Chagas, 2004; *T. mirabilis* Wagner, 1981; *T. pelliticornis* Enderlein, 1937; *T. protuberata* Quate & Brown, 2004; *T. psacadoptera* Quate & Brown, 2004; *T. rapiformis* Quate & Brown, 2004; *T. rectilata* Quate, 1999; *T. robusta* Bravo, Alves & Chagas, 2008; *T. sicilis* Quate & Brown, 2004; *T. spina* Chagas-Vieira, 2012; and *T. tripenis* Chagas-Vieira, 2012.

Incertae sedis. *Tonnoira sakhalinensis* Azar & Maksoud, 2020.

Azar and Maksoud (2020) present no strong evidence to place *Tonnoira sakhalinensis* in the genus *Tonnoira*, the description provided is consistent with placement in several tribes, the wing, on one hand, is consistent with Brunettiini and Maruinini, on the other hand, the genitalia looks more Brunettiini-like rather than Maruinini. The extraordinary number of tenacula present in this species

Table 1. Extant species of the genus *Tonnoira* Enderlein, 1937 distribution.

Species	Author, year	Known distribution
T. andradei	Santos & Curler, 2014	BRAZIL: Espirito Santo
T. bifida	Bravo & Chagas, 2004	BRAZIL: Bahia
T. bifurcata	Quate & Brown, 2004	BRAZIL: Rondônia
T. bitalea	Quate, 1999	PANAMA; BRAZIL: Amazonas
T. bitenacula	Quate, 1996	COSTA RICA
T. brisolaii	Santos & Curler, 2014	BRAZIL: Espirito Santo
T. castanea	Quate & Brown, 2004	BRAZIL: Amazonas; SURINAM: Raleighvallen
T. cavernicola	Quate & Brown, 2004	BOLIVIA: Chapare; FRENCH GUIANA: 23 km S St. Laurent de Maroni
T. conistylus	Jaume-Schinkel	COSTA RICA: Suiza de Turrialba
T. didyma	Quate & Brown, 2004	FRENCH GUIANA: Maripasoula; SURINAM: Raleighvallen
T. distincta	Bravo, Alves & Chagas, 2008	BRAZIL: Amazonas, ECUADOR: Morona-Santiago Province*
T. ferreirai	Santos & Curler, 2014	BRAZIL, Espirito Santo
T. fusiformis	Quate & Brown, 2004	COSTA RICA: Cartago; ECUADOR: E Santo Domingo
T. galatiae	Santos & Curler, 2014	BRAZIL: Espirito Santo
T. igrapiunensis	Bravo, Vilarinho & Araújo, 2020	BRAZIL: Bahia
T. longipennis	Bravo & Chagas, 2004	BRAZIL: Bahia
T. magna	Bravo & Chagas, 2004	BRAZIL: Bahia
T. mirabilis	Wagner, 1981	BRAZIL: Amazonas, Estirão do Ecuador; SURINAM: Raleighvallen
T. pelliticornis	Enderlein, 1937	PERU: Callanga
T. protuberata	Quate & Brown, 2004	VENEZUELA: Aragua
T. psacadoptera	Quate & Brown, 2004	VENEZUELA: 10 km NE of Macaray
T. rapiformis	Quate & Brown, 2004	BRAZIL: Estrada do Caripi; SURINAM: Brownsberg Nature Park
T. rectilata	Quate, 1999	BRAZIL: Amazonas; NICARAGUA; PANAMA: Nusagandi; SURINAM
T. robusta	Bravo, Alves & Chagas, 2008	BRAZIL: Amazonas
T. sicilis	Quate & Brown, 2004	FRENCH GUIANA: Maripasoula
T. spina	Chagas-Vieira, 2012	BRAZIL: Amazonas
T. tripenis	Chagas-Vieira, 2012	BRAZIL: Amazonas

* new records for the country

(six compared to 1–3 in extant species) makes it difficult to place it in *Tonnoira*, therefore I consider this species as *incertae sedis*.

Tonnoira conistylus **sp. nov**. (Figures 2A–E, 3 A–B)

Examined material

Holotype: m#, labeled 'COSTA RICA, 1921 // SUIZA DE TURRIALBA // *98*' 'PSYCHODIDAE // Tonnoira // conistylus // Jaume-Schinkel, S.' [NHM].

Differential diagnosis

Tonnoira conistylus **sp. nov**. can be easily differentiated from the other species in the genus by the combination of the following characters: first flagellomere cylindrical; gonostylus not bifurcate; surstyli with two tenacula; aedeagus straight and with parameres present. This species is similar to *Tonnoira cavernicola* but it can be easily recognized by the conical shape of the gonostylus in *T. conistylus* **sp. nov**. (tapered and curved in *T. cavernicola*) and the aedeagus with two parameres in *T. conistylus* **sp. nov**. (one paramere in *T. cavernicola*).

Type locality

COSTA RICA: Cartago, Turrialba, Suiza de Turrialba.

Description

Male. Head. Wider than long; eye bridge of four rows of facets; eyes separation resembling a sand-watch, with the upper part separated by less than one facet diameter and almost joined at the waisted part; interocular suture as an inverted 'Y,' short, length about 1.5 facet diameters; the frontal patch of alveoli trapezoidal slightly concave in upper and lower margins; palpus incomplete in the material examined, first segment of the palpus almost spherical; labium without strong sclerite; labella bulbous with two rows of setae on apical margin. Antennal scape 1.6 times the length of the pedicel, almost cylindrical, apical margin wider than basal margin; pedicel spherical, smaller than scape; flagellomeres cylindrical, about three times the length of the pedicel, with scattered setae on the surface, apical flagellomeres absent in examined material; ascoids missing in reviewed material.

Wing. Length two times its width; wing membrane hyaline; junction of R_{2+3} basal to M_{1+2}, 'stem' of R_{2+3} very short; R_5 ending at wing apex; CuA_2 with an almost 90° bend at the apex.

Terminalia (Figures 2C–E, 3). Gonocoxites are 1.2 times the length of gonostylus. Gonostylus covered with scattered alveoli, conical with rounded apex. Aedeagus with basiphallus longer than gonocoxite. Epandrium about width 1.4 times its length. Hypoproct triangular, not very prominent, covered in small setulae. Surstyli tapered from base to apex, curved dorsally, each with two tenacula, one inserted dorsoapically and one inserted dorsolaterally, separated by about the length of one tenaculum; apical tenacula with crow-like apex; dorsal tenacula spoon-like apex.

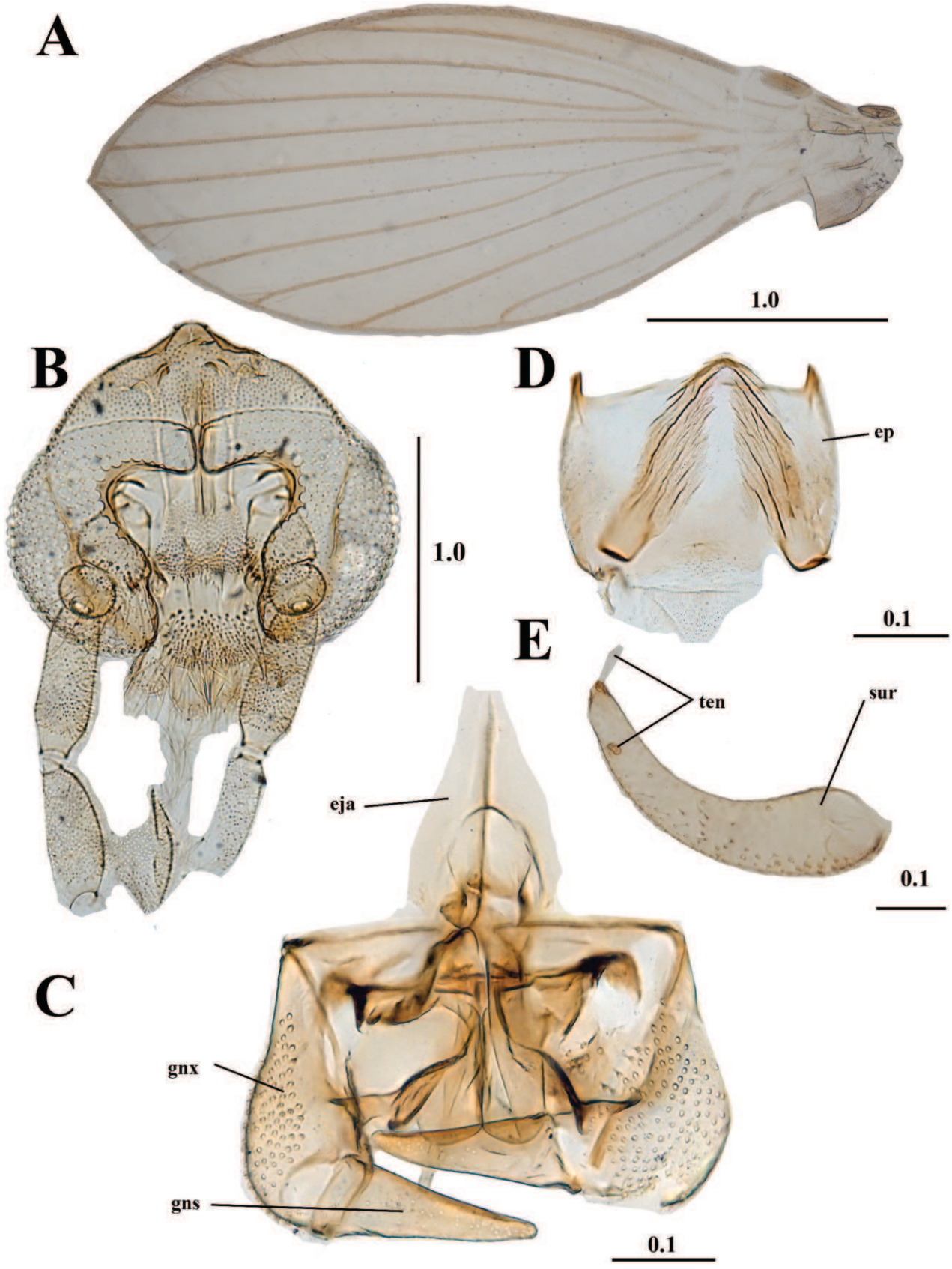

Figure 2. *Tonnoira conistylus* **sp. nov**. Holotype, Male. **A**. Wing, **B**. Head, frontal view, **C**. Aedeagus, gonocoxites and gonostylus, **C**. Epandrium, **D**. Surstylus lateral view. Abbreviations: eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; ep = epandrium; sur = surstylus; ten = tenacula. Scales in mm.

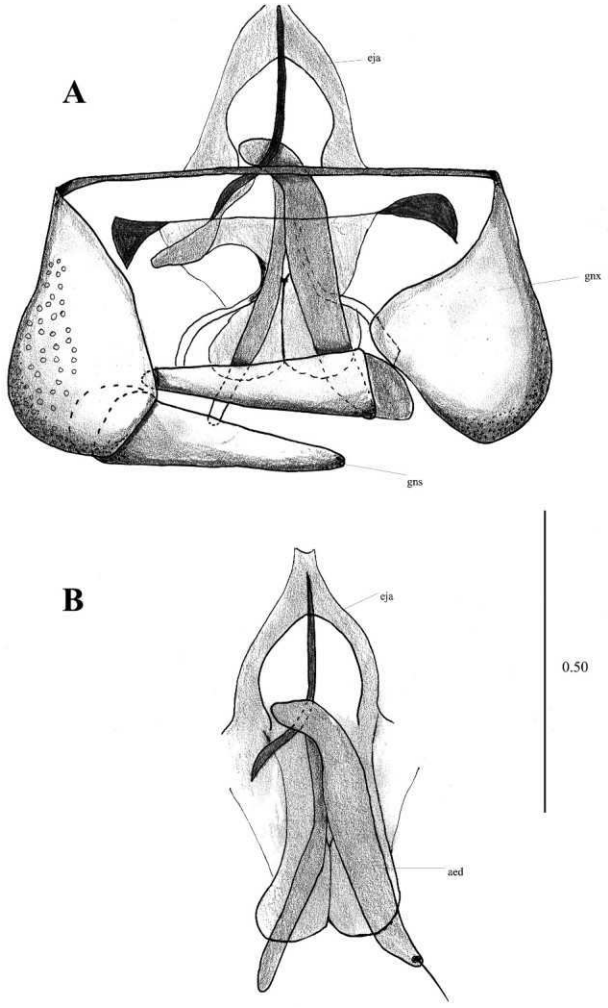

Figure 3. *Tonnoira conistylus* **sp. nov**. Holotype, male. Genitalia, Gonocoxites, Gonostylus, Aedeagus, and Parameres. Abbreviations: aed = aedeagal complex; eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite. Scales in mm.

Female. Unknown.

Etymology. From the combination of Latin '*cōnus*' (meaning cone) and surstylus, referencing the conical shape of the surstylus. Species name to be treated as a name in apposition.

Distribution. Only known from the type locality in Costa Rica.

Remarks. *Tonnoira conistylus* **sp. nov**. can be placed in the key provided by Bravo et al. (2020) by modifying it as follows:

24. Eyes separated by one facet diameter; aedeagus extending to half of gonostylus (see Bravo et al. 2008: fig. 6) … ***T. distincta*** **Bravo, Alves & Chagas**

-. Eyes separated by less than one facet diameter; aedeagus extending to the apex of gonocoxite or more, but never close to the half of gonostylus … **25**

25. aedeagus extending a little beyond tip of gonocoxite; gonostylus tapered at apex, incurved; aedeagus with one paramere; (see Quate & Brown 2004: fig. 72) … ***T. cavernicola*** **Quate & Brown**

-. Aedeagus extending to the apex of gonocoxites; gonostylus conical not curved; aedeagus with two parameres (Figure 2C, 3) … ***T. conistylus*** **sp. nov.**

Tonnoira distincta Bravo et al 2008 (Figures 4A–D, 5 A–D)

Tonnoira distincta Bravo, Alves & Chagas, 2008: 65. Type locality: BRAZIL, Amazonas, Presidente Figueiredo. (Bravo et al. 2008).

Examined material. 2 m#, 1 f# labeled '*ECUADOR, Morona- // Santiago province, // Rio Cuangos, Cuevas // de los Tayos, 3°07´S // 78°12´W, 16.viii-1976 // (A. M. Hutson)* // BM 1976–659.' 'PSYCHODIDAE // Tonnoira // distincta // Bravo et al. 2008 // det. Jaume-Schinkel, S.' [NHM]. This is the first record of the species for Ecuador.

Remarks. One of the males has the paramere bent toward the coverglass, giving it the appearance of not having a paramere and difficulting the visualization. The position of the aedeagus makes it look slightly different from the other male examined but, nonetheless, based on the locality and other characters compared favorably to the other specimen and the illustrations by Bravo et al. (2020); I have no doubts that both belong to the same species and that they can be positively identified as *T. distincta*.

Discussion

To date, only three species of *Tonnoira* have been collected in caves, namely: *T. cavernicola, T. distincta*, and *T. robusta*. The new report of *T. distincta* for Ecuador was also collected in a cave. Although the association of *Tonnoira* species with caves is poorly studied this new record collected in a similar environment as the type series in a different country leads me to believe that there is still a high amount of understudied interactions inside the Psychodinae subfamily in their natural environments.

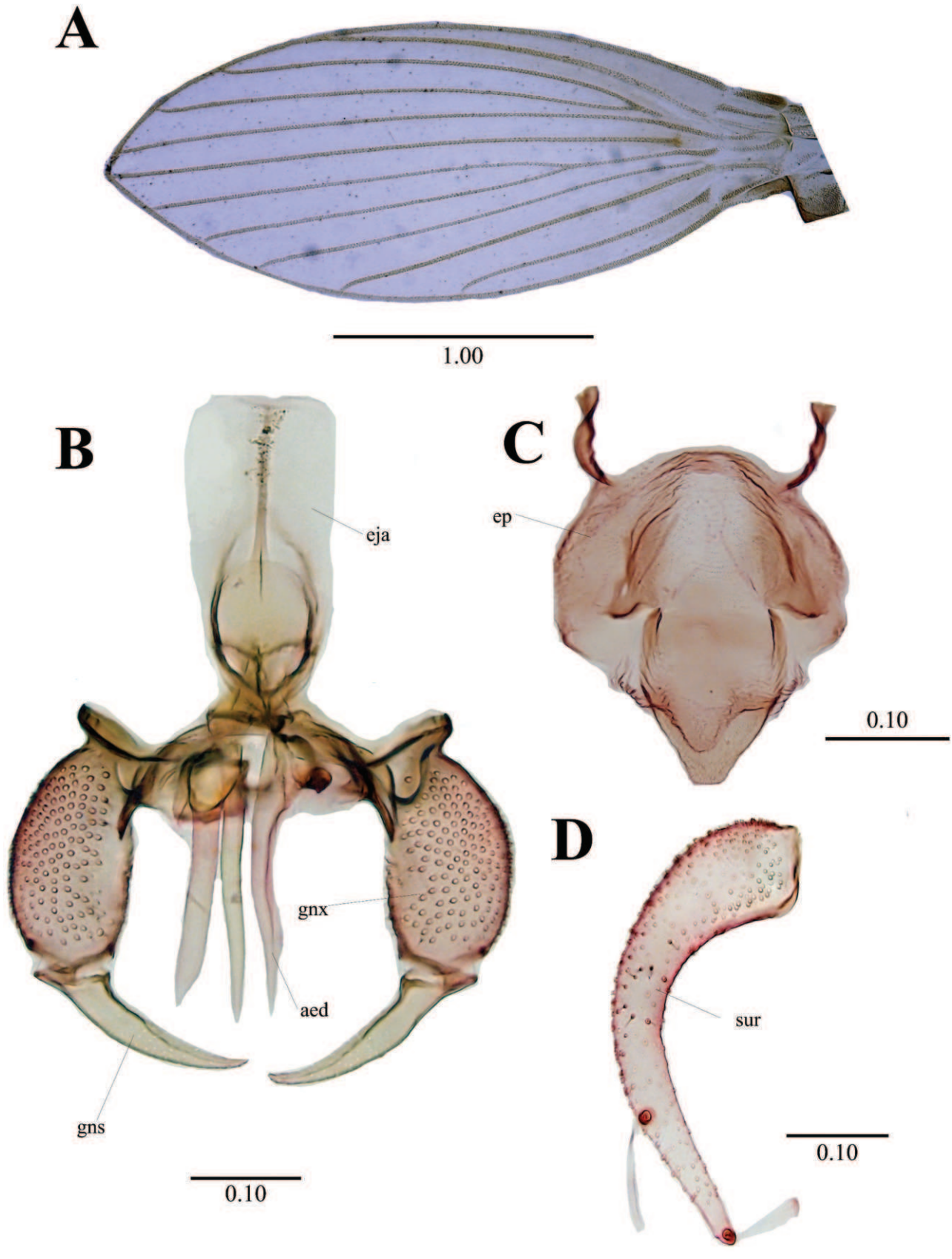

Figure 4. *Tonnoira distincta* Bravo, Alves & Chagas, Male. **A**. Wing, **B**. Aedeagus, gonocoxites, and gonostylus, **C**. Epandrium, **D**. Surstylus lateral view). Abbreviations: eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; hyp = hypandrium; sur = surstylus. Scales in mm.

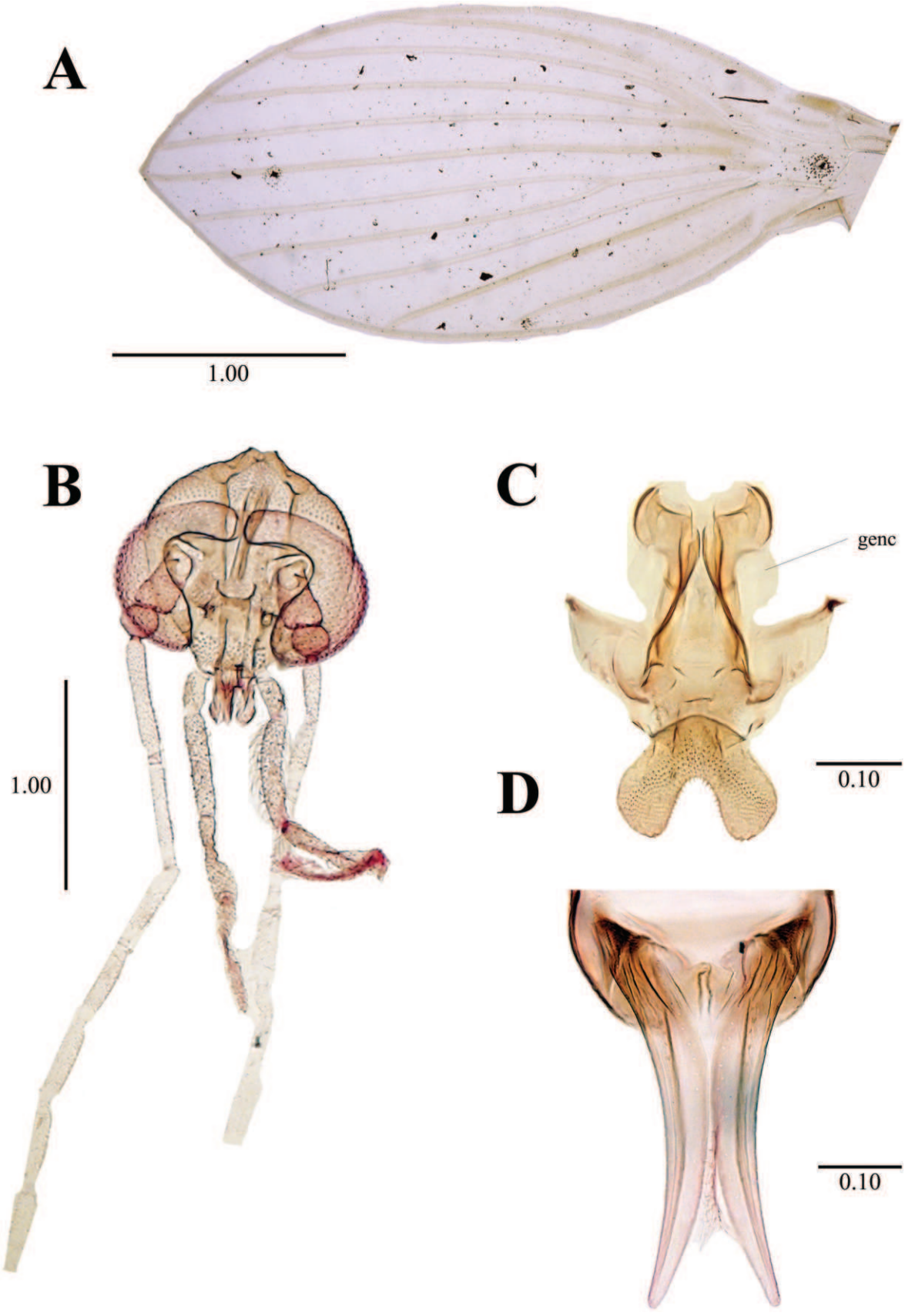

Figure 5. *Tonnoira distincta* Bravo, Alves & Chagas, Female. **A**. wing, **B**. Head, frontal view, **C**. Genital chamber, **D**. Genitalia. Scales in mm.

Acknowledgments

I am thankful to Duncan Sivell that hosted me at the NHM collection in the UK and for organizing the loan of the type material. I am grateful to Greg Curler for the talks that improved my understanding of *Tonnoira*. I extend my gratitude to Ximo Mengual and Gunnar M. Kvifte for their valuable comments that improved the earlier version of the manuscript, I am thankful with the reviewers that improved the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author.

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Appendix 10. (Publication chapter 12)

Chapter 12 – Publication

Jaume-Schinkel S (2023) Description of *Tonnoira chuki* sp. n. (Diptera: Psychodidae) from Ecuador with an updated taxonomical Key for the genus Tonnoira Enderlein, 1937. *Integrative Systematics*, 6(1), 51–57. https://doi.org/10.18476/2023.462484

Description of Tonnoira chuki sp. n. (Diptera: Psychodidae) from Ecuador, with an updated identification key for the genus Tonnoira

Author: Jaume-Schinkel, Santiago

Source: Integrative Systematics: Stuttgart Contributions to Natural History, 6(1) : 51-58

Published By: Stuttgart State Museum of Natural History

URL: https://doi.org/10.18476/2023.462484

RESEARCH ARTICLE

Description of *Tonnoira chuki* sp. n. (Diptera: Psychodidae) from Ecuador, with an updated identification key for the genus *Tonnoira*

Santiago Jaume-Schinkel

Abstract

Tonnoira chuki **sp. n.** is described based on specimens collected in a pre-mountain rainforest in Pichincha Province, northeastern Ecuador. Illustrations and photographs of the new species are provided. The identification key to males of the genus is updated. Furthermore, the first published DNA barcodes (*COI*) for the genus are provided.

Keywords: dark taxa, DNA barcoding, moth flies, new taxa, Psychodinae, taxonomy.

Zusammenfassung

Tonnoira chuki **sp. n.** wird anhand von Exemplaren beschrieben, die in einem Vorgebirgsregenwald der Provinz Pichincha im Nordosten Ecuadors gesammelt wurden. Die neue Art wird anhand Illustrationen und Fotos dargestellt. Der Bestimmungsschlüssel für die Männchen der Gattung wird aktualisiert. Zudem werden die ersten DNA-Barcodes (*COI*) für die Gattung zur Verfügung gestellt.

Introduction

The Neotropical genus *Tonnoira* Enderlein, 1937 is currently present in ten countries, not including the Caribbean islands, ranging from Nicaragua to Brazil (Bravo et al. 2008; Santos & Curler 2014). The genus was erected based on a single female, *Tonnoira pelliticornis* Enderlein, 1937, and to date, the male sex of the type species remains unknown. Since then, a total of 27 extant species have been described (Enderlein 1937; Wagner 1981; Quate 1996, 1999; Bravo & Chagas 2004; Quate & Brown 2004; Bravo et al. 2008, 2020; Chagas-Vieira 2012; Santos & Curler 2014; Jaume-Schinkel 2022).

The present work describes a new species from male individuals collected in Ecuador, and the first DNA barcodes (*COI* gene) for this genus are provided. Moreover, an updated identification key to the known males of *Tonnoira* species of the world is given.

Material and methods

Study area. Cantón Pedro Vicente Maldonado is located in Pichincha Province in the northeastern part of Ecuador (0.1667N, -79.0000E), with an average altitude of 600 m. a.s.l. Pedro Vicente Maldonado experiences a tropical climate characterized by warm to hot temperatures throughout the year. Average highs range from 25 °C to 30 °C, while average lows range from 18 °C to 22 °C. The wet season typically extends from December to May, with the highest rainfall occurring from January to April and an average annual precipitation of 4,341 millimeters. The region includes lowland rainforests, cloud forests, and the foothills of the Andes, but the main vegetation is pre-mountain rainforest (HPPC 2015).

General morphology follows Cumming & Wood (2017) and Kvifte & Wagner (2017).

Measurements. The length of the wing was measured from its base, at the start of the costal node, to its apex, while its width was roughly measured with an imaginary line crossing the wing at the apex of vein CuA_2. The width of the head was measured at its widest part, roughly above the insertion of the antennal scape, and its length was measured from the vertex to the lower margin of the clypeus. Palpal proportions are provided using the first segment's length as a unit of measurement (1.0).

All examined material was collected using double Malaise traps with 96% ethanol as a killing and preserving medium, and temporarily stored in 96% ethanol for DNA extraction. Whole specimens were used for DNA extraction and processed at Museum Koenig (ZFMK; previously known as Zoologisches Forschungsmuseum Alexander Koenig) in Bonn (Germany). Lysis and PCR were performed at ZFMK following the protocol by Astrin & Stüben (2008) and the primers from Folmer et al. (1994). After the PCR, samples were sent to BGI Group (formerly Beijing Genomics Institute) for bidirectional sequencing. Raw data were curated manually using Geneious (v. 7.1.9). Final *COI* sequences were 658 bp long. All sequences are publicly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and/or BOLD (https://www.boldsystems.org).

After DNA extraction, whole specimens were put back into 96% ethanol and then further dehydrated in absolute ethanol (100%) for five minutes, transferred to clove oil for 10 minutes, and mounted on microscope slides using Euparal.

The material examined for this study is deposited in the following natural history institutions, referred to in the text with the following acronyms:

INABIO: Instituto Nacional de Biodiversidad, Quito, Ecuador.
ZFMK: Museum Koenig, Bonn, Germany.

In the material examined section and at the end of each record, the holding institution is indicated in square brackets ([]). In the description of type labels, the contents of each label are enclosed in double quotation marks (“ ”), and a double forward slash (//) separates the individual lines of data.

Taxonomic account

Genus *Tonnoira* Enderlein, 1937

Type species: *Tonnoira pelliticornis* Enderlein, 1937: 106; type locality: Peru, Callanga.

Important references: Enderlein (1937: 106; original description); Quate (1963: 189; diagnosis); Quate (1996: 33; revised description); Quate & Brown (2004: 25; revised description); Santos & Curler (2014: 464; updated diagnosis); Bravo et al. (2020: 4; species list; identification key); Jaume-Schinkel (2022: 2–3; updated distribution map and species list).

To date, there are 28 described species in the genus, including the herein newly described species.

Tonnoira chuki **sp. n.**
(Figs. 1–2)

Differential diagnosis

The aedeagal shape of *Tonnoira chuki* **sp. n.** resembles those of *Tonnoira rapiformis* Quate & Brown, 2004 and *T. bitenacula* Quate, 1996, but both these species can be separated from *Tonnoira chuki* **sp. n.** by the number of tenacula present on the epandrial appendages (surstyli): one in *Tonnoira chuki* **sp. n.** and two in *T. rapiformis* and *T. bitenacula*. Furthermore, *Tonnoira chuki* **sp. n.** can be differentiated from *T. castanea* Quate & Brown, 2004 by the shape of the aedeagus (symmetrical and spear-shaped in *Tonnoira chuki* **sp. n.**, asymmetrical and not spear-shaped in *T. castanea*).

Type locality

Ecuador, Pichincha Prov., Parroquia Pedro Vicente Maldonado.

Type material

Holotype: “Ecuador, Pichincha Prov. // Parroquia Pedro Vicente // Maldonado, roadway to // Pachijal. // 0.11561100000000001, // -78.958053599999996. 750 m. // 1-9. February.2022 // ZFMK-TIS-2637090 // Leg. Kilian, Isabel” “SJS-00811” “ZFMK-DIP-00097120” “Psychodidae // Tonnoira // chuki” “HOLOTYPE [red]” [INABIO].

Paratypes: 2 ♂♂, same label information except: SJS-00824, ZFMK-DIP-00097121; SJS-00837, ZFMK-DIP-00097122 [INABIO]; 4 ♂♂, same label information except SJS-00838, ZFMK-DIP-00097123; SJS-00847, ZFMK-DIP-00097124; SJS-00852, ZFMK-DIP-00097125; SJS-881, ZFMK-DIP-00097126 [ZFMK]; 1 ♂, “Ecuador, Pichincha Prov., // Parroquia Pedro Vicente // Maldonado, 0.118626, -78.95802240, 770m. // 25-28.January.2020. // Leg. Kilian, Isabel.” “ZFMK-DIP-00081674” “Psychodidae // Tonnoira // chuki // det. Jaume-Schinkel, Santiago” “PARATYPE [yellow]” [ZFMK]; 1 ♂, same label information except “ZFMK-DIP-00081650” [ZFMK].

Description

Male. Measurements in mm (n=5) Wing length 2.20 (2.3–2.1), width 1.05 (1.1–0.9); head length 0.52 (0.56–0.50), width 0.52 (0.55–0.49); antennal segments: scape 0.12 (0.13–0.18), pedicel 0.07 (0.09–0.05), flagellomeres 1–5: 0.22 (0.25–0.20); palpomere 1: 0.08 (0.1–0.07), palpomere 2: 0.18 (0.20–0.17), palpomere 3: 0.18 (0.20–0.18), palpomere 4: 0.17 (0.19–0.16).

Head. Slightly longer than wide; eye bridge separated by less than one eye facet diameter, with four rows of facets, interocular suture as an inverted “Y”; frontal patch of alveoli almost divided in two but joined in the middle, upper margin M-shaped, lower margin rounded, with a concavity in the middle. Antennal scape about two times the length of the pedicel, almost cylindrical; pedicel spherical; flagellomeres cylindrical, at least four times longer than wide, with scattered setae on the surface, apical flagellomeres absent in the examined material, maximum number of flagellomeres present five; ascoids indistinguishable in the examined material. Palpal segments cylindrical, palpal proportions: 1.0:2.0:2.0:1.9; labium without any strong sclerite; labella bulbous, with seven setae scattered between the middle and the apical margin.

Thorax without allurement organs, with a single patch of alveoli on the paratergite and anteprontum; all coxae with a stripe of three to five rows of alveoli. Wing length about two times its width; wing membrane brown-infuscated; subcostal vein short, ending at level of origin of R_4; junction of R_{2+3} basal to M_{1+2}, stem of R_{2+3} very short; R_5 ending slightly below the wing apex; CuA_2 ending at wing margin.

Terminalia (Figs. 1D, E, 2A–C). Hypandrium a distinct band connecting the gonocoxites, narrow and arch-like; gonocoxites about the same length as gonostyli; gonostyli lightly incurved, with a sudden lateral and digitiform narrowing as in Fig. 2A, B, covered with scattered alveoli; ejaculatory apodeme shorter than aedeagus, rounded; aedeagus spear-tip-shaped, formed of two triangular and elongated phallomeres, joined at the apex; aedeagus with two digitiform parameres, parameres narrowing towards apex, resembling an inverted V; below the aedeagal complex is a triangular, semi-sclerotized structure that resembles an aedeagal sheath, here considered to be the subepandrial sclerite; epandrium about two times wider than long; hypoproct triangular, longer than epandrium and covered in small setulae; epiproct shorter than hypoproct; epandrial appendages (surstyli) conical, tapering towards the apex and curved dorsally, each with one apical tenaculum, tenacula with rounded apex.

Female. Unknown.

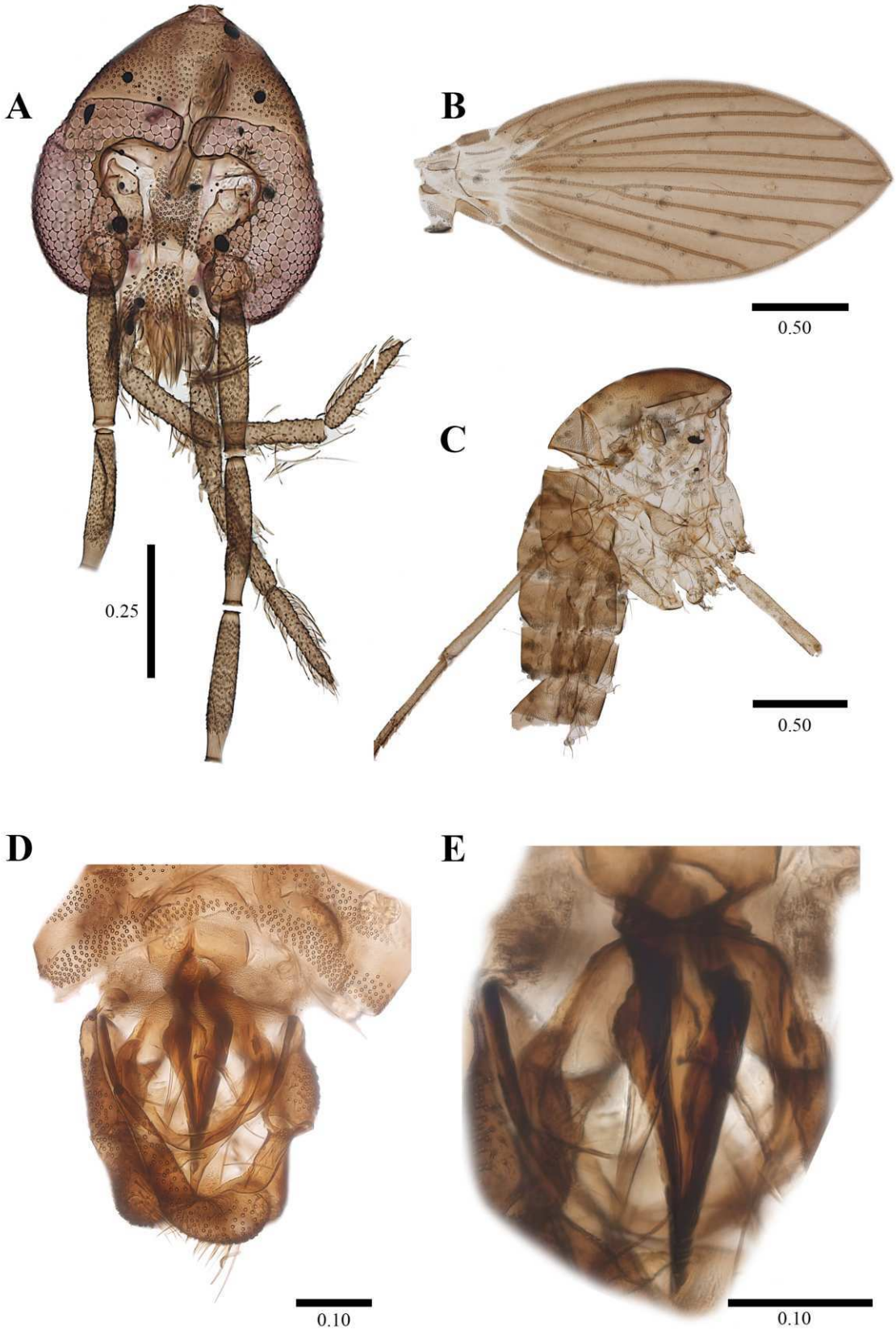

Fig. 1. *Tonnoira chuki* **sp. n.**, male holotype. **A**. Head. **B**. Wing. **C**. Thorax and abdomen. **D**. Genitalia. **E**. Aedeagus. Scales in mm.

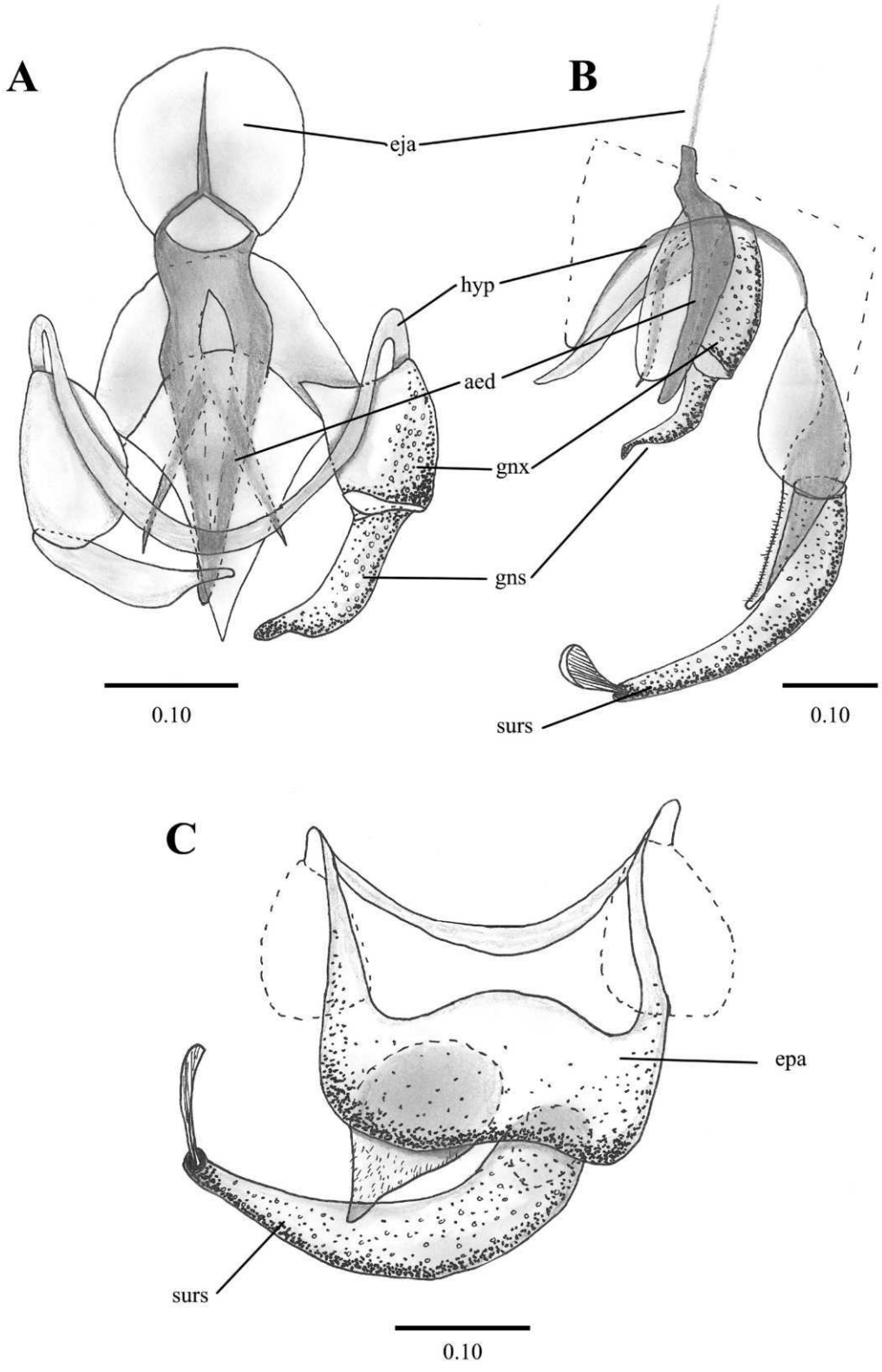

Fig. 2. *Tonnoira chuki* **sp. n.**, male paratype. **A**. Dorsal view of aedeagus, gonocoxites and gonostyli. **B**. Lateral view of terminalia. **C**. Ventral view of epiproct, hypoproct, epandrium, and surstylus. Abbreviations: aed: aedeagus, eja: ejaculatory apodeme, epa: epandrium, gns: gonostylus, gnx: gonocoxites, hyp: hypandrium, surs: epandrial appendage (surstylus). Scales in mm.

Etymology

The specific epithet is derived from the Quechuan word "chuki" meaning spear, in reference to the spear-shaped aedeagus.

Distribution

Only known from the type locality in Ecuador.

Genetics

Nine specimens were successfully sequenced: ZFMK-DIP-00097120, ZFMK-DIP-00097121, ZFMK-DIP-00097122, ZFMK-DIP-00097123, ZFMK-DIP-00097124, ZFMK-DIP-00097125, ZFMK-DIP-00097126, ZFMK-DIP-00081674, and ZFMK-DIP-00081650. The maximum intraspecific uncorrected pairwise distance between sequences was 1.82% or 12 bp. GenBank accession numbers are: ZFMK-DIP-00097120 (ZFMK-TIS-2637090): OQ685791; ZFMK-DIP-00097121 (ZFMK-TIS-2637103): OQ685797; ZFMK-DIP-00097122 (ZFMK-TIS-2637116): OQ685794; ZFMK-DIP-00097123 (ZFMK-TIS-2637117): OQ685792; ZFMK-DIP-00097124 (ZFMK-TIS-2637126): OQ685795; ZFMK-DIP-00097125 (ZFMK-TIS-2637131): OQ685793; ZFMK-DIP-00097126 (ZFMK-TIS-2637160): OQ685796; BOLD sequence accession numbers are: ZFMK-DIP-00081674 (ZFMK-TIS-2629869): GDIP21965-23; ZFMK-DIP-00081650 (ZFMK-TIS-2629888): GDIP21976-23.

Key to known males of extant species of *Tonnoira*

[modified from Bravo et al. (2020) and Jaume-Schinkel (2022)]

1 Gonostylus simple, not bifurcated **9**
- Gonostylus bifurcated **2**

2 Gonostylus with lateral and mesal branches of the same length; branches crossed (see Santos & Curler 2014: fig. 4) ..... ***T. brisolaii*** Santos & Curler, 2014
- Gonostylus with lateral and mesal branches of different lengths; branches subparallel or divergent **3**

3 Lateral branch of gonostylus shorter than mesal branch; mesal branch of gonostylus longer than gonocoxite (see Bravo & Chagas 2004: fig. 8) **6**
- Lateral branch of gonostylus longer than mesal branch; mesal branch of gonostylus shorter than gonocoxite (see Santos & Curler 2014: fig. 12) **4**

4 Eye bridge with 5 facet rows; epandrial appendages (surstyli) with one tenaculum ..... ***T. protuberata*** Quate & Brown, 2004
- Eye bridge with 4 facet rows; epandrial appendages (surstyli) with two tenacula **5**

5 Eyes separated by 0.5 facet diameter; parameres symmetrical (see Santos & Curler 2014: fig. 12) ..... ***T. andradei*** Santos & Curler, 2014
- Eyes separated by almost 1.0 facet diameter; parameres asymmetrical (see Bravo et al. 2020: fig. 9) ..... ***T. igrapiunensis*** Bravo, Vilarinho & Chagas, 2004

6 Lateral branch of gonostylus very short, 0.2 times the length of mesal branch (see Santos & Curler 2014: fig. 8) ..... ***T. ferreirai*** Santos, 2014
- Lateral branch of gonostylus 0.4–0.6 times the length of mesal branch (see Bravo & Chagas 2004: fig. 8) **7**

7 Aedeagus bipartite, basally U-shaped, with one of the branches longer than the other (see Quate & Brown 2004: fig. 69 a) ..... ***T. bifurcata*** Quate & Brown, 2004
- Aedeagus bipartite, with neither of the branches basally U-shaped, or tripartite **8**

8 Lateral and mesal branches of gonostylus with the same degree of sclerotization; hypandrium narrower than gonocoxite, stripe-like (see Quate & Brown 2004: fig. 70) ..... ***T. didyma*** Quate & Brown, 2004
- Lateral branch of gonostylus appearing more sclerotized than mesal branch; hypandrium wider than gonostylus (see Bravo & Chagas 2004: fig. 6) ..... ***T. bifida*** Bravo & Chagas, 2004

9 Epandrial appendages (surstyli) with 2 or 3 tenacula **14**
- Epandrial appendages (surstyli) with 1 tenaculum **10**

10 Base of wing vein R_3 with a cluster of black "granules" (see Quate & Brown 2004: fig. 63) ..... ***T. psacadoptera*** Quate & Brown, 2004
- Base of wing vein R_3 normal, without a cluster of black "granules" **11**

11 Hypandrium broad, of same length as width of gonostylus; gonocoxite 0.8 times as long as gonostylus (see Chagas-Vieira 2012: fig. 16) ..... ***T. tripenis*** Chagas-Vieira, 2012
- Hypandrium narrow, stripe-like, shorter than width of gonostylus; gonocoxite about as long as gonostylus **12**

12 Aedeagus tripartite with a short spur/like branch, a sickle-shaped lateral branch, and a broad, twisted and paddle-shaped central branch (see Quate & Brown 2004: fig. 68) ..... ***T. sicilis*** Quate & Brown, 2004
- Aedeagus bipartite, branches may be joined at apex **13**

13 Aedeagus bipartite, one branch with basal three-quarters broad and asymmetrically narrowing at distal one-quarter, other branch digitiform, blunt, about half as long as other branch (see Quate & Brown 2004: fig. 67a) ..... ***T. castanea*** Quate & Brown, 2004
- Aedeagus with two triangular sclerites joining towards the apex, resembling the tip of a spear (Figs. 1D, E, 2A), with two additional spine-like parameres ..... ***T. chuki* sp. n.**

14 Epandrial appendages (surstyli) with 2 tenacula **16**
- Epandrial appendages (surstyli) with 3 tenacula **15**

15 Aedeagus ending at same level as apex of gonostylus; ejaculatory apodeme shorter than gonostylus (see Wagner 1981: fig. 3) ..... ***T. mirabilis*** Wagner, 1981
- Aedeagus ending beyond apex of gonostylus; ejaculatory apodeme longer than gonostylus (see Chagas-Vieira 2012: fig. 12) ..... ***T. spina*** Chagas-Vieira, 2012

16 Aedeagus symmetrical or asymmetrical, long, subcylindrical, pointed or rounded at apex; parameres present **18**
- Aedeagus symmetrical, base broad, apex sagittate; parameres absent (see Quate 1996: fig. 13 b; Quate & Brown 2004: fig. 71) **17**

17 Hypandrium present as a single stripe-like structure; gonocoxites as wide as long (see Quate 1996: fig. 13 b) ..... ***T. bitenacula*** Quate, 1996
- Hypandrium vestigial, sclerotization interrupted and broken in the middle; gonocoxites longer than wide (see Quate & Brown 2004: fig. 71) ... ***T. rapiformis*** Quate & Brown, 2004

18 Aedeagus ending far beyond apex of gonostylus (see Bravo & Chagas 2004: fig. 1) ..... ***T. longipennis*** Bravo & Chagas, 2004
- Aedeagus ending near or just beyond apex of gonocoxites, never beyond apex of gonostylus **19**

19 Aedeagus sickle-shaped; ejaculatory apodeme triangular and shorter than aedeagus (see Quate & Brown 2004: fig. 77) ..... ***T. rectilata*** Quate, 1999
- Aedeagus straight or slightly curved; ejaculatory apodeme sub-oval, longer than wide, of same length or longer than aedeagus **20**

20 First flagellomere cylindrical **24**
- First flagellomere fusiform **21**

21 Gonocoxal apodeme expanded posteriorly, with a pair of slender, acute processes flanking the aedeagus and extending nearly to tip of aedeagus (see Quate 1999: fig. 5k)..... ..... ***T. bitalea*** Quate, 1999
- Gonocoxal apodeme not expanded posteriorly, without processes flanking the aedeagus **22**

22 Hypandrium wide, posteriorly bilobed (see Bravo & Chagas 2004: fig. 23) ..... ***T. magna*** Bravo & Chagas, 2004
- Hypandrium narrow, posteriorly without lobes **23**

23 Eyes separated by 0.5 facet diameters; gonostyli shorter than gonocoxites; ejaculatory apodeme shorter than gonocoxites (see Bravo et al. 2008: figs. 11, 16)..... ..... ***T. robusta*** Bravo, Alves & Chagas, 2008
- Eyes separated by 0.2 facet diameters; gonostyli longer than gonocoxites; ejaculatory apodeme longer than gonocoxites; hypandrium with triangular projections at posterolateral margin; aedeagus not bipartite, with two parameres (see Santos & Curler 2014: fig. 16) .... ***T. galatiae*** Santos & Curler, 2014

24 Aedeagus bipartite, with one branch abruptly narrowing to acute apex, other branch digitiform, evenly narrowing towards the apex; aedeagus without parameres (see Quate & Brown 2004: fig. 74) .... ***T. fusiformis*** Quate & Brown, 2004
- Aedeagus not bipartite, evenly narrowing to an acute apex... **25**

25 Eyes separated by one facet diameter; aedeagus extending to middle of gonostyli, with two parameres ending at the same level as apex of aedeagus (see Bravo et al. 2008: fig. 6)..... ..... ***T. distincta*** Bravo, Alves & Chagas, 2008
- Eyes separated by less than one facet diameter; aedeagus extending to apex of gonocoxite or more, but never close to middle of gonostyli, one or two parameres present..... **26**

26 Aedeagus extending a little beyond apex of gonocoxite; gonostyli digitiform, tapering towards apex, incurved; aedeagus with one paramere (see Quate & Brown 2004: fig. 72) .... ***T. cavernicola*** Quate & Brown, 2004
- Aedeagus extending to apex of gonocoxites; gonostyli conical, straight; aedeagus with two parameres (see Jaume-Schinkel 2022: figs. 2c, 3) .... ***T. conistylus*** Jaume-Schinkel, 2022

Discussion

The distribution of *Tonnoira* is restricted to the Neotropical Region, ranging from Nicaragua to Brazil (Bravo et al. 2008, 2020; Santos & Curler 2014; Jaume-Schinkel 2022). Currently, Brazil has the highest recorded diversity with 18 species, followed by Suriname with five species, and Ecuador, now with four species. This discrepancy in the numbers of recorded and/or described species throughout the Neotropics highlights the lack of taxonomic surveys in this biogeographic realm, and there is no doubt that new species are still waiting to be described.

The general biology and larval stages of *Tonnoira* remain greatly understudied. Three species have been found to be associated with caves (Bravo et al. 2020; Jaume-Schinkel 2022); nonetheless, for the majority of species, the microhabitat preferences and life cycle remain unknown.

Tonnoira is placed in the tribe Maruinini (see Kvifte 2018). To date, the only publicly available *COI* barcodes of other Maruinini genera belong to *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 and *Platyplastinx ibanezbernali* Jaume-Schinkel & Kvifte, 2022 (Jaume-Schinkel et al. 2022; Jaume-Schinkel & Kvifte 2022). The maximum intraspecific uncorrected pairwise distance for *COI* sequences in *Alepia viatrix* is 5.71%, while specimens of *Platyplastinx ibanezbernali* present a maximum intraspecific uncorrected pairwise distance of 1.35%; therefore, the intraspecific distance of 1.82% in *Tonnoira chuki* **sp. n.** does not differ greatly from the intraspecific uncorrected pairwise distances reported for related genera. Nonetheless, further DNA barcodes from different genera and species are required to properly assess the intraspecific and interspecific uncorrected pairwise distances for the tribe.

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Chapter 13 – Publication

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A revision of the genus *Armillipora* Quate (Diptera: Psychodidae) with the descriptions of two new species

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Abstract. The genus *Armillipora* Quate is recorded for the first time in Ecuador, with a new geographical record for *Armillipora selvica* Quate, 1996 and the descriptions of two new species, namely *Armillipora muyu* sp. nov. and *Armillipora imitata* sp. nov., doubling the total number of species in the genus. In addition, we make available the first DNA barcodes for the genus, providing a sequence of the 5′-end of the cytochrome *c* oxidase subunit I (COI) gene for *A. imitata*, *A. muyu*, and, *A. selvica.* Moreover, we describe the second known female of the genus and we provide a taxonomical key for the known males of the world. Finally, we build Species Distribution Models and discuss the potential distribution of the genus in the Neotropical region.

Keywords. Moth flies, DNA barcode, integrative taxonomy, Neotropical region, new species.

Jaume-Schinkel S. & Mengual X. 2024. A revision of the genus *Armillipora* Quate (Diptera: Psychodidae) with the descriptions of two new species. *European Journal of Taxonomy* 925: 161–178.
https://doi.org/10.5852/ejt.2024.925.2459

Introduction

The moth fly genus *Armillipora* Quate, 1996 (Diptera: Psychodidae) has only been recorded in the Neotropical region (Quate 1996, 1999; Ježek *et al.* 2020). For many years a single species was known, namely *Armillipora selvica* Quate, 1996. This species was described from Costa Rica (Quate 1996) and recorded in Panama three years later (Quate 1999). More recently, Ježek *et al.* (2020) described a second species from Bolivia, *Armillipora suapiensis* Ježek, Oboňa & Le Pont, 2020, breaking the monotypy of the genus and adding a new geographical record to *A. selvica* from Nicaragua. Up to now, *A. selvica* has been recorded from Central America (Nicaragua, Costa Rica, Panama) and *A. suapiensis* is known only from Bolivia. Besides its scattered known distribution, we do not have information about the larval stages or the adult biology of this genus.

In the present study, we report for the first time the genus *Armillipora* from Ecuador, and we describe two new species based on morphological and molecular characters, bringing the total number of

species of *Armillipora* to four. We also describe the second known female of this genus, which belongs to *Armillipora muyu* sp. nov., and provide a new geographical record for *Armillipora selvica* from Ecuador. Moreover, we make available the first DNA barcodes (the sequences of the 5′-end of the cytochrome c oxidase subunit I or COI gene) for *A. selvica*, *A. imitata* sp. nov., and *A. muyu* and provide an identification key for the world males of *Armillipora*. Finally, we discuss the potential distribution of the genus in the Neotropical region based on our species distribution model.

Material and methods

Study area

The Cantón Pedro Vicente Maldonado is located in the Pichincha Province in the northern part of Ecuador, (0°10′00″ N, 79°00′00″ W) with an average altitude of 600 m a.s.l. The climate is warm-humid with an average annual temperature of 24.5°C and an annual precipitation of 4341 mm. The main vegetation is characterized as pre-mountain rainforest (HPPC 2015).

Terminology

We follow the general terminology proposed by Cumming & Wood (2017) and Kvifte & Wagner (2017).

Collection and preparation of specimens

Specimens were collected using a Malaise trap, euthanized and preserved in 96% ethanol, and later stored at -20°C. Specimen preparation was done following the protocol explained by Jaume-Schinkel & Kvifte (2022), with the modification of using the whole specimen for DNA extraction instead of just the thorax.

In the material examined section and at the end of each record the holding institution is stated, and between square brackets ([ ]) the number of the specimen is indicated. The abbreviations used for holding institutions and their equivalents are given below:

INABIO = Instituto Nacional de Biodiversidad, Quito, Ecuador.
LACM = Natural History Museum of Los Angeles County, Los Angeles, California, USA.
ZFMK = Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels (previously known as Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany.

Genetics

A non-destructive methodology from complete specimens was performed in the facilities of ZFMK with the following workflow: A Qiagen (Hilden, Germany) BioSprint 96 magnetic bead extractor, and the corresponding kits were used following the manufacturers' specifications. We amplified from the 5′-end of the cytochrome c oxidase subunit I (COI) gene using the primers HCO2198-JJ (forward) and LCO1490-JJ (reverse) (Astrin & Stüben 2008). PCR was carried out using a TouchDown PCR (TD-PCR) as proposed by Korbie & Mattick (2008) using a QIAGEN Multiplex PCR Kit. Later the PCR products were shipped to Beijing Genomic Institute (BGI) (China, Hong Kong) for bidirectional sequencing. DNA sequences were assembled, aligned, and cleaned using Geneious Prime ver. 2022.1.1 (Biomatters, Auckland, New Zealand). The total sequence length was set to 658 bp.

We downloaded all the available sequences of the tribe Maruinini from BOLD and we used Geneious Prime ver. 2023 to perform a distance-based neighbor-joining (NJ) analysis using the Jukes-Cantor model. All of the sequences generated for this study can be accessed in BOLD under the Dataset DS-ARMI (available at: https://doi.org/10.5883/DS-ARMI). Bootstrap support (BS) values were estimated from 1000 replicates as calculated in Geneious.

Species Distribution Models

We built a distribution model for the genus using the software MaxEnt ver. 3.4.4 (Phillips *et al.* 2023) with the species' geographical records to infer the potential distribution in the Americas. In MaxEnt ver. 3.4.4 we used all the records of the genus as a single biological entity to evaluate the distribution model for the genus, instead of using each species separately. The resulting map was trimmed to North America, Central America, and South America. Climate variables were obtained from WorldClim (Fick & Hijmans 2017). Geographic coordinates used for the analysis were extracted from the localities reported in the literature and museum collection databases. Localities are summarized in Table 1. For the records without exact coordinates in the literature, we used Google® Earth to search for the reported locality and obtain proxy coordinates and compared them with the coordinates in the collection data base. After comparison, if coordinates did not match, we adjusted them to have at most a difference of ± 50 meters between both.

Results

Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Suborder Psychodomorpha Hennig, 1968
Family Psychodidae Newman, 1834
Subfamily Psychodinae Newman, 1834

Genus ***Armillipora*** Quate, 1996

Armillipora Quate, 1996: 29. Type species *Armillipora selvica* Quate, 1996 (by original designation).

Armillipora – Jezek *et al.* 2020: 418 (updated diagnosis, redescription of type species, and description of new species).

Differential diagnosis

The genus *Armillipora* has been placed in the tribe Maruinini Enderlein, 1937 based on the presence in the wing of a radial fork being basal to the medial fork and both forks located basally on the wing, as well as the broad and dorsally flattened shape of the ejaculatory apodeme (Quate 1996; Kvifte 2018; Ježek *et al.* 2020). At first glance, species of *Armillipora* resemble those of *Alepia* Enderlein, 1937 and *Platyplastinx* Enderlein, 1937 mainly by the wing maculation and the presence of two different types of tenacula on the epandrial appendage (see Quate 1996: fig. 11; Ježek *et al.* 2020: figs 1–20; Jaume-Schinkel *et al*. 2022: figs 1–15; Jaume-Schinkel & Kvifte 2022: figs 1–16). But species of *Armillipora* can be easily differentiated using characteristics of the male genitalia as follows: the characteristic shape of the irregularly-asymmetrical epandrial appendage (not irregularly asymmetrical in *Alepia* and *Platyplastinx*), the long accessory tenacula (long in *Alepia,* but short in *Platyplastinx*) with a group of short cylindrical tip-folded tenacula (not present in *Alepia* and *Platyplastinx*), with the absence of apical tenacula (usually none, one or more apical tenacula in *Alepia* and *Platyplastinx*), the lack of gonostyli and the gonocoxites fused in *Armillipora* (gonostyli present and gonocoxites usually not fused in *Alepia* and *Platyplastinx*), and the absence of the aedeagal sheath (present in *Alepia*, but absent in *Platyplastinx*) (see Ježek *et al.* 2020). Females of *Armillipora* can be differentiated of those of *Alepia* and *Platyplastinx* by the following characters: antennal flagellomeres with double circle of teardrop-shaped pores in the center, although less conspicuous than males they are present in *Armillipora* (absent in *Alepia* and *Platyplastinx*); *Armillipora* with the subgenital plate longer than wide (subgenital plate length variable in *Alepia*, usually about the same length as its width in *Platyplastinx*), with apical lobes separated by a broad concavity (concavity is broader than twice the length of the apical lobe) with a pair of long spines

on apical margin of concavity (apical lobes not separated by broad concavity (concavity being less than the length of apical lobe) and pair of long spines on apical margin of concavity absent in *Alepia* and *Platyplastinx*).

Biology

To date, nothing is known about the immature stages and the biology of the species of *Armillipora*. Given the known information about the tribe Maruinini, it is expected that larvae of *Armillipora* breed in some aquatic or semi-aquatic environment.

Species included

Armillipora imitata sp. nov., *A. muyu* sp. nov., *A. selvica* Quate, 1996, *A. suapiensis* Ježek, Oboňa & Le Pont 2020. Species distribution is shown in Table 1.

Armillipora imitata sp. nov.

urn:lsid:zoobank.org:act:41F1459A-4F5D-41EF-B205-BE0EB30AC37E

Figs 1–2

Differential diagnosis

Armillipora imitata sp. nov. is very similar to *A. muyu* sp. nov. and *A. selvica* but the three can be differentiated as follows: in *A. selvica* the interocular suture has a short posterior spur (posterior spur absent in *A. imitata* and in *A. muyu*); one conical apical and one spiniform tooth at the apex of labella in *A. imitata* (only one conical apical teeth in *A. selvica*; one preapical spiniform and one apical claw-shaped in *A. muyu*); *A. imitata* has six apical setae at the apex of gonocoxites (three to four setae placed on a preapical lump in *A. muyu*, and three to four preapical setae in *A. selvica*); the gonocoxal condyles is not triangular and not protruding beyond the base of the ejaculatory apodeme in *A. imitata* and *A. muyu* (the sclerite is triangular and protruding beyond the base of ejaculatory apodeme in *A. selvica*).

Etymology

The species epithet '*imitata*' derives from the Latin word '*imitātus*' (feminine '*imitāta*') referring to its similarity with other species. It is to be treated as an adjective.

Material examined

Holotype

ECUADOR – **Pichincha** • ♂; Parroquia Pedro Vicente Maldonado, Roadway to Pachijal; 0.11561° N, 78.95805° W; alt. 750 m; 1–9 Feb. 2022; Kilian and Isabel leg.; INABIO [ZFMK-DIP-00097935, ZFMK-TIS-2637091].

Paratypes

ECUADOR • 1 ♂; same collection data as for holotype; ZFMK [ZFMK-DIP-00097934, ZFMK-TIS-2637173] • 1 ♂; same collection data as for holotype; ZFMK [ZFMK-DIP-00097931, ZFMK-TIS-2637132].

Description

Measurements in mm (n = 3). Wing length: 2.19 (2.20–2.18), width: 1.00 (1.05–0.98). Head length: 0.50 (0.52–0.48), width: 0.55 (0.56–0.52). Antennal segments: scape: 0.10 (0.10–0.10); pedicel: 0.06 (0.06–0.06); flagellomeres 1–5: 0.15 (0.15–0.11), flagellomeres 6–10: 0.12 (0.15–0.13). Palpal segment 1: 0.07 (0.07–0.07); palpal segment 2: 0.08 (0.09–0.08); palpal segment 3: 0.08 (0.08–0.08); palpal segment 4: 0.14 (0.15–0.14).

Male

Head. About 1.10 times as wide as long; eye bridge separated by l facet's diameter, with four rows of facets, interocular suture as sclerotized, almost straight line; frontal patch of alveoli divided. Antennal scape about 1.5 times as long as pedicel, almost cylindrical; pedicel spherical, smaller than scape; flagellomeres fusiform and longer than scape, with scattered setae on surface, setae almost as long as flagellomere bearing them, each flagellomere with two rings of teardrop-shaped pores, apical flagellomeres absent in examined material, maximum number of flagellomeres present: five; ascoids indistinguishable in examined material. Palpal segments cylindrical, palpal proportions: 1.0:1.1:1.1:1.8, last palpal segment corrugated; labium without any strong sclerite; labella elongated and irregularly shaped scattered setae on surface, with one apical spiniform tooth on each.

Thorax. Without allurement organs. With single patch of alveoli in praterguite and antepronotum; all coxae with stripe of one to two rows of alveoli. Wing length about two times its width; wing membrane brown-infuscated, with lightened spots in between apex of longitudinal veins, and with light triangular-shaped spot between origin of R_1 and R_{2+3} (Fig. 1B); subcostal vein short ending beyond origin of R_5; junction of R_{2+3} basal to junction of M_{1+2}, not joining R_4, origin of M_{1+2} basal to origin of R_{2+3}; R_5 ending at wing apex; CuA_2 faintly ending at wing margin.

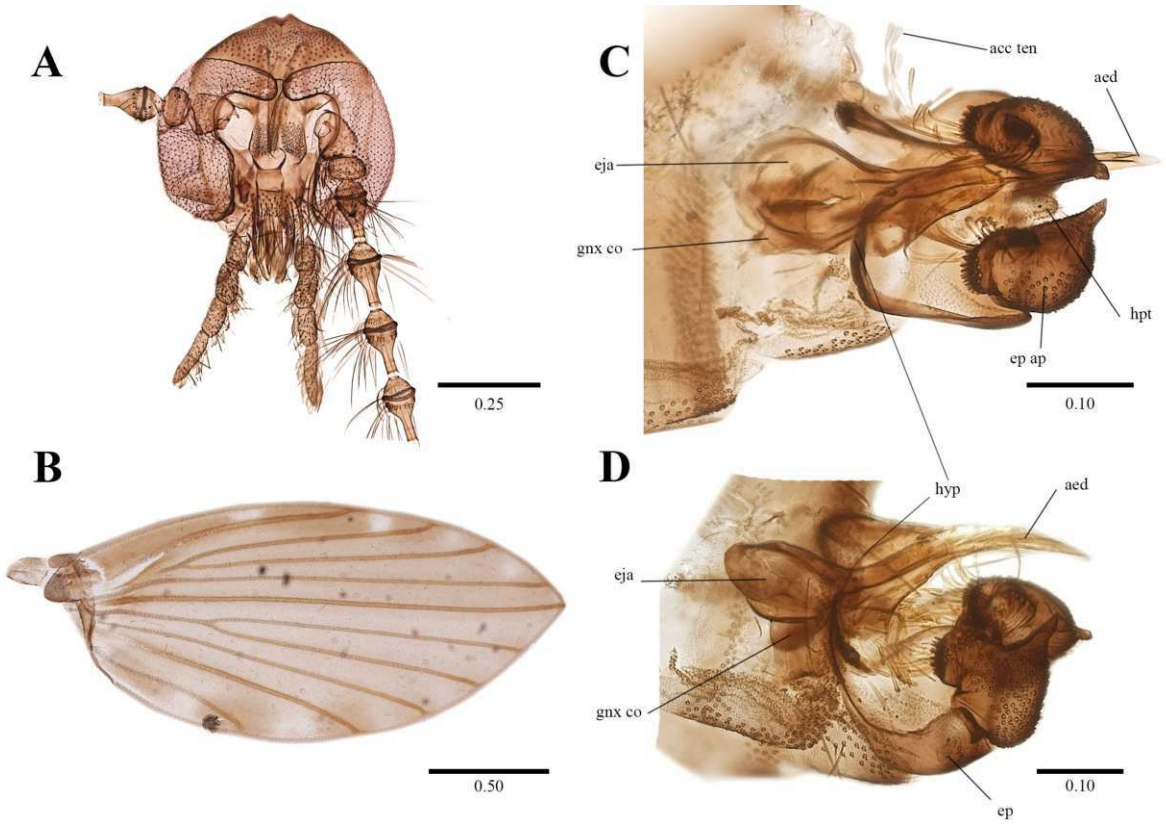

Fig. 1. *Armillipora imitata* sp. nov. **A**–**C**. Holotype, ♂ (ZFMK-DIP-00097935). **D**. Paratype, ♂ (ZFMK-DIP-00097934). **A**. Head. **B**. Wing. **C**. Genitalia in dorsal view. **D**. Genitalia in ventral view. Scale bars in mm. Abbreviations: acc ten = accessory tenacula; aed = aedeagus; eja = ejaculatory apodeme; ep = epandrium; ep ap = epandrial appendages; gnx co = gonocoxal condyles; hpt = hypoproct; hyp = hypandrium.

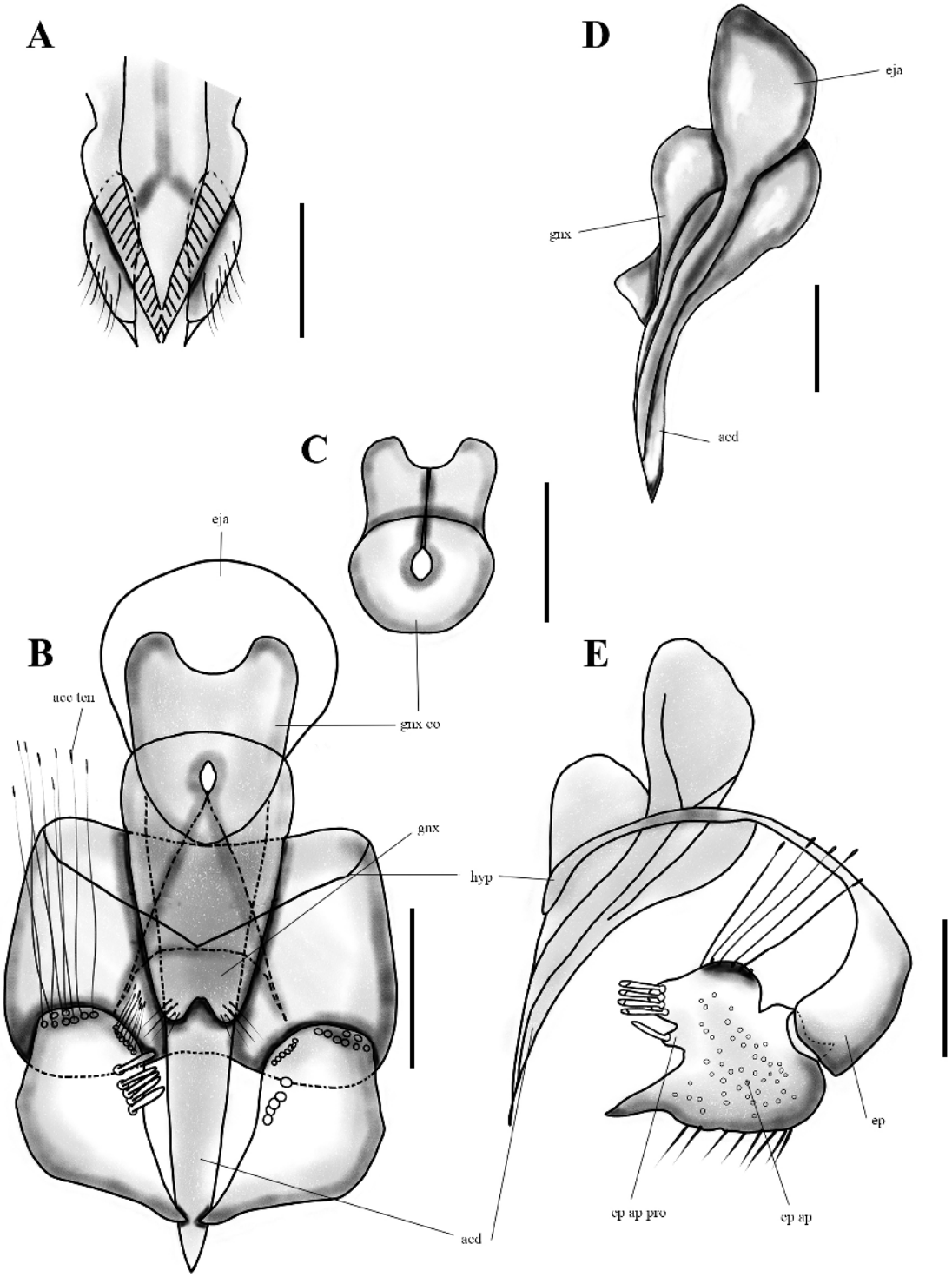

Fig. 2. *Armillipora imitata* sp. nov. **A**–**C**. Holotype, ♂ (ZFMK-DIP-00097935). **D**–**E**. Paratype, ♂ (ZFMK-DIP-00097934). **A**. Mouthparts. **B**. Genitalia in ventral view. **C**. Gonocoxal condyles. **D**. Aedeagus in lateral view. **E**. Genitalia in lateral view. Scale bars = 0.10 mm. Abbreviations: acc ten = accessory tenacula; aed = aedeagus; eja = ejaculatory apodeme; ep = epandrium; ep ap = epandrial appendage; ep ap pro = epandrial appendage projection; gnx co = gonocoxal condyles; hyp = hypandrium.

Terminalia (Figs 1C–D, 2B–E). Hypandrium in dorsal view V-shaped, sclerotized and joining base of gonocoxites, in lateral view hypandrium looks membranous (Figs 1C, 2B, E) with sclerotized margin; gonocoxites joining at apex forming U-shaped sclerite, with concavity at lower margin. Gonocoxite sclerite placed above aedeagal complex. On each side of sclerite is a preapical cluster of six setae (Figs 1C, 2B); gonostyli absent; aedeagus in dorsal view straight, as single sclerite, no discernible parameres. In lateral view, aedeagus apex curved towards epandrial appendage (Figs 1D, 2E); ejaculatory apodeme about half length of aedeagus, in dorsal view basal margin rounded and slightly concave in middle, in lateral view, ejaculatory apodeme looks like half-circle, with basal margin convex; gonocoxal condyles fitting in concavity on underside of ejaculatory apodeme, not triangular-shaped and not protruding beyond base of ejaculatory apodeme; epandrium rectangular, wider than long, with more sclerotization at margins, anterior and posterior margins with medial concavity; hypoproct tongue-shaped (Fig. 1C), shorter than epandrium and covered with small setulae, epiproct not visible in examined material; epandrial appendage barely hemispherical, prolonged and tapering distally, covered with small setae; epandrial appendage lacking apical tenacula. In dorsal view (Figs 1C, 2B), line of five short and cylindrical tenacula, with folded tips; in lateral view, (Figs 1D, 2E) first four tenacula close to each other, last tenaculum separated and located in projection of epandrial appendage, this projection not visible in dorsal view; epandrial appendage possesses additional patch of long accessory tenacula basally concentrated in darkened patch, these accessory tenacula being as long as or longer than epandrium (Figs 1C, 2B).

Female

Unknown.

Distribution

Only known from the type locality in Ecuador.

Genetics

Three specimens were successfully sequenced (ZFMK-TIS-2637091, ZFMK-TIS-2637173, and ZFMK-TIS-00097931). The maximum intraspecific uncorrected pairwise distance for COI sequences was 1.06 % or 7 bp. GenBank accession numbers are: OQ706375; OQ706387; OQ706388.

Armillipora muyu sp. nov.

urn:lsid:zoobank.org:act:F00EDE09-9B2D-4114-971F-6168D54DC6AF

Figs 3–5

Differential diagnosis

Male: see differential diagnosis under *A. imitata* sp. nov.

Etymology

The species epithet ‘muyu’ derives from the Quechuan word ‘*muyu*’, meaning circle and referring to the circular shape of the base of the ejaculatory apodeme. It is to be treated as a name in apposition.

Material examined

Holotype

ECUADOR – **Pichincha** • ♂; Parroquia Pedro Vicente Maldonato, Roadway to Pachijal; 0.11882° N, 78.95802° W; alt. 750 m; 1–9 Feb. 2022; Kilian, Isabel leg.; INABIO [ZFMK-DIP-00081675, ZFMK-TIS-2636967] .

Paratypes

ECUADOR • 1 ♂; same collection data as for holotype; ZFMK [ZFMK-DIP-00081976, ZFMK-TIS-636968] • 1 ♂; same collection data as for holotype; ZFMK [ZFMK-DIP-00081977 ZFMK-TIS-2636969] • 1 ♂; same collection data as for holotype; ZFMK [ZFMK-DIP-00097932, ZFMK-TIS-2637146] • 1 ♀; same collection data as for holotype; ZFMK [ZFMK-DIP-00081836, ZFMK-TIS-2636973] • 1 ♂; same collection data as for holotype; 25–28 Jan. 2020; 0.11561° N, 78.95805° W; ZFMK [ZFMK-DIP-00081975, ZFMK-TIS-2636967] • 1 ♂; same data as for preceding; INABIO [ZFMK-DIP-00081667, ZFMK-TIS-2629905] .

Description

Male

Measurements in mm (n = 5). Wing length 1.85 (2.00–1.65), width 0.84 (0.90–0.71); head length 0.40 (0.45–0.37), width 0.47 (0.52–0.41). Antennal segments: scape: 0.09 (0.10–0.08); pedicel: 0.05 (0.06-0.05); flagellomeres 1: 0.12 (0.12–0.11), flagellomeres 2–5: 0.12 (0.13–0.11). Palpal segment 1: 0.05 (0.06–0.05); palpal segment 2: 0.06 (0.08–0.07); palpal segment 3: 0.04 (0.07–0.06); palpal segment 4: 0.12 (0.13–0.12).

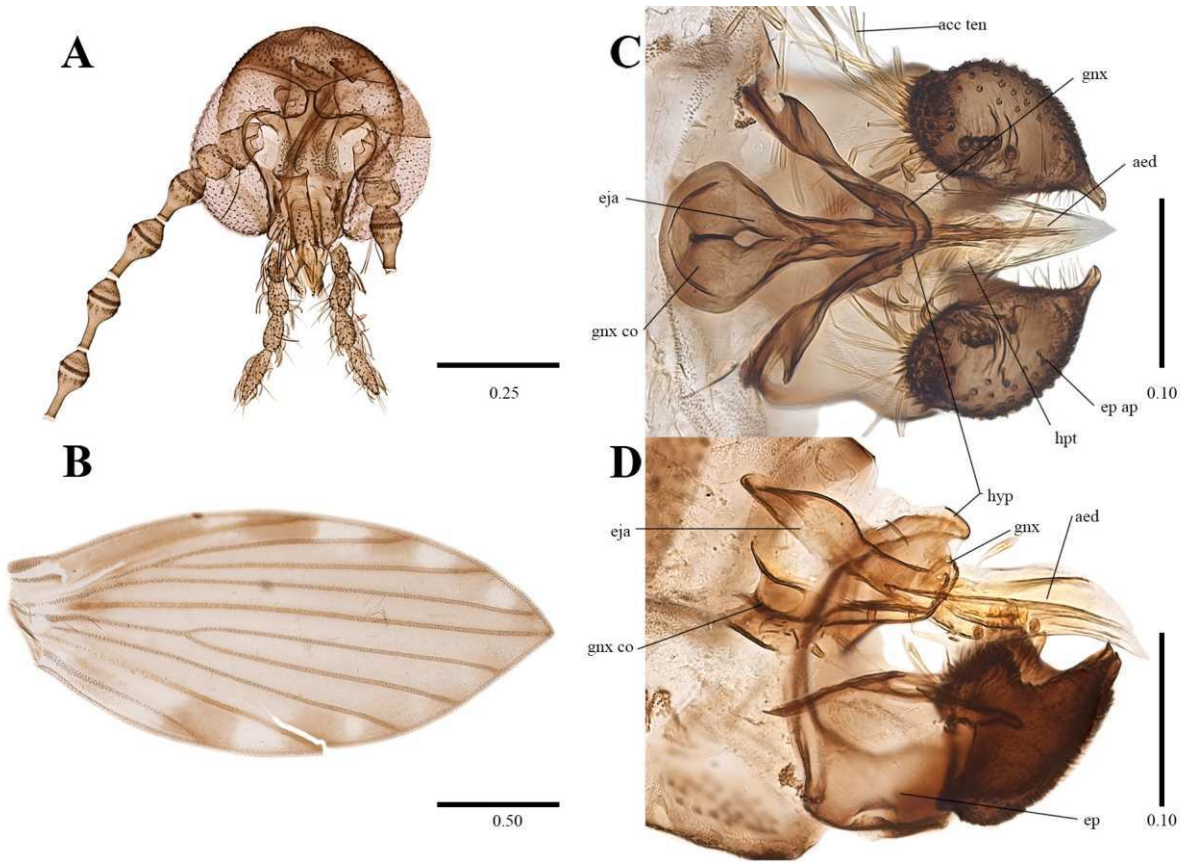

Fig. 3. *Armillipora muyu* sp. nov. **B**, **C**. Holotype, ♂ (ZFMK-DIP-00081675). **A**, **D**. Paratype, ♂ (ZFMK-DIP-00081976). **A**. Head. **B**. Wing. **C**. Genitalia in ventral view. **D**. Genitalia in lateral view. Scale bars in mm. Abbreviations: acc ten = accessory tenacula; aed = aedeagus; eja = ejaculatory apodeme; ep = epandrium; ep ap = epandrial appendage; gnx = gonocoxite; gnx co = gonocoxal condyles; hpt = hypoproct; hyp = hypandrium.

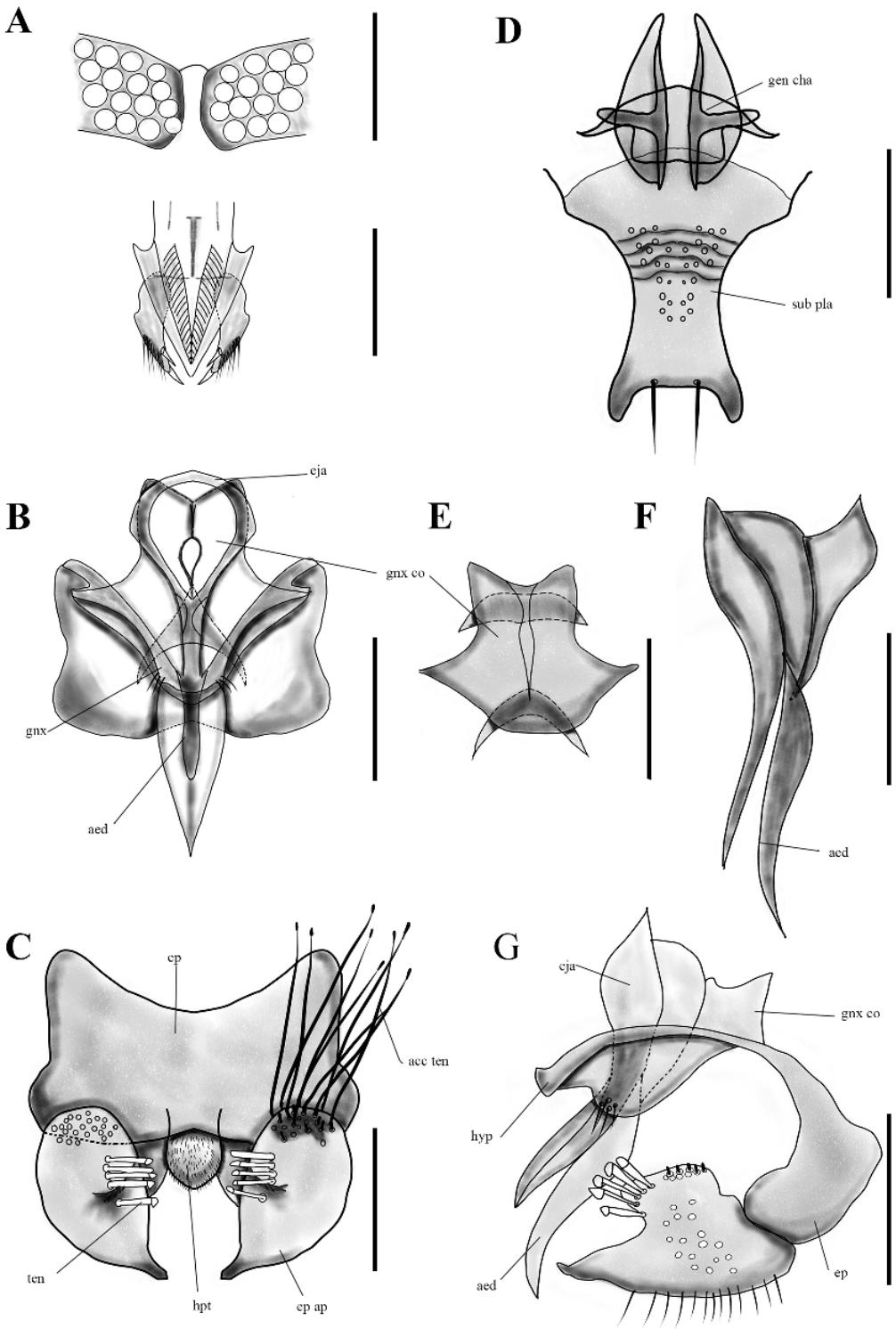

Fig. 4. A–C, E–G: ♂. D: ♀. *Armillipora muyu* sp. nov. **A**. Eye bridge and mouthparts. **B**. Aedeagal complex. **C**. Epandrium and epandrial appendages. **D**. Female genitalia. **E**. Gonocoxal condyles. **F**. Aedeagus in lateral view. **G**. Genitalia in lateral view. Scale bars = 0.10 mm. Abbreviations: acc ten = accessory tenacula; add scl = additional sclerite of ejaculatory apodeme; aed = aedeagus; eja = ejaculatory apodeme; ep = epandrium; gen cha = genital chamber; gnx = gonocoxite; gnx co = gonocoxal condyles; hpt = hypoproct; hyp = hypandrium; sub pla = subgenital plate; ten = tenacula.

Head. About 1.15 times as wide as long; eye bridge separated by one or less than one facet diameters, with four rows of facets, five on broadest part of eye bridge in some specimens; interocular suture as sclerotized, slightly curved line, without posterior spur; frontal patch of alveoli divided. Antennal scape about 1.8 times as long as pedicel, almost cylindrical; pedicel

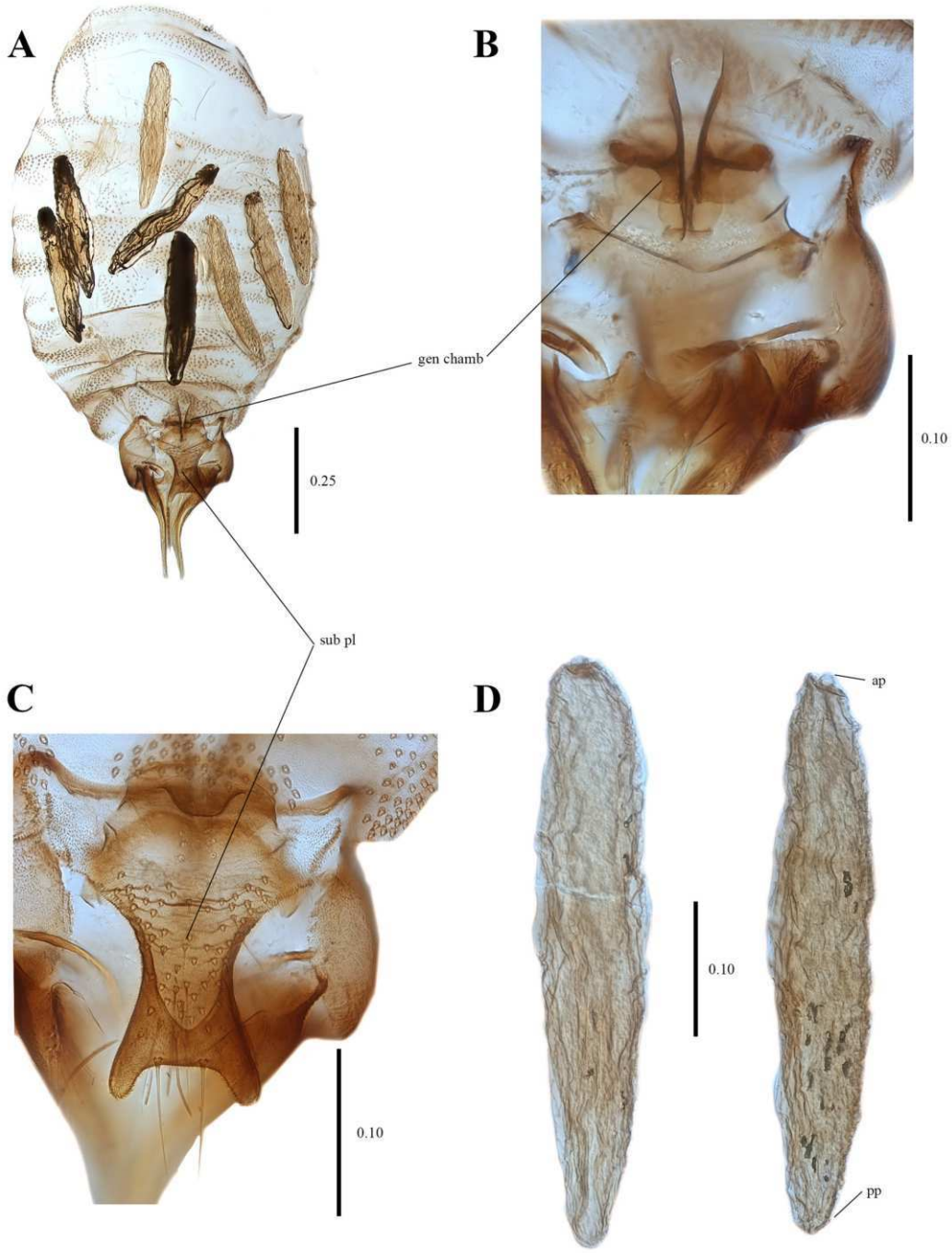

Fig. 5. *Armillipora muyu* sp. nov., paratype, ♀ (ZFMK-TIS-2636973). **A**. Abdomen. **B**. Genital chamber. **C**. Subepandrial plate. **D**. Eggs. Scale bars in mm. Abbreviations: ap = anterior pole; gen chamb = genital chamber; pp = posterior pole; sub pl = subgenital plate.

spherical, smaller than scape; flagellomeres fusiform and longer than scape, with scattered setae on surface, setae almost as long as flagellomere bearing them, each flagellomere with two rings of teardrop-shaped pores, apical flagellomeres absent in examined material, maximum number of flagellomeres present: 5; ascoids indistinguishable in reviewed material. Palpal segments cylindrical, palpal proportions: 1.0:1.2:1.1:2.0, last palpal segment not corrugated; labium without any strong sclerite; labella elongated and irregularly shaped with six to eight setae concentrated in darkened spot at lower outer margin, with one pre-apical spiniform and one apical spiniform tooth on each.

Thorax. Allurement organs absent, with single patch of alveoli in paraterguite and antepronotum; all coxae with stripe of one to two rows of alveoli. Wing length about two times its width; wing membrane brown-infuscated, with darkened spots on apex of longitudinal veins (Fig. 1B); subcostal vein short ending beyond origin of R_5; junction of R_{2+3} basal to junction of M_{1+2}, not joining R_4, origin of M_{1+2} basal to origin of R_{2+3}; R_5 ending at wing apex; CuA_2 faintly ending at wing margin.

Terminalia (Figs 3C–D, 4B–C, E–G). Hypandrium in dorsal view U-shaped, and sclerotized, joining base of gonocoxites. In lateral view hypandrium looks membranous (Fig. 3D) with sclerotized margin; gonocoxites joining at apex forming V-shaped sclerite placed above aedeagal complex, each with preapical lateral lump with cluster of three to four setae (Figs 3C, 4B), gonostyli absent; aedeagus in dorsal view straight, as single sclerite, no discernible parameres. In lateral view, aedeagus has curved apex towards hypandrium (Fig. 4G); ejaculatory apodeme about same length as aedeagus, in dorsal view, basal margin rounded and slightly concave in middle, in lateral view, ejaculatory apodeme looks like half-circle, with basal margin concave; gonocoxal condyles fitting in concavity on underside of ejaculatory apodeme, not triangular-shaped and not protruding beyond base of ejaculatory apodeme; epandrium rectangular, slightly wider than long, with more sclerotization at margins, lateral margins with slight concavity in middle; hypoproct tongue-shaped, shorter than epandrium and covered with small setulae, epiproct shorter than hypoproct; epandrial appendage barely hemispherical, prolonged and tapering distally, covered with small setae, lacking apical tenacula but with line of five short and cylindrical tenacula, with folded tips, and additional patch of long accessory tenacula basally concentrated in darkened patch, these accessory tenacula being as long as or longer than epandrium (Figs 3C, 4C).

Female (Figs 4D, 5A–C)

Similar to male except for following characteristics: two rings of teardrop-shaped pores in flagellomeres not as well defined as in males being more scattered and smaller, flagellomeres smaller than male flagellomeres. Wing length equals 2.55 times its width. Subgenital plate long, lateral margins concave in middle, and apical margin has rectangular concavity, with two setae at margin of concavity in addition to scattered setae on surface (Figs 4D, 5A, C); cerci about 1.5 times as long as subgenital plate (Fig. 5A), each with scattered setae on basal surface; genital chamber appears asymmetrical; however, this might be due to bad slide preparation, nonetheless, structures can be seen in Figs 4D, 5B. Female of *Armillipora muyu* sp. nov. can be easily differentiated from female of *Armillipora selvica* by following characters: apical concavity in subgenital plate rectangular (rounded in *A. selvica*); genital chamber with two anterior lobes in *Armillipora muyu* as in Figs 4D, 5B (genital chamber quadrate without anterior lobes in *A. selvica*, see Quate 1996: fig. 11d).

Egg (Fig. 5D)

Female specimen contained eggs inside abdomen, shape of eggs long-ovoid, being five times as long as wide; general appearance of membrane corrugated, with irregular folds across entire surface; anterior pole of eggs has semi-circular small projection.

Remarks

In the paratype ZFMK-DIP-00081667, the palpal segments are missing; the thorax and right wing were used for DNA extraction and are not present in the slide.

Distribution

Only known from the type locality in Ecuador.

Genetics

Six specimens were successfully sequenced (ZFMK-TIS-2636967, ZFMK-TIS-636968, ZFMK-TIS-2636969, ZFMK-TIS-2636973, ZFMK-TIS-2637146, ZFMK-TIS-2629905). The maximum intraspecific uncorrected pairwise distance for COI sequences was 3.04 % or 20 bp. Genbank accession numbers are: OQ706383, OQ706381, OQ706385, OQ706386, OQ706378, OQ706376.

Armillipora selvica Quate, 1996

Fig. 6

Armillipora selvica Quate, 1996: 29 (description). – Quate 1999: 427 (a new geographical record). – Ježek *et al*. 2020: 419 (redescription and a new geographical record).

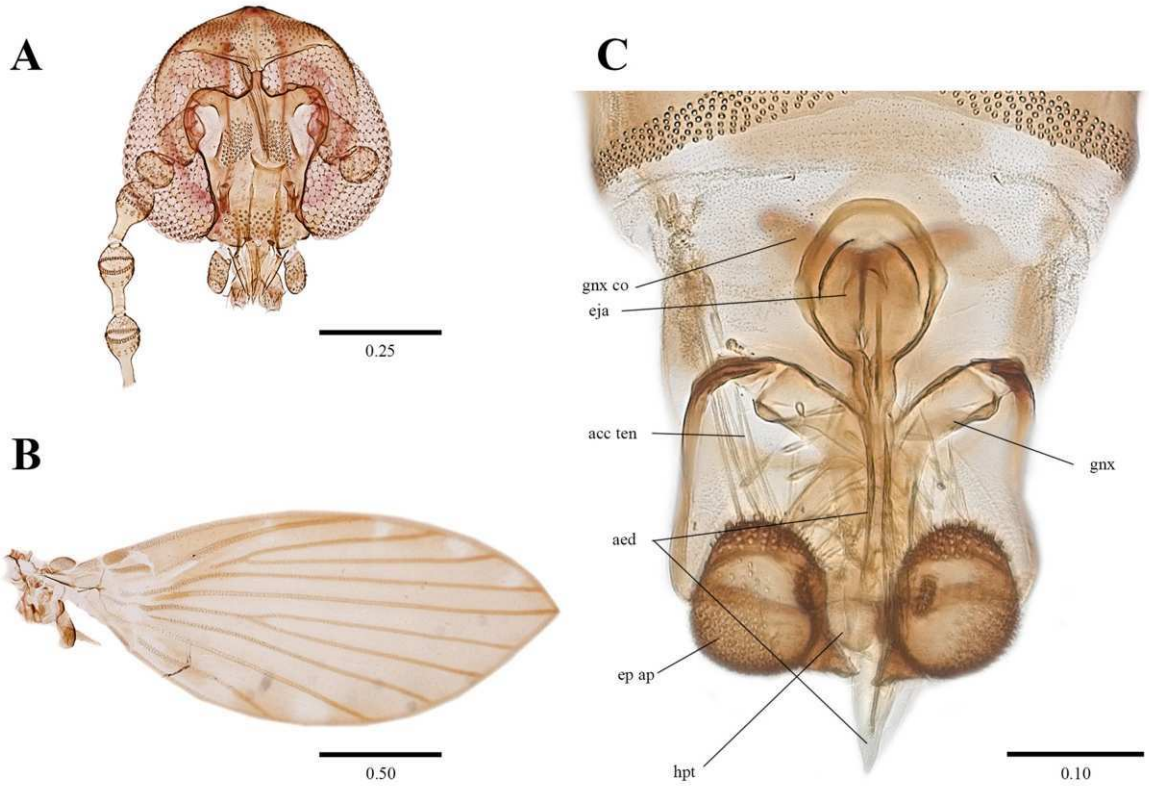

Fig. 6. *Armillipora selvica* Quate, 1996, ♂ (ZFMK-TIS-2629865). **A**. Head. **B**. Wing. **C**. Genitalia in ventral view. Scale bars in mm. Abbreviations: acc ten = accessory tenacula; aed = aedeagus; eja = ejaculatory apodeme; ep ap = epandrial appendage; gnx = gonocoxite; gnx co = gonocoxal condyles; hpt = hypoproct.

Differential diagnosis

See differential diagnosis under *A. imitata* sp. nov.

Material examined

ECUADOR – **Pichincha** • 1 ♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonado, Roadway to Pachijal; 0.11882° N, 78.95802° W; alt. 750 m; 1–9 Feb. 2022; Kilian and Isabel leg.; ZFMK [ZFMK-DIP-00097930, ZFMK-TIS-2637093] • 1 ♂; same collection data as for preceding; INABIO [ZFMK-DIP-00097933, ZFMK-TIS-2637154] • 1 ♂; same collection data as for preceding; ZFMK [ZFMK-DIP-00081837, ZFMK-TIS-2636974] • 1 ♂; same data as for preceding; 30 Dec. 2021–5 Jan. 2022; ZFMK [ZFMK-DIP-00081968, ZFMK-TIS-2636960] • 1 ♂; same collection data as for preceding; 25–28 Jan. 2020; ZFMK [ZFMK-DIP-00081670, ZFMK-TIS-2629865].

Distribution

Nicaragua (Ježek *et al.* 2020), Costa Rica (Quate 1996), Panama (Quate 1999), and Ecuador (this publication, new record).

Genetics

Five specimens were successfully sequenced (ZFMK-TIS-2637093, ZFMK-TIS-2637154, ZFMK-TIS-2636960, ZFMK-TIS-2629865, and, ZFMK-TIS-2636974). The maximum intraspecific uncorrected pairwise distance for COI sequences was 4.10 % or 27 bp. Genbank accession numbers are: OQ706382; OQ706380; OQ706377; OQ706379; OQ706384.

Armillipora suapiensis Ježek, Oboňa & Le Pont, 2020

Armillipora suapiensis Ježek, Oboňa & Le Pont, 2020: 422 (description).

Differential diagnosis

Based on the original description of Ježek *et al*. (2020), *Armillipora suapiensis* can be easily separated from all the other species of the genus by the following characteristics: eye bridge with five rows of facets (four rows of facets in other species); more than six cylindrical tenacula present in epandrial appendage in *A. suapiensis* (six or less cylindrical tenacula present in other species); gonocoxites not fused in *A. suapiensis* (fused in other species); parameres not fused and outwardly curved in *A. suapiensis* (fused in other species), aedeagus around twice as long as ejaculatory apodeme in *A. suapiensis* (about same length of ejaculatory apodeme in other species); ejaculatory apodeme with pointed anterior margin in *A. suapiensis* (rounded anterior margin in other species) (see Ježek *et al.* 2020).

Material examined

None.

Distribution

Bolivia (Ježek *et al*. 2020).

Key to the males of Armillipora

1. Eye bridge with five facet rows; more than six tenacula on the epandrial appendage; gonocoxites not fused..... ***A. suapiensis*** Ježek, Oboňa & Le Pont, 2020
– Eye bridge with four facet rows; six or fewer tenacula on the epandrial appendage; gonocoxites fused..... 2

2. The interocular suture with short posterior spur (Fig. 6A); gonocoxal condyles triangular-shaped and protruding beyond base of ejaculatory apodeme ..... ***A. selvica*** Quate, 1996
– Interocular suture without posterior spur (Figs 1A, 3A); gonocoxal condyles not triangular-shaped and not protruding beyond base of ejaculatory apodeme 3

3. Gonocoxites fused, forming U-shaped sclerite, with concavity at lower margin, gonocoxites without preapical lumps, each containing six preapical setae (Figs 1C, 2B); epandrial appendage in lateral view with line of four short and cylindrical tenacula, with additional tenaculum placed on separate projection of epandrial appendage (Fig. 2E) ..... ***A. imitata*** sp. nov.
– Gonocoxites fused, forming V-shaped sclerite (Figs 3C, 4B), each with preapical lump, each containing three to four setae; epandrial appendage in lateral view with line of five short and cylindrical tenacula without additional projections on epandrial appendage (Fig. 4G) .... ***A. muyu*** sp. nov.

Genetics

Barcoded specimens of *Armillipora imitata* sp. nov. have an intraspecific uncorrected pairwise distance for COI sequences of 1.06 %. Similarly, specimens of *A. muyu* sp. nov. have an uncorrected pairwise distance of 3.04 %, and specimens of *A. selvica* show an uncorrected pairwise distance 3.80 %. The interspecific uncorrected pairwise distances are higher, for instance *A. muyu* has a maximum interspecific uncorrected pairwise distance of 9.57 % (9.57–9.11 %) with *A. imitata* and 10.03 % (10.03–8.81 %) with *A. selvica*. In a similar manner, *A. imitata* has a maximum uncorrected pairwise distance of 5.92 % (5.92–5.02) compared to *A. selvica* (Table 2). All sequenced specimens cluster well into morphological taxa in the NJ tree (Fig. 7).

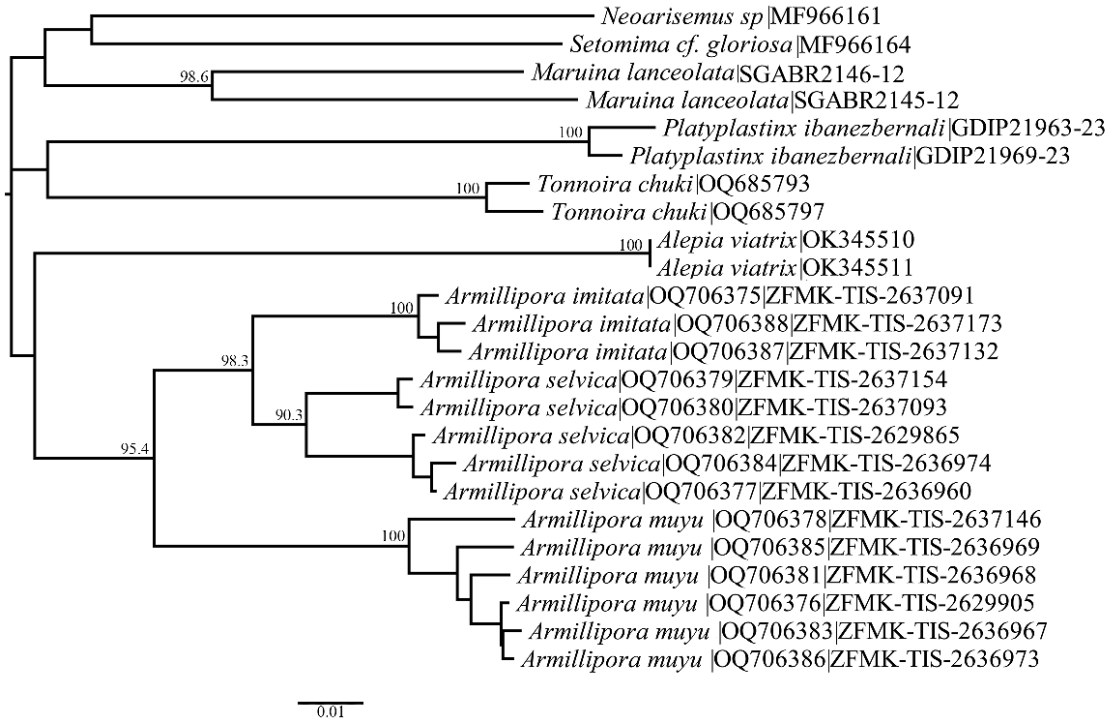

Fig. 7. Neighbor-joining tree using Jukes-Cantor model based on the COI sequences of the examined material and sequences downloaded from BOLD. The name for each specimen has the name of the species | BOLD/GenBank accession number | sample ID. Bootstrap support values are given at the nodes.

Species distribution model

We gathered a total of 76 geographical records for all the species of *Armillipora* (*A. imitata* sp. nov. n = 3; *A. muyu* sp. nov. n = 7; *A. selvica* n = 55; and *A. suapiensis* n = 11), mainly from the original descriptions. Thirty-six records had exact geographical coordinates, while 40 records were lacking the exact geographical coordinates and these were adjusted based on the reported locality (Table 1).

The species of *Armillipora* are currently reported in five countries, namely Bolivia, Costa Rica, Ecuador, Nicaragua, and Panama (Table 1), and our model shows that the genus can be present in several more countries in the Neotropical region, extending towards North America (Fig. 8). Still, the highest probability of presence is concentrated in Central America and northern South America (Fig. 8).

Discussion

Species of *Armillipora* are morphologically very similar-looking to each other and the differences are subtle even with slide-mounted specimens. Our results with DNA barcodes prompt us to think that morphological determination can be complemented using COI barcodes with good species delimitation

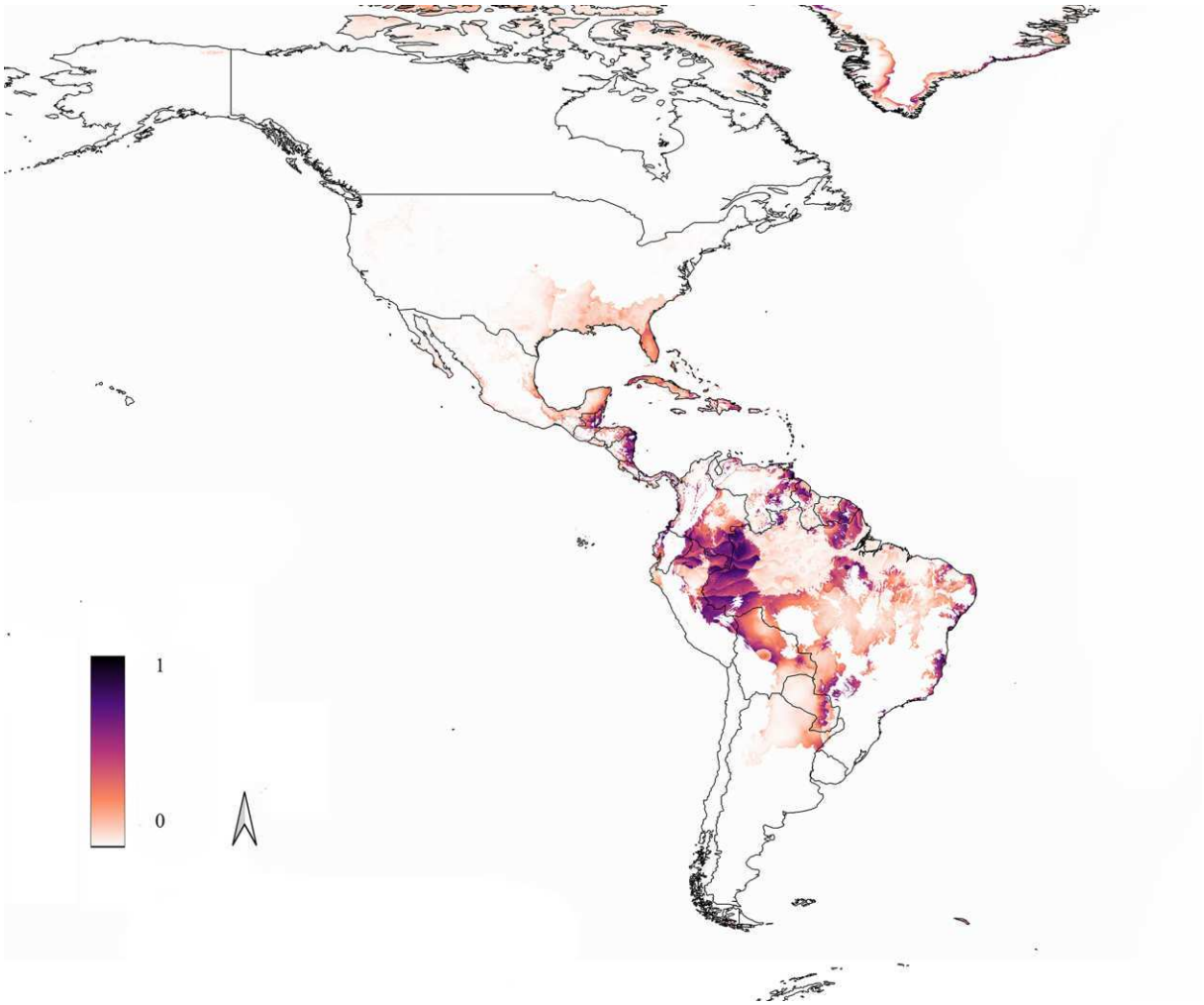

Fig. 8. Heat map resulting from the Species Distribution Model using MaxEnt, where 1 is equal to the highest probability of distribution, while 0 is the lowest probability.

results. In other words, we think that morphological determination in combination with DNA barcodes is a good way to determine species of the genus *Armillipora*. In the Neotropical region, however, there is a gap in the number of known species (Linnean shortfall) in combination with a poorly documented geographic distribution (Wallacean shortfall). Both shortfalls with the combination of an absent DNA barcode reference library and only a handful of DNA barcodes generated from the Neotropical region lead to a lack of important information for an integrative taxonomy approach, and future species delimitation techniques could provide valuable information for the genus and for the subfamily Psychodinae in the Neotropics, in general.

Species distribution models do well in predicting the occurrence of many species (Lee-Yaw *et al*. 2021), even dealing at genus level (Stas *et al.* 2020), and our inferred distribution model (Fig. 7) shows that *Armillipora* could be found in several other countries than the ones from which the genus is currently reported (e.g., Brazil, Colombia, French Guyana, Guyana, Peru, Surinam, Venezuela). Following the proposed biogeographic regionalization of Morrone (2014: fig. 12) it can be expected to find the genus in the Mexican Transition Zone, the Antillean subregion, Brazilian subregion, Mesoamerican dominion, Pacific dominion, Boreal Brazilian dominion, South Brazilian dominion and the Chacoan subregion, with a potential distribution mainly restricted to the Neotropics. There is no doubt that the known range of the species lacks information and further records will be found. As more information about the immature stages of Psychodinae is known it will become easier to find specific habitats to find new records and new species (e.g., searching for specific microhabitats).

Biodiversity loss in the Neotropical region is mainly due to the high fragmentation of the habitats, and many species are doomed to disappear (Antonelli 2021). Hence, the importance to fill the distribution gaps in order to increase our understanding of the natural environments and the relationships with the species inhabiting them (Santos & Hoppe 2018). Moreover, these knowledge gaps are usually encumbered by insufficient taxonomical information (e.g., lack of taxonomists, lack of funding, lack of species surveys, and taxonomic impediments) and highlight the importance of both the need for taxonomical works and the usual apathy of governments to support taxonomical initiatives. This is why projects such as "Diversidad de moscas florícolas (Insecta: Diptera) del Ecuador", together with international collaborations, are crucial to fill the taxonomical gaps in the Neotropical region aiming for a more complete species list and their distribution in the Neotropics.

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Corrigendum

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A revision of the genus *Armillipora* Quate (Diptera: Psychodidae) with the descriptions of two new species – Corrigendum

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https://doi.org/10.5852/ejt.2024.925.2473

The present corrigendum rectifies issues in Jaume-Schinkel & Mengual (2024), notably the missing tables cited in the article are presented here.

Table 1. Distribution of species of *Armillipora*. For the coordinates status, adjusted means no exact coordinates were presented in the references and coordinates needed to be obtained by searching the reported locality, on the contrary, exact means exact coordinates were provided in the reference.

Species	Distribution	Coordinates status	Lat (°N)	Long (°W)	Reference
Armillipora selvica Quate, 1996	Costa Rica	adjusted	10.4304	-84.0063	Quate 1996
	Panama	adjusted	9.1551	-79.8432	Quate 1999
	Nicaragua	exact	13.67229	-84.5059	Jezek *et al*. 2020
	Ecuador	exact	0.11883	-78.958	
A. suapiensis Jezek *et al*., 2020	Bolivia	exact	-16.1	-67.7667	Jezek *et al*. 2020
A. imitata sp. nov.	Ecuador	exact	0.11561	-78.9581	Current manuscript
A. muyu sp. nov.	Ecuador	exact	0.11883	-78.958	Current manuscript

Table 2 (contined on the next page)**.** Uncorrected pairwise distance for COI sequences (% of similarity) of the COI sequences between specimens. The name of each specimen has: the name of the species | BOLD process ID.

Platyplastinx ibanezbernali |GDIP21963-23|ZFMK-TIS-2629867											
Platyplastinx ibanezbernali |GDIP21969-23|ZFMK-TIS-2629874	98.5										
Pericoma rivularis | GMFID118-12	84.5	85									
Maruina lanceolata |SGABR2146-12	83.9	84.5	86.4								
Maruina lanceolata |SGABR2145-12	83.8	83.6	86.6	90							
Tonnoira chuki |OQ685793 |ZFMK-TIS-2637131	85.1	85.4	83.3	85.5	85.7						
Tonnoira chuki |OQ685797 |ZFMK-TIS-2637103	85	85.1	83.3	85.3	85.1	98.5					
Alepia viatrix |OK345510|ZFMK-TIS-2628057	83.9	83.3	82.8	83.1	82.8	84.3	84.2				
Alepia viatrix |OK345511|ZFMK-TIS-2628055	83.9	83.3	82.8	83.1	82.8	84.3	84.2	100			
Armillipora muyu |OQ706378|ZFMK-TIS-2637146	84	84.7	85.1	87	85.3	86.2	85.7	85	85		
Armillipora muyu |OQ706385|ZFMK-TIS-2636969	84.2	84.5	85	86.7	85.9	86.6	86.2	84.7	84.7	96.8	
Armillipora muyu |OQ706381|ZFMK-TIS-2636968	83.9	84.2	85	85.6	85.4	85.7	85.7	84.7	84.7	96.8	98.5

Table 2 (continued)**.** Uncorrected pairwise distance for COI sequences (% of similarity) of the COI sequences between specimens. The name of each specimen has: the name of the species | BOLD process ID.

Armillipora muyu |OQ706376|ZFMK-TIS-2629905	84.3	85	85.4	85.6	85.6	86	85.9	85	85	96.8	98.5	98.8										
Armillipora muyu |OQ706386|ZFMK-TIS-2636973	84.2	84.8	85.3	85.5	85.3	85.7	85.6	84.8	84.8	96.8	98.2	98.8	99.7									
Armillipora muyu |OQ706383|ZFMK-TIS-2636967	84.3	85	85.4	85.9	85.7	86.2	86	85	85	97	98	98.6	99.5	99.5								
Armillipora imitata |OQ706375|ZFMK-TIS-2637091	85.1	85.6	83.7	85.9	84.3	85.9	85.7	86.8	86.8	90.9	90.9	90.9	90.9	90.7	90.7							
Armillipora imitata |OQ706388|ZFMK-TIS-2637173	84.8	85	83.3	85.8	83.8	85.7	85.6	86.5	86.5	90.4	90.7	90.7	90.7	90.6	90.6	99.1						
Armillipora imitata |OQ706387|ZFMK-TIS-2637132	84.8	85	83.3	85.6	83.8	85.4	85.3	86.6	86.6	90.6	90.6	90.6	90.9	90.7	90.7	98.9	99.2					
Armillipora selvica |OQ706379|ZFMK-TIS-2637154	85.1	85.6	83.7	86.3	84.8	86.8	86.8	86.9	86.9	90.7	91	90.9	91.2	91	90.9	94.5	94.2	94.4				
Armillipora selvica |OQ706380|ZFMK-TIS-2637093	85	85.4	83.7	86.3	84.9	86.9	86.8	87.2	87.2	90.4	90.9	90.7	91	90.9	91	94.4	94.1	94.2	99.5			
Armillipora selvica |OQ706382|ZFMK-TIS-2629865	85.4	86.2	84	85.8	84.6	86	86.2	86.6	86.6	90.6	91	90.9	91.2	91	90.9	95	94.7	94.8	96.8	96.5		
Armillipora selvica |OQ706384|ZFMK-TIS-2636974	85.9	86.3	84.3	85.6	84.1	85.6	85.9	86.5	86.5	90	90.1	90.3	90.6	90.4	90.3	94.7	94.1	94.2	96.2	95.9	99.1	
Armillipora selvica |OQ706377|ZFMK-TIS-2636960	85.6	86.3	84.5	85.8	84.3	85.6	85.7	86.5	86.5	90.4	90.6	90.4	91	90.9	90.7	95.1	94.5	94.7	96.7	96.4	99.5	99.5

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Appendix 12. (Publication chapter 14)

Chapter 14 – Publication

Jaume-Schinkel S, Kilian I, Pazmiño-Palomino A, Mengual X (submitted) Revision of the genus *Bryopharsos* Quate, 1996 (Diptera: Psychodidae) with the description of nine new species. European Journal of Taxonomy.

Revision of the genus *Bryopharsos* Quate, 1996 (Diptera: Psychodidae) with the description of nine new species

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Running title: Revision of *Bryopharsos*

'The present paper has not been submitted to another journal, nor will it be in the 6 months after initial submission to EJT. All co-authors are aware of the present submission.'

Abstract

In this study, we revise the genus *Bryopharsos* and describe nine new species. These include *B. curvum* Jaume-Schinkel sp. nov., *B. gorgona* Jaume-Schinkel sp. nov., and *B. tetracanthus* Jaume-Schinkel sp. nov. from Colombia; *B. insperatus* Jaume-Schinkel sp. nov. from Costa Rica; *B. asymmetricum* Jaume-Schinkel sp. nov. and *B. septenacula* Jaume-Schinkel sp. nov. from Ecuador; *B. bitenacula* Jaume-Schinkel sp. nov. and *B. chuspi* Jaume-Schinkel sp. nov. from Peru; and *B. bifidum* Jaume-Schinkel sp. nov. from Venezuela. Additionally, we report new geographical records of *B. amazonensis* Bravo & Araújo, 2019 from Colombia and Ecuador and new records of *B. clavigum* Quate, 1996, *B. claviformosum* Quate, 1996 and *B. palpiculum* Quate, 1996 from Ecuador. These new records correspond to the first report of *Bryopharsos* from Colombia, Ecuador, Peru, and Venezuela. Furthermore, we provide the first description of a *Bryopharsos* egg along with the redescription of the female of *B. palpiculum*. We also make available the first DNA barcodes for several species including *Bryopharsos asymmetricum* sp. nov., *B. amazonensis, B. clavigum, B. claviformosum*, *B. palpiculum,* and *B. septenacula* sp. nov. Lastly, we update the identification key to the known species, present a distribution map of the known taxa, and discuss the potential distribution of the genus in the Neotropical Region using a species distribution model.

Keywords. Integrative taxonomy; DNA barcoding; Neotropical Region; Psychodinae; new taxa; new record.

Introduction

Bryopharsos is a small genus of Neotropical moth flies (Diptera: Psychodidae) described by Quate (1996) to include four species from Costa Rica, namely *Bryopharsos*

claviformosum Quate, 1996, *B. clavigum* Quate, 1996, *B. palpiculum* Quate, 1996, and *B. tritaleum* Quate, 1996. Posteriorly, Quate (1999) recorded *B. palpiculum* in Panama and described the female of that species, which remains the only known female of this genus. Recently, Bravo & Araújo (2019) described three new *Bryopharsos* species from Brazil, i.e., *B. paulistensis* Bravo & Araújo, 2019*, B. uncinatum* Bravo & Araújo, 2019, and *B. amazonensis* Bravo & Araújo, 2019, bringing the total number of *Bryopharsos* species to seven and expanding the previously known distribution from Central America to South America.

In the present study, we describe nine new species based on material from Colombia, Costa Rica, Ecuador, Peru, and Venezuela, namely, *B. asymmetricum* Jaume-Schinkel sp. nov., *B. bifidum* Jaume-Schinkel sp. nov., *B. bitenacula* Jaume-Schinkel sp. nov., *B. curvum* Jaume-Schinkel sp. nov., *B. chuspi* Jaume-Schinkel sp. nov., *B. gorgona* Jaume-Schinkel sp. nov., *B. insperatus* Jaume-Schinkel sp. nov., *B. septenacula* Jaume-Schinkel sp. nov., and *B. tetracanthus* Jaume-Schinkel sp. nov. Moreover, we record the genus for the first time in Colombia, Ecuador, Peru, and Venezuela. In addition, we redescribe the female of *B. palpiculum* and provide the first description of a *Bryopharsos* egg. Moreover, we make available the first DNA barcodes (Cytochrome *c* Oxidase subunit 1 gene or *COI*) for several species recorded in Ecuador (i.e. *Bryopharsos asymmetricum* Jaume-Schinkel sp. nov., *B. amazonensis*, *B. clavigum*, *B. claviformosum*, *B. palpiculum*, and *B. septenacula* Jaume-Schinkel sp. nov.). Lastly, we update the diagnosis of the genus and provide an identification key for the world species.

Material and methods

Zoological Collections

The examined material is deposited in different entomological collections referred to by their abbreviations in the text. The abbreviations used for collections and their equivalents are given below:

MECN: Entomological Collection, Instituto Nacional de Biodiversidad, Quito, Ecuador.

INBio: Instituto Nacional de Biodiversidad, Heredia, Costa Rica.

LACM: Natural History Museum of Los Angeles County, California, United States.

MZFS: Entomological Collection Prof. Johann Becker, Museu de Zoologia da Universidade Estadual de Feira de Santana, Bahia, Brazil.

ZFMK: Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels (previously known as Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany.

Genetics

For 54 of the specimens deposited in ZFMK, we employed a non-destructive approach to analyze complete specimens. The process involved the utilization of a Qiagen (Hilden, Germany) BioSprint 96 magnetic bead extractor and the associated kits, following the guidelines provided by the manufacturer. To amplify the 5'-end of the Cytochrome *c* Oxidase subunit I (COI) gene, the primers HCO2198-JJ (forward) and LCO1490-JJ (reverse) were utilized (Astrin & Stüben 2008). PCR amplification was carried out using a QIAGEN Multiplex PCR Kit and a TouchDown PCR (TD-PCR) method, as described by Korbie & Mattick (2008). Subsequently, the PCR products were sent to the Beijing Genomic Institute (BGI) in Hong Kong, China, for bidirectional sequencing. The obtained DNA sequences were then assembled, aligned, and cleaned using Geneious

Prime ver. 2022.1.1 (Biomatters, Auckland, New Zealand). The final sequence length was 658 bp.

The Geneious software was used to conduct a distance-based Neighbor-Joining (NJ) analysis (Saitou & Nei 1987) using the Jukes-Cantor model. For the NJ tree, we constrained the COI sequence of *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022, (GenBank accession number: OK345511) as the root to help with the visualization. The sequences used in the analysis can be accessed on BOLD under the dataset DS-BRYO (available at: To be added). Bootstrap support (BS) values were estimated through 1000 replicates generated in Geneious.

Species records

A map was generated to visualize the distribution of the species of *Bryopharsos* based on the geographic coordinates extracted from the localities reported in the literature. For those records without exact reported coordinates we looked for the reported locality and adjusted the coordinates using Google® Earth. As some species have overlap in their distribution we used the Rstudio (ver. 2023.03.1+446) package "ggplot", with the "geom_jitter" and "theme_bw" functions to enhance the visualization and apply a black-and-white theme to the map. This approach facilitated the visualization of the spatial distribution of *Bryopharsos* when species distribution overlap.

Genus distribution model

The genus distribution model was built using the software MaxEnt 3.4.4 (Phillips *et al.* 2023) using species geographical records to model the potential distribution of the genus. Since the known species are restricted to the Neotropical Region, the resulting map was

111 trimmed to the American continent. Climate variables were obtained from WorldClim
112 (Fick & Hijmans 2017).

113 **Terminology**

114 We follow the general terminology proposed by Cumming & Wood (2017) and Kvifte &
115 Wagner (2017a). For the reasons mentioned in the discussion, we use the term 'surstyli'
116 to refer to the caudal appendages, which are also referred to as cercopods, surstyli, or
117 hypopods (also see the discussion in Kvifte & Wagner 2017b).

118 Within the male genitalia of *Bryopharsos* species, there is a structure located on the
119 gonocoxal lobes that resembles a spine-like projection. This structure is called appendage
120 by Quate (1996), and Bravo & Araujo (2019) refer to it in the description of *B.*
121 *paulistensis* as “lateral lobes projected posteriorly in the posterior expansion of the
122 gonocoxal apodemes”. We herein refer to it as the spine of the gonocoxal lobes (see
123 Figure 3).

124 **Results**

125 **Taxonomic account**

126 Class Insecta Linnaeus, 1758

127 Order Diptera Linnaeus, 1758

128 Suborder Psychodomorpha Hennig, 1968

129 Family Psychodidae Newman, 1834

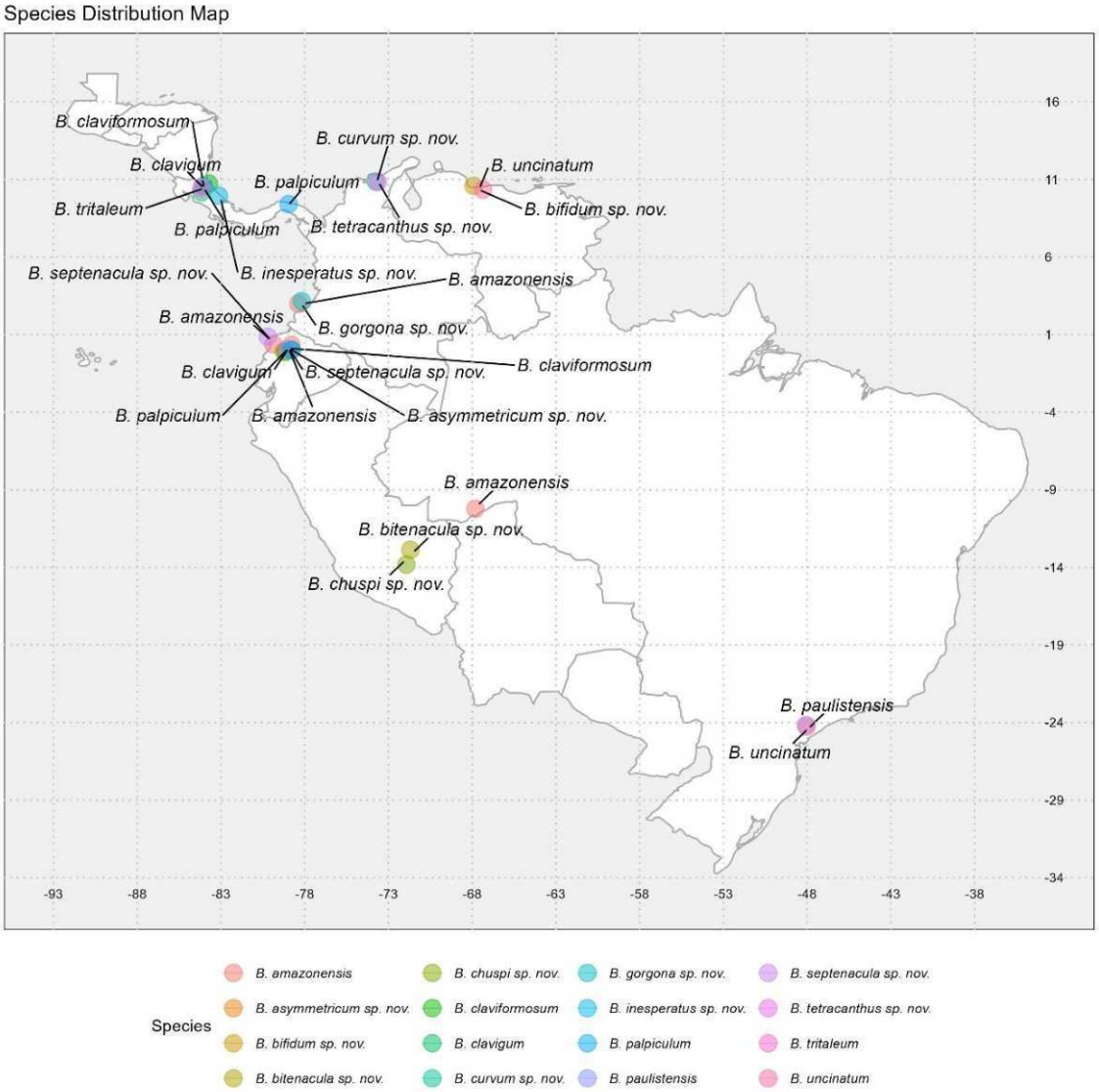
130 Subfamily Psychodinae Newman, 1834

Genus *Bryopharsos* Quate, 1996

Bryopharsos Quate, 1996: 40. Type species: *Bryopharsos palpiculum* Quate, 1996 by original designation.

Bryopharsos: Quate (1999): 434 (female description and updated distribution); Ježek (2010): 237 (discussion of characters); Kvifte (2018): 603 (tribal classification); Bravo & Araújo (2019): 364 (description of new species and key to world species).

Species distribution shown in Figure 1.



138

139 **Fig. 1.** Species distribution map. Species dots do not represent the actual localities; jitter
140 was added for visualization as the species' real distributions overlap.

141

142 **Diagnosis**

143 Building upon Quate's (1996) original diagnosis, Bravo and Araújo (2019) proposed a
144 broader diagnosis to fit the newly described Brazilian species. I n this study, we update
145 the genus diagnosis, including previously and herein described species. **Male.** Vertex
146 short, up to twice the width of the eye bridge; eye bridge contiguous conformed by 3–5

facet rows; antennae with 14 flagellomeres, 1–13 asymmetrically-nodiform, flagellomere 14 with long digitiform apiculus; flagellomeres 1–13 with a pair of broad leaf-shaped ascoids; ascoids width at least the same as the length of the flagellomere carrying them; palpi short reaching the level of the third flagellomere; labellum short and not bulbous; thorax with or without allurement organs; wing membrane with alveoli between longitudinal veins; wing vein R_5 ends at wing apex; gonocoxal apodemes fused or contiguous at midline; gonocoxal lobes projected anteriorly, with or without a spine; gonostyli digitiform to club-shaped with rounded apex; surstyli with 1-7 tenacula; aedeagal complex asymmetrical; ejaculatory apodeme width, half or more than its length in dorsal view.

Bryopharsos was considered by Ježek (2010) to be morphologically close to the Palearctic/Oriental genus *Saximormia* Ježek, 1984 as they share the following characters: ascoids as broad or broader than the flagellomere carrying them, eyes contiguous in the eye bridge, and the general wing structure of the wing. Nevertheless, they can be easily differentiated from each other by the following characters: alveoli on the wing membrane present in *Bryopharsos* (absent in *Saximormia*), wing vein radial and medial forks being at the same level and basal to the wing center in *Bryopharsos* (forks not being at the same level and apical to the wing center in *Saximormia*), vein R_5 ending at wing apex in *Bryopharsos* (vein R_5 ending at wing margin posterior to the apex in *Saximormia*), the ejaculatory apodeme is conspicuously enlarged in dorsal view in *Bryopharsos* (ejaculatory apodeme not enlarged in *Saximormia*), gonostyli club-shaped with a rounded blunt apex in *Bropharsos* (gonostyli tapering towards the apex and with a pointed apex in *Saximormia*), (Ježek 2010; Bravo & Araújo 2019).

***Bryopharsos amazonensis* Bravo & Araújo, 2019**

(Figs 1–3)

Bryopharsos amazonensis Bravo & Araújo, 2019: 368. Type locality: Brazil, 15 km SE Rio Branco, EMBRAPA [Holotype male, MZFS]

Diagnosis

Male

Eye bridge with four facet rows; wing 1.8 times longer than its width; ejaculatory apodeme paddle-shaped in dorsal view; surstyli with five tenacula of equal length; aedeagus long, triangular, and with a pointed apex. According to Bravo & Araújo (2019), *B. amazonensis* is morphologically similar to *B. clavigum* and *B. claviformosum*, but it can be differentiated by the number of tenacula (four in *B. clavigum;* five in *B. amazonensis* and *B. claviformosum*) and the length of the tenacula (four long and one short in *B. claviformosum;* five of equal length in *B. amazonensis;* four of the same length in *B. clavigum*).

Female

Unknown.

Material examined

ECUADOR – **Pichincha** • 9 ♂♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonado, near San Pancracio, roadway to Pachijal; 0.11862° N, -78.95802° E; alt. 750 m; 25–28 Jan. 2022; Kilian, Isabel leg.; ZFMK [ZFMK-DIP-00082154 = ZFMK-TIS-2628286, ZFMK-DIP-00082155 = ZFMK-TIS-2628287, ZFMK-DIP-00082156 = ZFMK-TIS-2628288, ZFMK-DIP-00082157 = ZFMK-TIS-2628289, ZFMK-DIP-

00082166 = ZFMK-TIS-2628298, ZFMK-DIP-00082167 = ZFMK-TIS-2628299, ZFMK-DIP-00082171 = ZFMK-TIS-2628303, ZFMK-DIP-00082172 = ZFMK-TIS-2628304, ZFMK-DIP-00082175 = ZFMK-TIS-2628307] • 2 ♂♂; same data as for preceding; 0.11561° N, -78.95805° E; 1–9 Feb. 2022; ZFMK [ZFMK-DIP-00081941 = ZFMK-TIS-2636932, ZFMK-DIP-00081944 = ZFMK-TIS-2636935] • 6 ♂♂; same data as for preceding; MECN [ZFMK-DIP-00081852 = ZFMK-TIS-2636989, ZMFK-DIP-00102094 = ZFMK-TIS-2637097, ZMFK-DIP-00102095 = ZFMK-TIS-2637102, ZMFK-DIP-00102096 = ZFMK-TIS-2637110, ZMFK-DIP-00102097 = ZFMK-TIS-2637122, ZMFK-DIP-00102098 = ZFMK-TIS-2637145].

COLOMBIA – **Cauca** • 5 ♂♂ Guapí, Gorgona island, alta El Mirador; alt. 180 m; 4 to 24 Mar. 2000; R. Doque leg.; LACM [LACM ENT 279390, LACM ENT 279391, LACM ENT 279392, LACM ENT 279399, LACM ENT 279398].

Distribution

Brazil (Bravo & Araújo 2019), Colombia (this publication, new record), and Ecuador (this publication, new record) (Fig. 1).

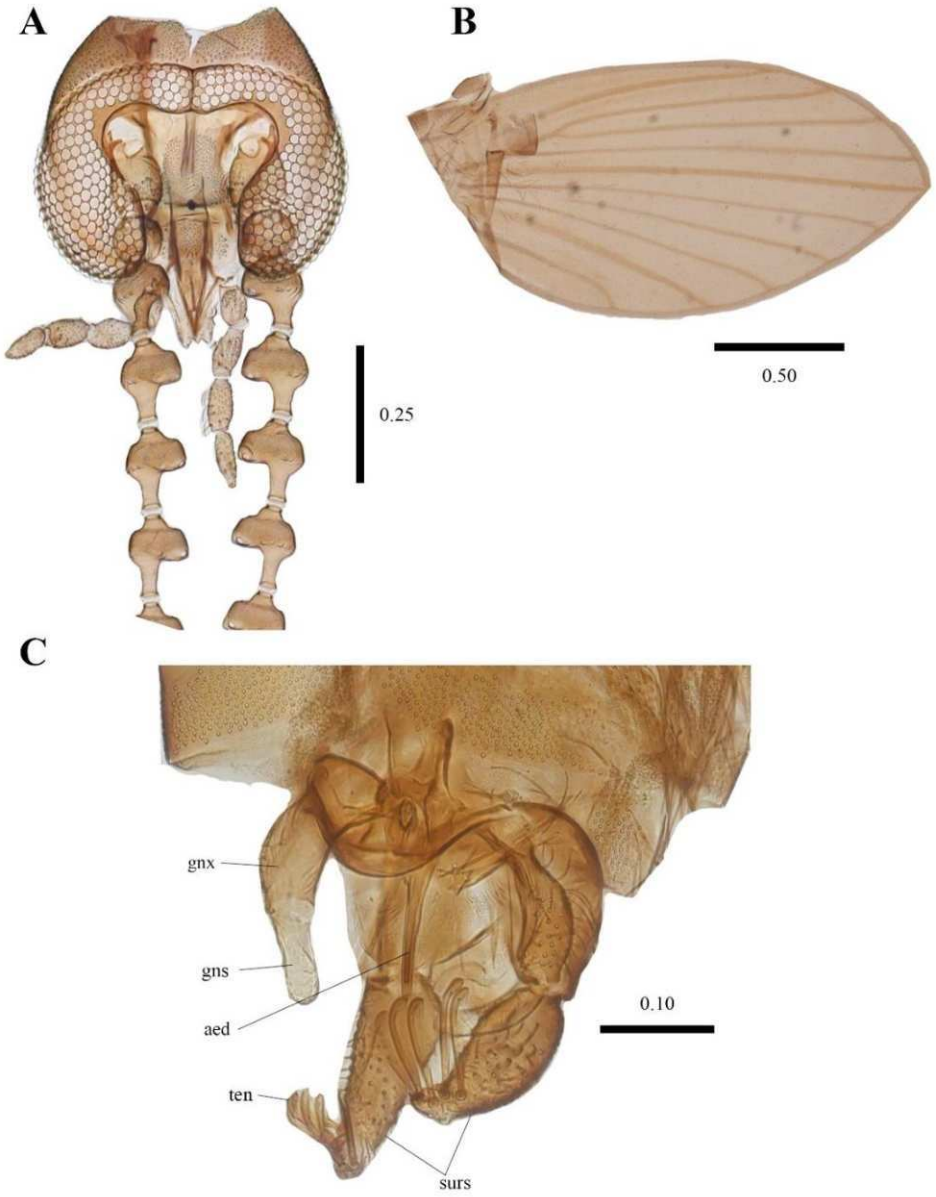
Genetics

22 specimens were successfully sequenced: ZFMK-TIS-2628286, ZFMK-TIS-2628288, ZFMK-TIS-2628289, ZFMK-TIS-2628298, ZFMK-TIS-2628303, ZFMK-TIS-2628304, ZFMK-TIS-2628307, ZFMK-TIS-2636932, ZFMK-TIS-2636935, ZFMK-TIS-2636989, ZFMK-TIS2637067, ZFMK-TIS-2637069, ZFMK-TIS-2627070, ZFMK-TIS-2627072, ZFMK-TIS-2627074, ZFMK-TIS-2627075, ZFMK-TIS-2627076, ZFMK-TIS-2627078, ZFMK-TIS-2637097, ZFMK-TIS-2637102, ZFMK-TIS-2637110, ZFMK-TIS-2637122. The maximum intraspecific uncorrected pairwise

distance for COI sequences was 0.37 % or 3 bp. GeneBank accession numbers are: To be added.

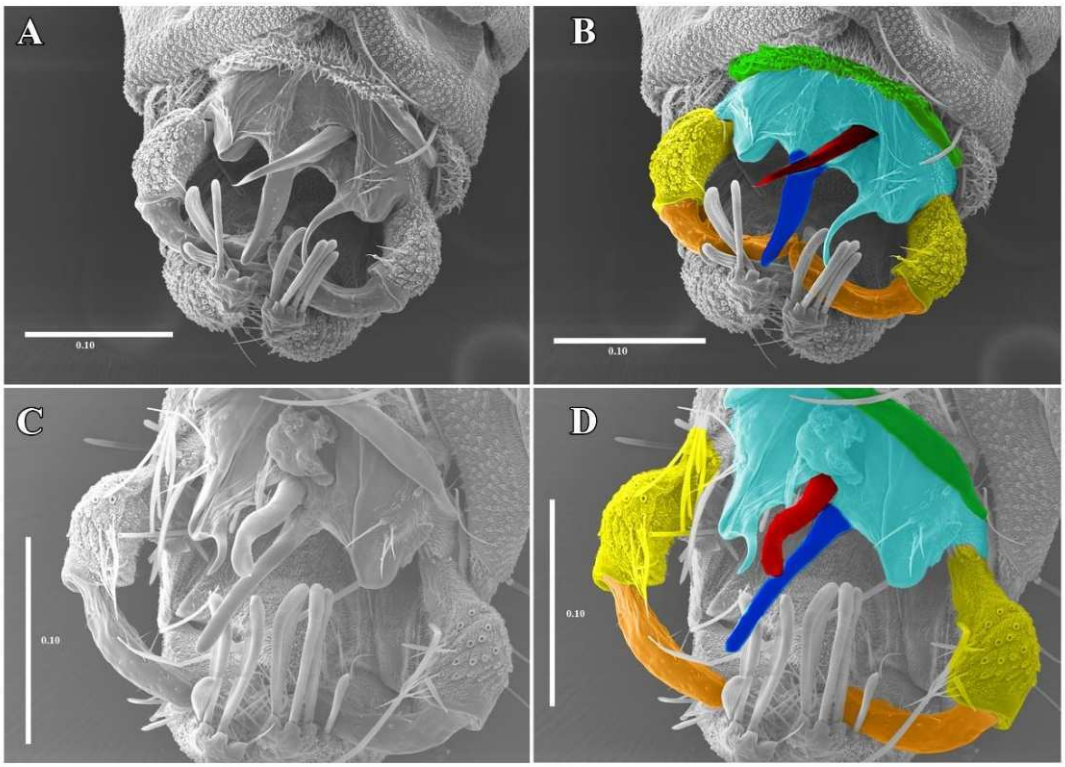
Remarks

Three specimens (ZFMK-DIP-00081944, ZFMK-DIP-00081852, ZMFK-DIP-00102094) have a small morphological variation as they present a spine in the gonocoxal apodeme, while the remaining specimens do not present the spine. The molecular evidence and the remaining morphological characters suggest all specimens belong to the same species. The original differentiation of *B. amazonensis* and *B. claviformosum* by Bravo & Araújo (2019) based on the difference in tenacula lengths (five of the same length in *B. amazonensis*; four of equal length and one shorter in *B. claviformosum*) appears to be a good morphological character to separate them. This is further supported by molecular data in this study.



227

228 **Fig. 2** *Bryopharsos amazonensis* Bravo & Araújo, 2019, ♂. **A.** Head. **B.** Wing. **C.**
229 Genitalia. Abbreviations: aed = aedeagus; gns = gonostylus; gnx = gonocoxite; surs =
230 surstyli; ten = tenacula. All scale bars are in millimeters (mm).



231

232 **Fig. 3** SEM pictures of male genitalia **A–B.** *Bryopharsos amazonensis* Bravo & Araújo,
233 2019. **C–D.** *Bryopharsos claviformosum* Quate, 1996. Colors: blue = paramere. cyan =
234 gonocoxal lobes, green = hypandrium, orange = gonostyli, red = aedeagus, yellow =
235 gonocoxites. All scale bars are in millimeters (mm).

236

237 ***Bryopharsos asymmetricum*** **Jaume-Schinkel sp. nov.**

238 (Figs 1, 4–5)

239 **Diagnosis**

240 Male

241 Eye bridge with five facet rows; wing 2.2 times longer than its width; ejaculatory
242 apodeme convex in dorsal view; gonocoxal apodeme with spine projection; surstyli with
243 2 or 3 tenacula; aedeagus digitiform and evenly tapering towards apex. This species is

similar to *B. tritaleum*, but they can be differentiated by the number of tenacula (three in *B. tritaleum*; some specimens with three tenacula on one surstyli in *B. asymmetricum* sp. nov. but the remaining with two tenacula), the number of facet rows in the eye bridge (four in *B. tritaleum*; five in *B. asymmetricum* sp. nov.), and the length of the ejaculatory apodeme (about the same length as the length of the aedeagus in *B. tritaleum*; shorter than the aedeagus in *B. asymmetricum* sp. nov.).

Female

Unknown.

Etymology

The species epithet *asymmetricum* derives from the Greek word συμμετρικός (symetrikós) with the Greek prefix ἀ- (without). Refering to the asymmetrical number of apical tenacula on the surstyli. Species epithet to be treated as an adjective.

Material examined

Holotype

ECUADOR – **Pichincha** • 1 ♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonado, near San Pancracio, roadway to Pachijal; 0.11561° N, -78.95805° E; alt. 750m; 1–9 Feb. 2022; Kilian, Isabel leg.; MECN [ZFMK-TIS-2637130].

Paratypes

ECUADOR – **Pichincha** • 5 ♂♂; same data as for holotype; ZFMK [ZFMK-DIP-00081943 = ZFMK-TIS-2636934, ZFMK-DIP-00081673 = ZFMK-TIS-2629868, ZFMK-DIP-00081664 = ZFMK-TIS-2629902, ZFMK-DIP-00102105 = ZFMK-TIS-

2637115, ZFMK-DIP-00102106 = ZFMK-TIS-2637127] • 1 ♂; same data as for preceding; MECN [ZFMK-TIS-2637149].

Description

Holotype male. Measurements in mm (n=6): Wing length: 1.90 (1.95–1.80), wing width: 0.85 (0.90–0.80); head length: 0.50 (0.50–0.45), head width: 0.40 (0.47–0.38); antennal segments: scape: 0.11 (0.11–0.10), pedicel: 0.06 (0.06–0.05), flagellomeres 1–5: 0.1 (0.12–0.11); palpal segment 1: 0.05 (0.5–0.05), palpal segment 2: 0.07 (0.08–0.06), palpal segment 3: 0.07 (0.08–0.07), palpal segment 4: 0.08 (0.08–0.07).

Head. Slightly longer than its width; eye bridge contiguous with five rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, upper margin with a concavity in the middle, lower margin rounded. Antennal scape about two times the length of the pedicel, almost cylindrical; pedicel spherical, smaller than scape; flagellomeres asymmetrical and nodiform, with scattered setae on the basal half surface, apical flagellomeres absent in examined material, the maximum number of flagellomeres present five; ascoids rectangular and broad, about the same length, and about two times the width of the flagellomere carrying them. Palpal segments cylindrical, palpal segment 4 with pointed apex, palpal proportions: 1.0:1.5:1.5:1.6; labium without any strong sclerite; labella bulbous with seven three setae on outer margin and two setae on inner margin.

Thorax. without allurement organs; all coxae with a stripe of three to five rows of alveoli. Wing length about 2.2 times its width; wing membrane brown-hyaline; alveoli distributed uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork

of R_{2+3} at the same level of M_{1+2} and joining R_4; fork of M_{1+2} weak; R_5 ending at the wing apex; CuA_2 ending at wing margin.

Terminalia (Figs. 4 C; 5). Hypandrium is a distinct band that connects the gonocoxites, broad and plate-like; gonocoxites about the same length as gonostyli, gonostyli slightly incurved, with rounded-blunt apex; aedeagus digitiform, , apex rounded, with an additional digitiform paramere, paramere evenly narrowing towards the apex, longer than aedeagus; ejaculatory apodeme shorter than aedeagus, basal margin convex; gonocoxal apodemes projected anteriorly as a trapezoidal plate, fused; epandrium narrow, about three times wider than its length, with posterior margin concave; hypoproct tongue-shaped, longer than epandrium and covered in small setulae, epiproct shorter than hypoproct; surstyli evenly tapering towards the apex and curved , one with three apical tenacula, the other with two tenacula, tenacula with rounded apex.

Distribution

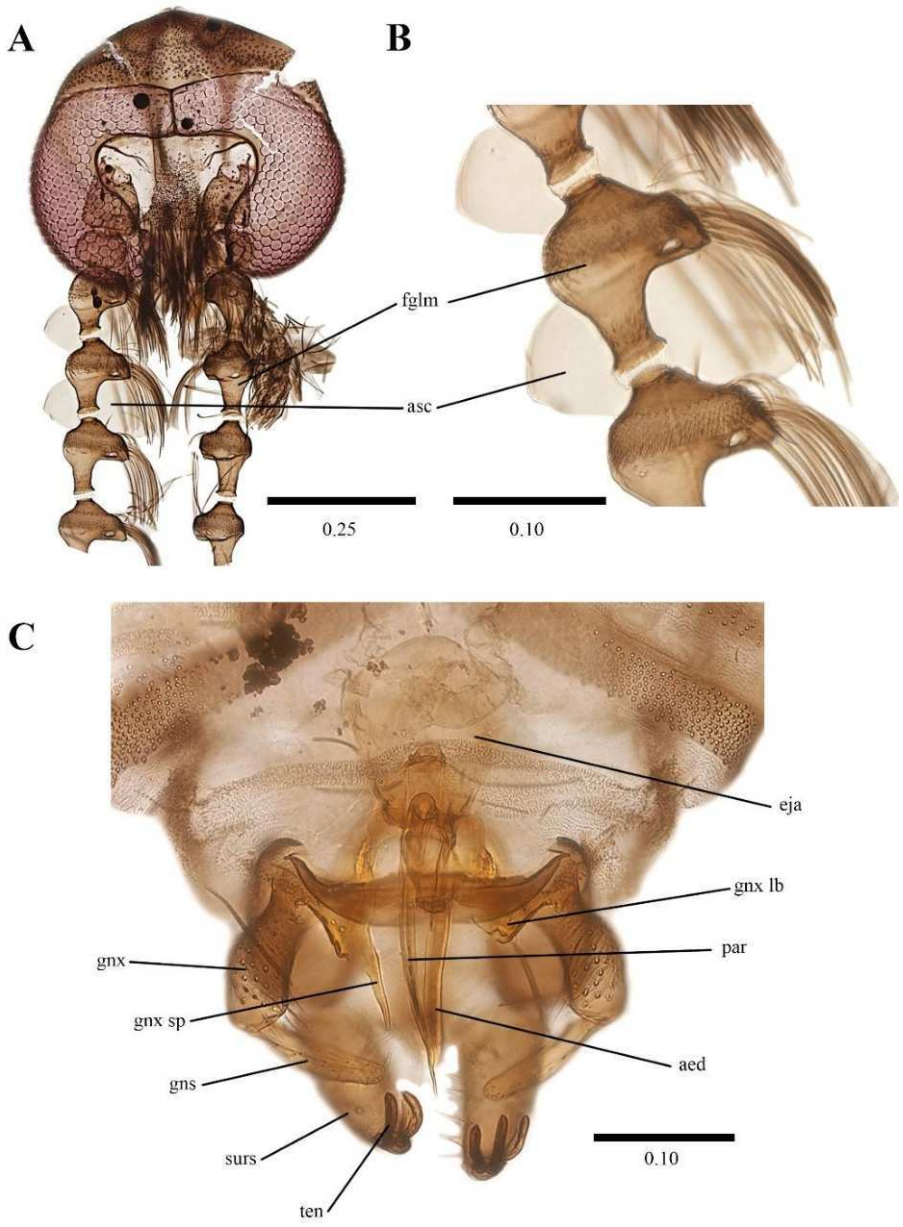
Only known from the type locality in Ecuador (Fig. 1).

Genetics

11 specimens were successfully sequenced: ZFMK-TIS-2629868, ZFMK-TIS-2629902, ZFMK-TIS-2636934, ZFMK-TIS-2637062, ZFMK-TIS-2637064, ZFMK-TIS-2637068, ZFMK-TIS-2637077, ZFMK-TIS-2637115, ZFMK-TIS-2637127, ZFMK-TIS-2637130, ZFMK-TIS-2637149. All eleven obtained sequences are identical. GeneBank accession numbers are: To be added.

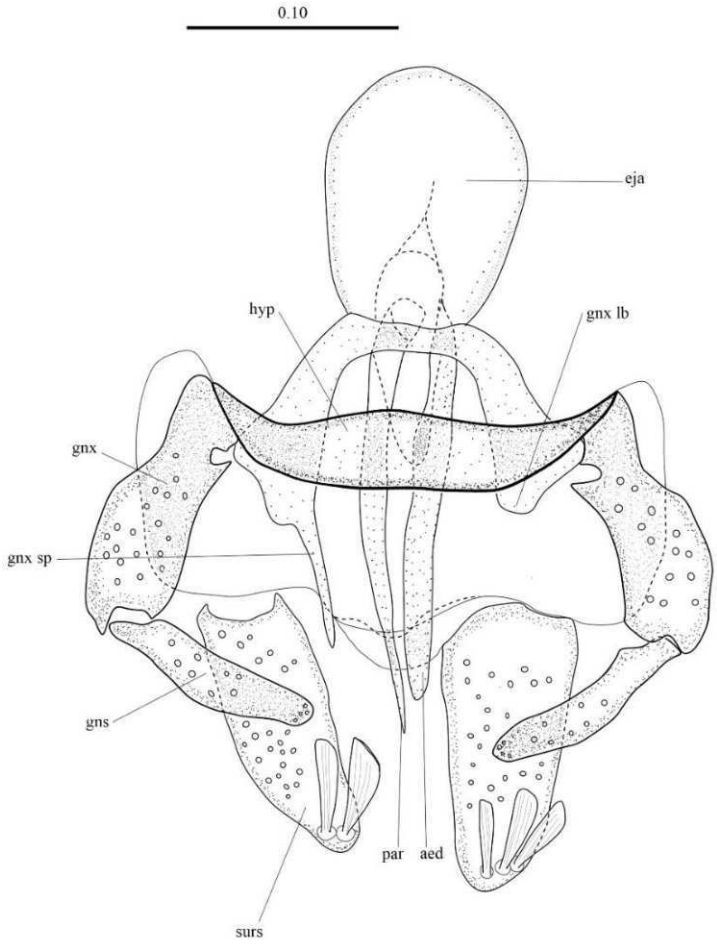
Remarks

309 The holotype and paratypes ZFMK-DIP-00081673, ZFMK-DIP-00102105, and ZFMK-
310 TIS-2637149 have three tenacula in one surstylus and two in the other, while the
311 paratypes ZFMK-DIP-00081664 and ZFMK-DIP-00102106 only present two tenacula on
312 both surstyli (no empty alveoli for a missing tenaculum is distinguishable), which leads
313 us to believe this character is variable. The presence of one tenaculum in one surstylus
314 and two on the other has also been recorded in the holotype of *B. paulistensis* (Bravo &
315 Araujo 2019, fig. 24). Furthermore, the spine in the gonocoxal apodeme is present either
316 on the right side or on the left side, which is also a variable character inside the examined
317 material. Nonetheless, the COI sequence of all specimens is identical, supporting our
318 hypothesis that all the specimens belong to the same species.



319

320 **Fig. 4** *Bryopharsos asymmetricum* Jaume-Schinkel sp. nov., holotype ♂. **A.** Head. **B.**
321 Antennal segments. **C.** Genitalia. Abbreviations: aed = aedeagus; asc = ascoids; eja =
322 ejaculatory apodeme; fglm = flagellomere; gns = gonostylus; gnx = gonocoxite; gnx lb =
323 gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; surs = surstyli; ten = tenacula.
324 All scale bars are in millimeters (mm).



325

326 **Fig. 5** *Bryopharsos asymmetricum* Jaume-Schinkel sp. nov., holotype ♂. Genitalia.
327 Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx =
328 gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; hyp =
329 hypandrium; par = paramere; surs = surstyli. Scale bar in millimeters (mm).

330

331 ***Bryopharsos bifidum* Jaume-Schinkel sp. nov.**

332 (Figs 1, 6–7)

333 **Diagnosis**

Male

Eye bridge with three facet rows; wing 1.9 times longer than its width; ejaculatory apodeme ovoid, with anterior margin straight, slightly longer than the aedeagus; gonocoxal apodeme fused; surstyli with a single apical tenaculum; aedeagus digitiform, curved, with rounded apex. This species shares the same number of apical tenacula in the surstyli but it can be easily differentiated by having the bifurcated gonostyli (no other known *Bryopharsos* species has bifurcated gonostyli).

Female

Unknown.

Etymology

The species epithet *bifidum* derives from from Latin *bifidus* meaning split into two parts, referring to the bifurcate gonostyli. Name to be treated as a noun in apposition.

Material examined

Holotype

VENEZUELA – **Aragua** • 1 ♂; 19 km North of Maracay; 14–17 Oct. 1993; alt. 1280 m; L.W. Quate leg.; Malaise Trap; Mounted in Euparal; LACM [LACM-ENT-279295].

Paratypes

VENEZUELA – **Choroní** • 1 ♂; 22 km South of Choroní, 17 Oct. 1993; alt. 1000 m.; L.W. Quate leg.; Malaise Trap; Mounted in Euparal; LACM [LACM-ENT-279294].

Description

Holotype male. Measurements in mm (n=2) Wing length 2.34 (2.24–2.45), wing width 1.19 (1.17–1.22); head length 0.60 (0.60–0.60), head width 0.79 (0.71–0.86); antennal segments: scape: 0.10 (0.10–0.10), pedicel: 0.08 (0.07–0.08), flagellomere 1 0.14 (0.13–0.14), flagellomeres 2-12: 0.15 (0.14–0.15), flagellomere 13 0.06 (0.06–0.06), flagellomere 14 0.08 (0.08–0.08); palpal segment 1: 0.05 (0.05–0,05), palpal segment 2: 0.05 (0.05–0.05), palpal segment 3: 0.05 (0.05–0.05), palpal segment 4: 0.04 (0.04–0.04).

Head. A little wider than its length; eye bridge contiguous, with three rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, triangular with the lower margin rounded. The antennal scape is slightly longer the length of the pedicel, cylindrical; the pedicel is spherical, shorter than the scape; 14 flagellomeres asymmetrical and nodiform, with scattered setae on the basal half surface, apical flagellomere with terminal apiculus; ascoids absent in examined material. Palpal segments cylindrical, palpal segment 4 apically pointed, palpal proportions: 1.0:1.0:1.0:0.8; labium without any strong sclerite; labella not bulbous with 3–4 setae on outer margin.

Thorax. Without allurement organs; all coxae with a stripe of three to five rows of alveoli. Wing length about 2.1 times its width; wing membrane brown-hyaline; alveoli distributed uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork of R_{2+3} basal to the level of M_{1+2} and joining R_4; fork of M_{1+2} weakly sclerotized; R_5 ending at the wing apex; CuA_2 ending at wing margin.

Terminalia (Figs 6 B–D; 7 A–B). Hypandrium is a distinct band that connects the gonocoxites, plate-like; gonocoxites are cylindrical, about the same length of gonostyli; gonostyli bifurcated, bifurcation occurs around half, both rami have rounded apex, mesal ramus digitiform, lateral ramus ovate; gonocoxal apodeme without anterior projections;

gonocoxal lobes with six setae on each side; aedeagus digitiform, curved, apex rounded, ending beyond the apex of the paramere, paramere digitiform, almost thumb-like, with rounded apex; ejaculatory apodeme with rounded margin, about the same length of the aedeagus; epandrium plate-like, about two times wider than its length; hypoproct tongue-shaped, and covered in small setulae, epiproct broader and shorter than hypoproct; surstyli conical, abruptly narrowing towards the apex, curved dorsally, with a single apical tenaculum, tenaculum with rounded apex.

Distribution

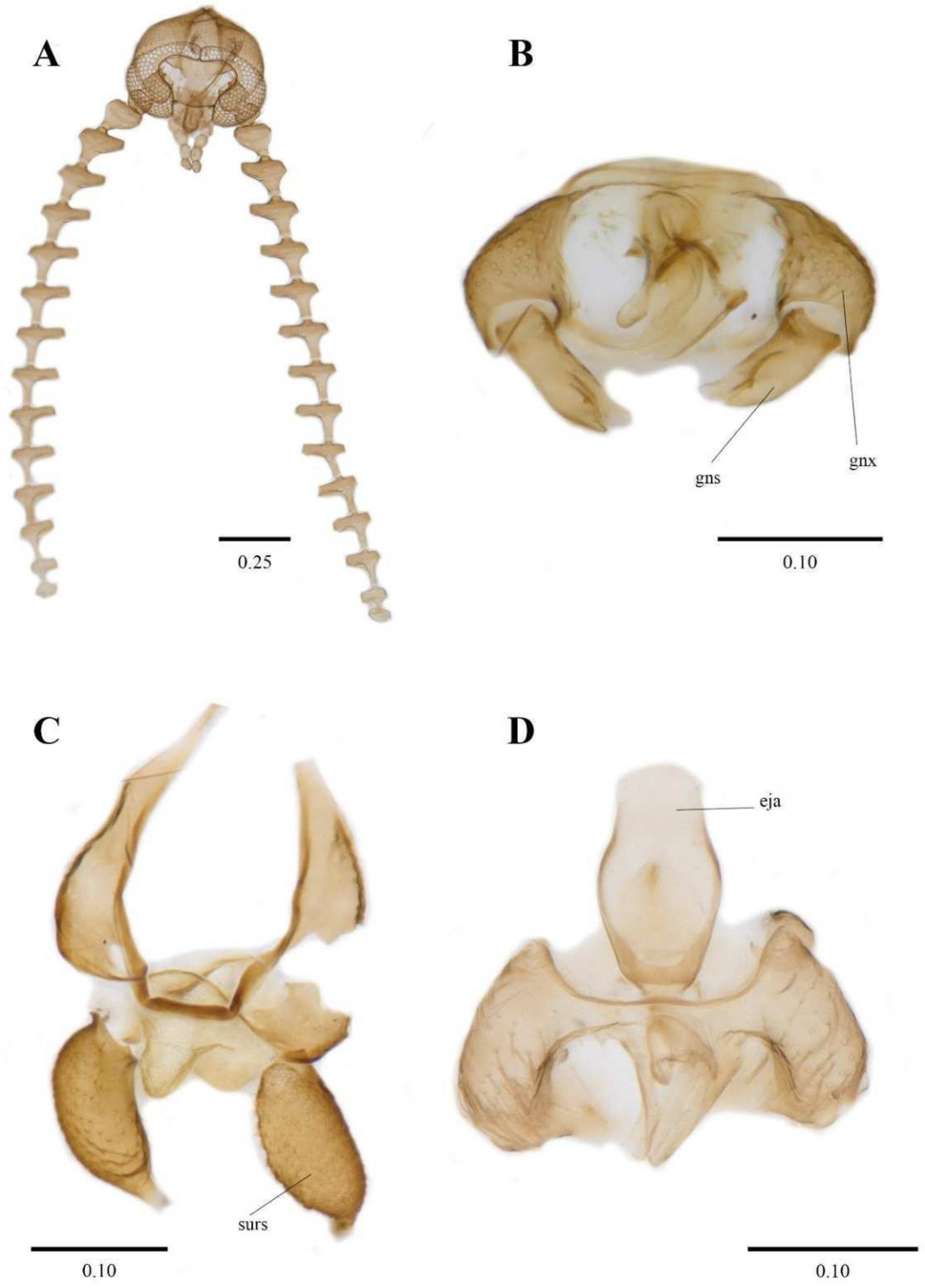
Only known from the type locality in Venezuela (Fig. 1).

Genetics

No specimens were available for DNA extraction.

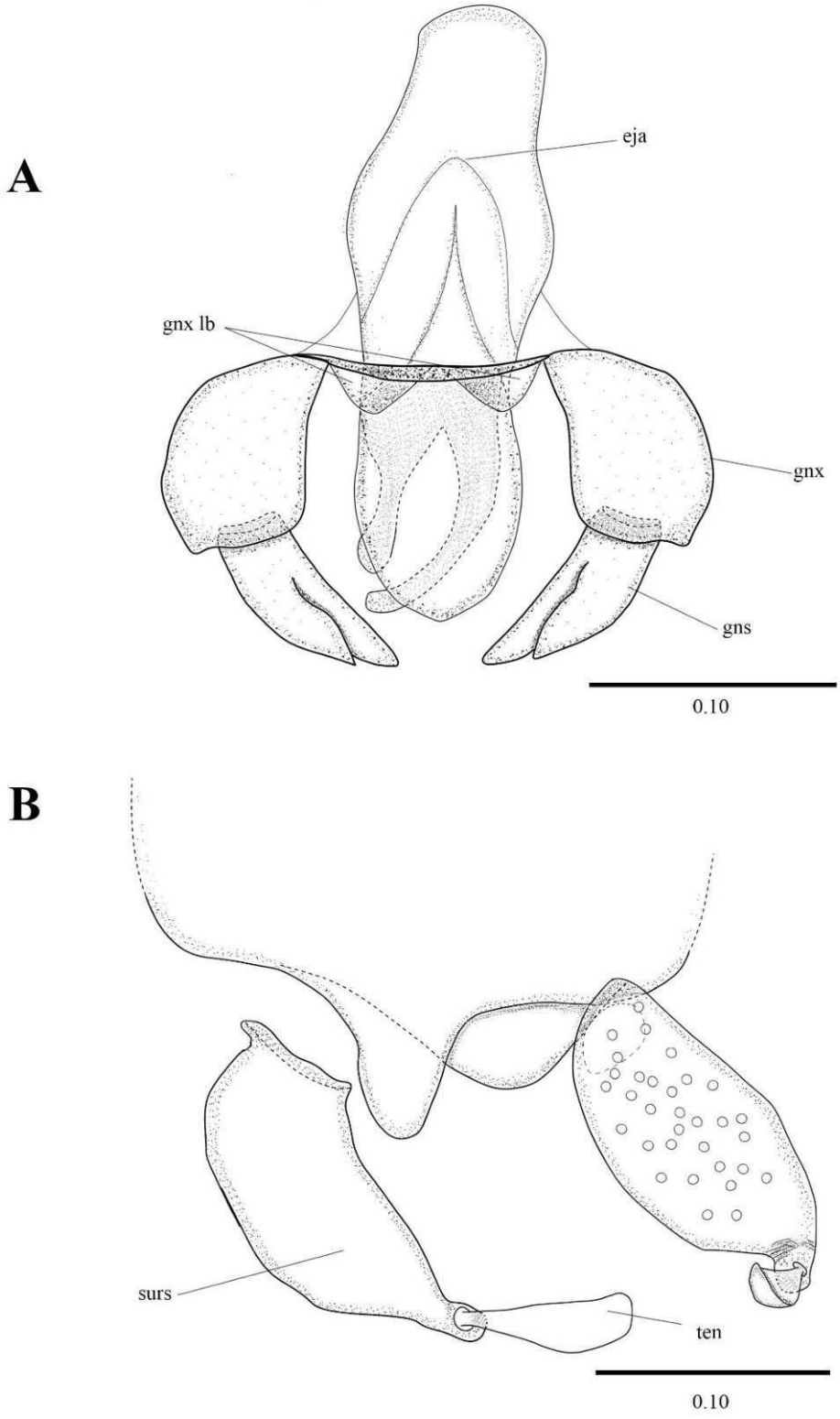
Remarks

Both specimens presented a label with L. W. Quate's handwriting reading "*Bryopharsos bifidum*", this name was never published nor was the description of the species, so we decided to keep the name chosen by Quate as it is fitting for the species.



393

394 **Fig. 6** *Bryopharsos bifidum* Jaume-Schinkel sp. nov., holotype ♂. **A.** Head **B.** Genitalia
395 **C.** Surstyli **D.** Genitalia. Abbreviations: eja = ejaculatory apodeme; gns = gonostylus; gnx
396 = gonocoxite; surs = surstyli. All scale bars are in millimeters (mm).



397

398 **Fig. 7** *Bryopharsos bifidum* Jaume-Schinkel sp. nov., holotype ♂. **A.** Genitalia **B.**
399 Surstyli. Abbreviations: eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite;
400 gnx lb = gonocoxal lobes; surs = surstyli; ten = tenacula. All scale bars are in millimeters
401 (mm).

402

403 ***Bryopharsos bitenacula*** **Jaume-Schinkel sp. nov.**

404 (Figs 1, 8–9)

405 **Diagnosis**

406 Male

407 Eye bridge with three facet rows; wing 2.1 times longer than its width; ejaculatory
408 apodeme ovoid, with anterior margin straight, shorter than the aedeagus; gonocoxal
409 apodeme projecting anteriorly; surstyli with two apical tenacula; aedeagus digitiform,
410 with rounded apex, paramere digitiform and longer than aedeagus. This species shares
411 the same number of apical tenacula in the surstyli as *B. asymmetricum* sp. nov. but it can
412 be easily differentiated by the shape of the paramere (digitiform with pointed apex in *B.*
413 *asymmetricum* sp. nov., digitiform and with rounded apex in *B. bitenacula* sp. nov.), and
414 the length of the paramere (shorter than the aedeagus in *B. bitenacula* sp. nov., longer
415 than the aedeagus in *B. asymmetricum* sp. nov.).

416 Female

417 Unknown.

418 **Etymology**

419 The species epithet derives from the Latin word *bi* meaning two, and tenacula. It makes
420 reference to the two apical tenacula present in the surstyli. To be treated as a noun in
421 apposition.

422 **Material examined**

Holotype

PERU – **Cuzco** • 1 ♂; 26 km wst of Pilcopata; 24 Jul. - 02 Aug. 1997; alt. 1500 m; L.W. Quate leg.; -13.055° N, -71.546667° E; Malaise Trap; Cloud Forest; Mounted in Euparal. LACM [LACM-ENT-279272].

Paratypes

PERU – **Cuzco** • 12 ♂♂; same data as for holotype, LACM [LACM-ENT-279266, LACM-ENT-279267, LACM-ENT-279268, LACM-ENT-279269, LACM-ENT-279270, LACM-ENT-279273, LACM-ENT-279274, LACM-ENT-279275, LACM-ENT-279277, LACM-ENT-279278, LACM-ENT-279290, LACM-ENT-279291] • 1 ♂, same data as for preceding; 25 Jul. - 03 Aug. 1997; LACM[LACM-ENT-279381].

Description

Holotype male. Measurements in mm (n=8) Wing length 2.38 (2.24–2.45), wing width 1.23 (1.17–1.22); head length 0.43 (0.42–0.50), head width 0.54 (0.52–0.68); antennal segments: scape: 0.10 (0.10–0.10), pedicel: 0.08 (0.07–0.08), flagellomere 1: 0.12 (0.12–0.13), flagellomeres 2-12: 0.15 (0.14–0.15), flagellomere 13 0.06 (0.06–0.06), flagellomere 14 0.08 (0.08–0.08); palpal segment 1: 0.04 (0.04–0.04), palpal segment 2: 0.08 (0.07–0.08), palpal segment 3: 0.07 (0.07–0.07), palpal segment 4: 0.06 (0.06–0.06).

Head. A little wider than its length; eye bridge contiguous, with five rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, rectangular with the lower margin straight, upper margin convex. The antennal scape is slightly longer the length of the pedicel, cylindrical; the pedicel is spherical, shorter than the scape; 14

flagellomeres asymmetrical and nodiform, with scattered setae on the basal half surface, apical flagellomere with terminal apiculus; ascoids about the same length as the flagellomere carrying them, and about two times wider than the width of flagellomere carrying them. Palpal segments cylindrical, palpal segment 4 apically pointed, palpal proportions: 1.0:1.8:1.6:1.4; labium without any strong sclerite; labella not bulbous with 3–4 setae on outer margin.

Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli. Wing length about 2.1 times its width; wing membrane brown-hyaline; alveoli distributed uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork of R_{2+3} basal to the level of M_{1+2} and joining R_4; fork of M_{1+2} normally sclerotized; R_5 ending at the wing apex; CuA_2 ending at wing margin.

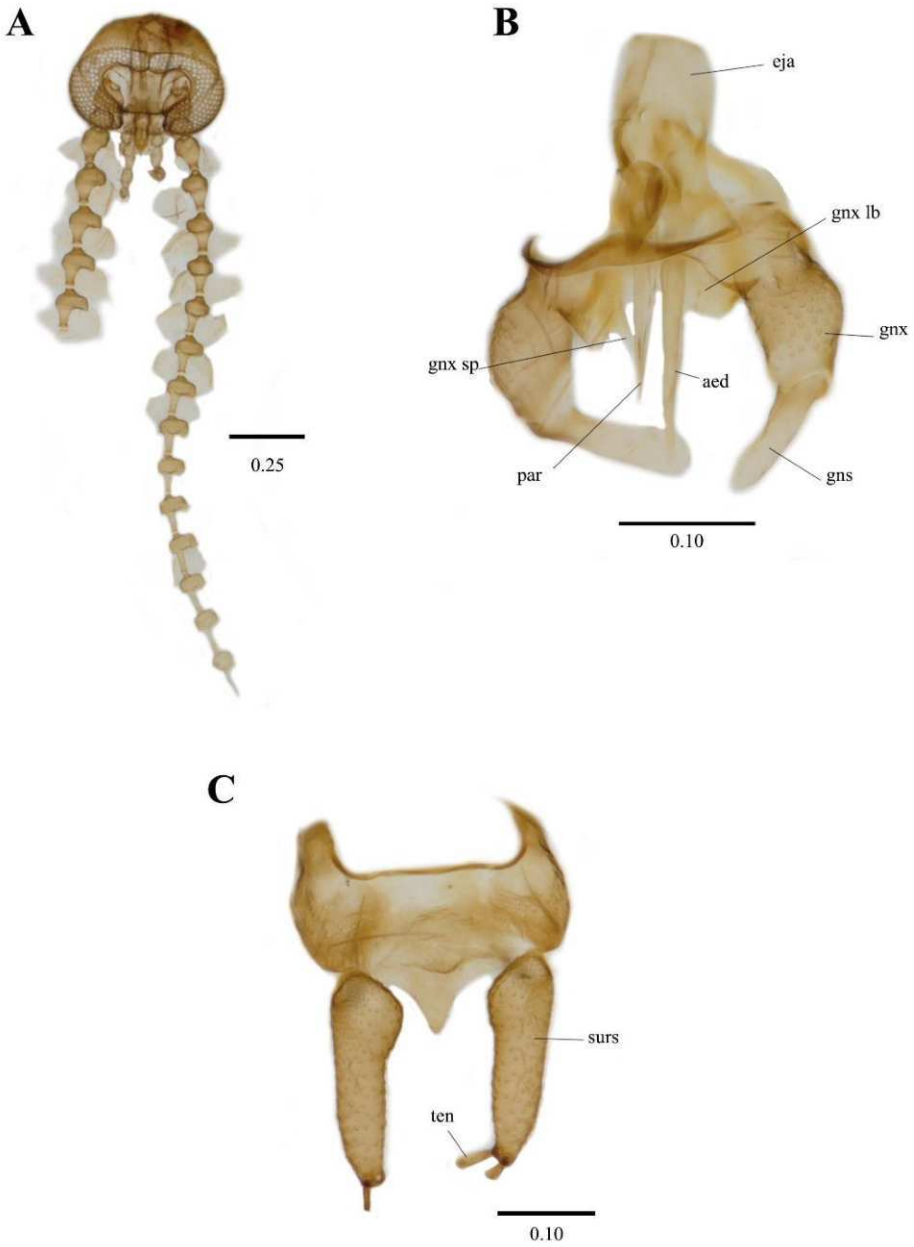
Terminalia (Figs 8 B–C; 9 A–B). Hypandrium is a distinct band that connects the gonocoxites, plate-like; gonocoxites are cylindrical, about the same length as the gonostyli, gonostyli digitiform; gonocoxal apodeme fused; gonocoxal lobes without anterior projections, with 3-5 setae on each side, with gonocoxal spine; aedeagus digitiform with rounded apex, ending beyond the apex of the paramere, paramere digitiform, with rounded apex, about ¾ the length of aedeagus; ejaculatory apodeme with anterior margin straight, shorter than the aedeagus; epandrium rectangular, about two times wider than its length; hypoproct v-shaped, and covered in small setulae, epiproct broader and shorter than hypoproct; surstyli conical narrowing towards the apex, curved dorsally, with two apical tenacula, tenacula with rounded apex.

Distribution

Only known from the type locality in Peru (Fig. 1).

468 **Genetics**

469 No specimens were available for DNA extraction.



470

471 **Fig. 8** *Bryopharsos bitenacula* Jaume-Schinkel sp. nov., holotype ♂. **A.** Head **B.** Genitalia
472 **C.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus;

gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; par = paramere; surs = surstyli; ten = tenacula. All scale bars are in millimeters (mm).

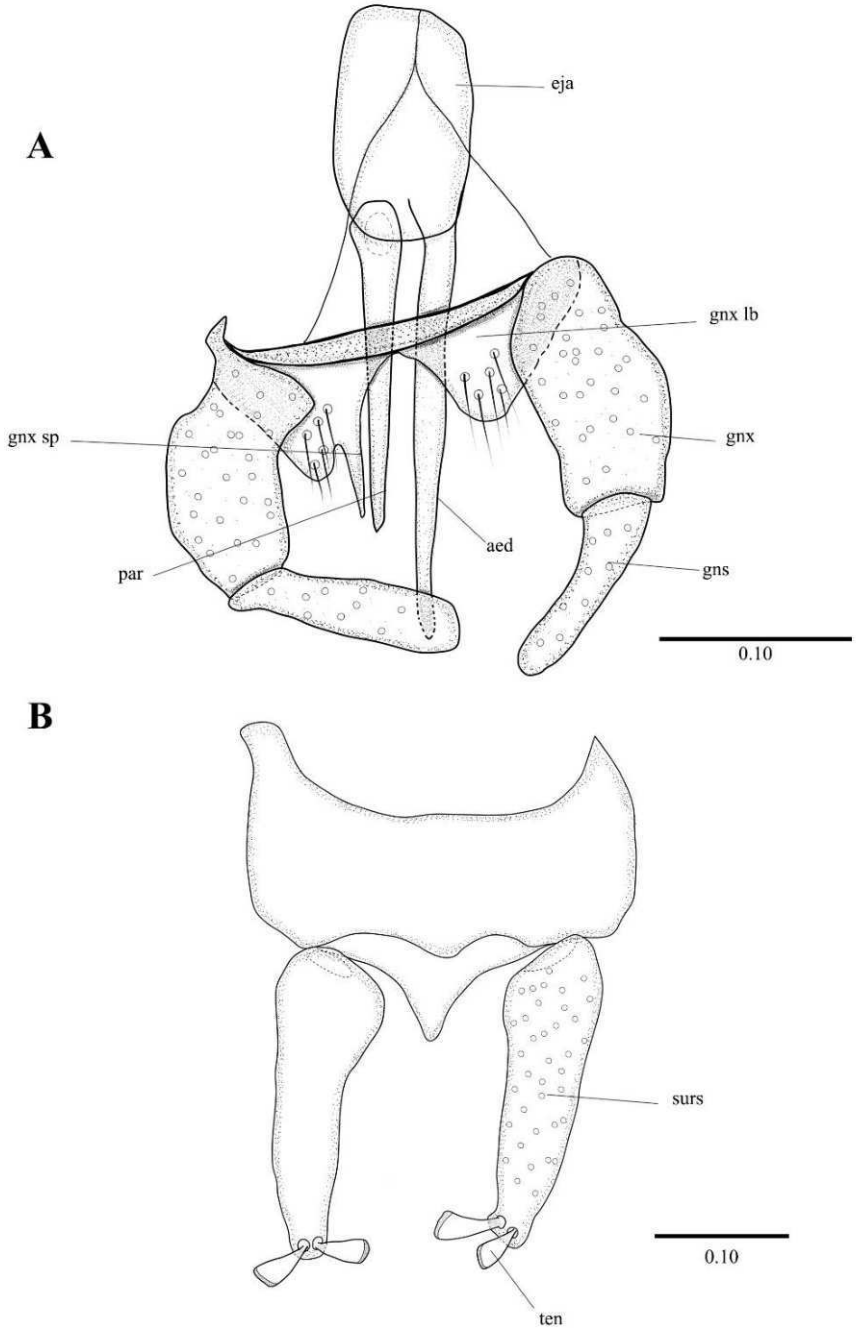


Fig. 9 *Bryopharsos bitenacula* Jaume-Schinkel sp. nov., holotype ♂. **A.** Genitalia **B.** Surstyli.). Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; par = paramere; surs = surstyli; ten = tenacula. All scale bars are in millimeters (mm

480

481 ***Bryopharsos chuspi* Jaume-Schinkel sp. nov.**

482 (Figs 1, 10–11)

483 **Diagnosis**

484 Male

485 Eye bridge with four facet rows; wing 2.8 times longer than its width; ejaculatory
486 apodeme about the same length as aedeagus, with anterior margin rounded, gonocoxal
487 apodeme projecting anteriorly; surstyli with six apical tenacula; aedeagus digitiform,
488 tapering towards apex, paramere digitiform, tapering towards the apex and longer than
489 aedeagus. This species shares the same number of apical tenacula in the surstyli as *B.*
490 *gorgona* **sp. nov.** Still, it can be easily differentiated by the length of the hypandrium
491 (hypandrium length is shorter than aedeagal width in *B. chuspi* **sp. nov.**, hypandrium
492 lenth is longer than aedeagal width) *B. gorgona* **sp. nov.**) and the shape of the epandrium
493 (U-shaped in *B. chuspi* **sp. nov.**, rectangular in *B. gorgona* **sp. nov.**).

494 Female

495 unkown

496 **Etymology**

497 The species epithet *chuspi* derives from the Quechuan word *chuspi* meaning fly. Specific
498 epithet to be treated as a name in apposition.

499 **Material examined**

500 **Holotype**

PERU – **Cuzco** • 1 ♂; 26 km west of Pilcopata; alt. 1500 m; 24 Jul. - 02 Aug. 1997; L. W. Quate leg.; LACM [LACM-ENT-279279].

Paratypes

PERU – **Cuzco** • 12 ♂♂; same data as for holotype; LACM [LACM-ENT-279286, LACM-ENT-279285, LACM-ENT-279284, LACM-ENT-279276, LACM-ENT-279281, LACM-ENT-279282, LACM-ENT-279283, LACM-ENT-279280, LACM-ENT-279202, LACM-ENT-279289, LACM-ENT-279288, LACM-ENT-279287].

Description

Holotype male. Measurements in mm (n=13) Wing length 2.76 (2.50–3.00), wing width 0.94 (0.90–1.00); head length 0.44 (0.42–0.45), head width 0.46 (0.46–0.48); antennal segments: scape: 0.12 (0.11–0.13), pedicel: 0.07 (0.07–0.08), flagellomere 1: 0.12 (0.11–0.12), flagellomeres 2-13: 0.14 (0.14–0.15), flagellomere 14 0.16 (0.16–0.16); palpal segment 1: 0.06 (0.05–0.06), palpal segment 2: 0.06 (0.06–0.07), palpal segment 3: 0.08 (0.08–0.08), palpal segment 4: 0.11 (0.10–0.12).

Head. A little wider than its length; eye bridge contiguous, with four rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, rectangular with concave lateral margins, lower margin straight, upper margin partially divided in the middle. The antennal scape is about two times longer the length of the pedicel, cylindrical; the pedicel is spherical, shorter than the scape; flagellomeres are asymmetrical and nodiform, with scattered setae on the basal half surface, flagellomere 14 with terminal apiculus being two times longer than the elongated part of the flagellomere; ascoids about the same length as the flagellomere carrying them, and about two times wider than the

524 width of flagellomere carrying them. Palpal segments cylindrical, palpal segment 4
525 apically pointed, palpal proportions: 1.0:1.0:1.3:1.8; labium without any strong sclerite;
526 labella not bulbous with 3–4 setae on outer margin.

527 Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli.
528 Wing length about two times its width; wing membrane brown-hyaline; alveoli
529 distributed uniformly on wing membrane; subcostal vein short ending beyond the origin
530 of R_4; fork of R_{2+3} basal to the level of M_{1+2} and joining R_4; fork of M_{1+2} weakly
531 sclerotized; R_5 ending at the wing apex; CuA_2 ending at wing margin.

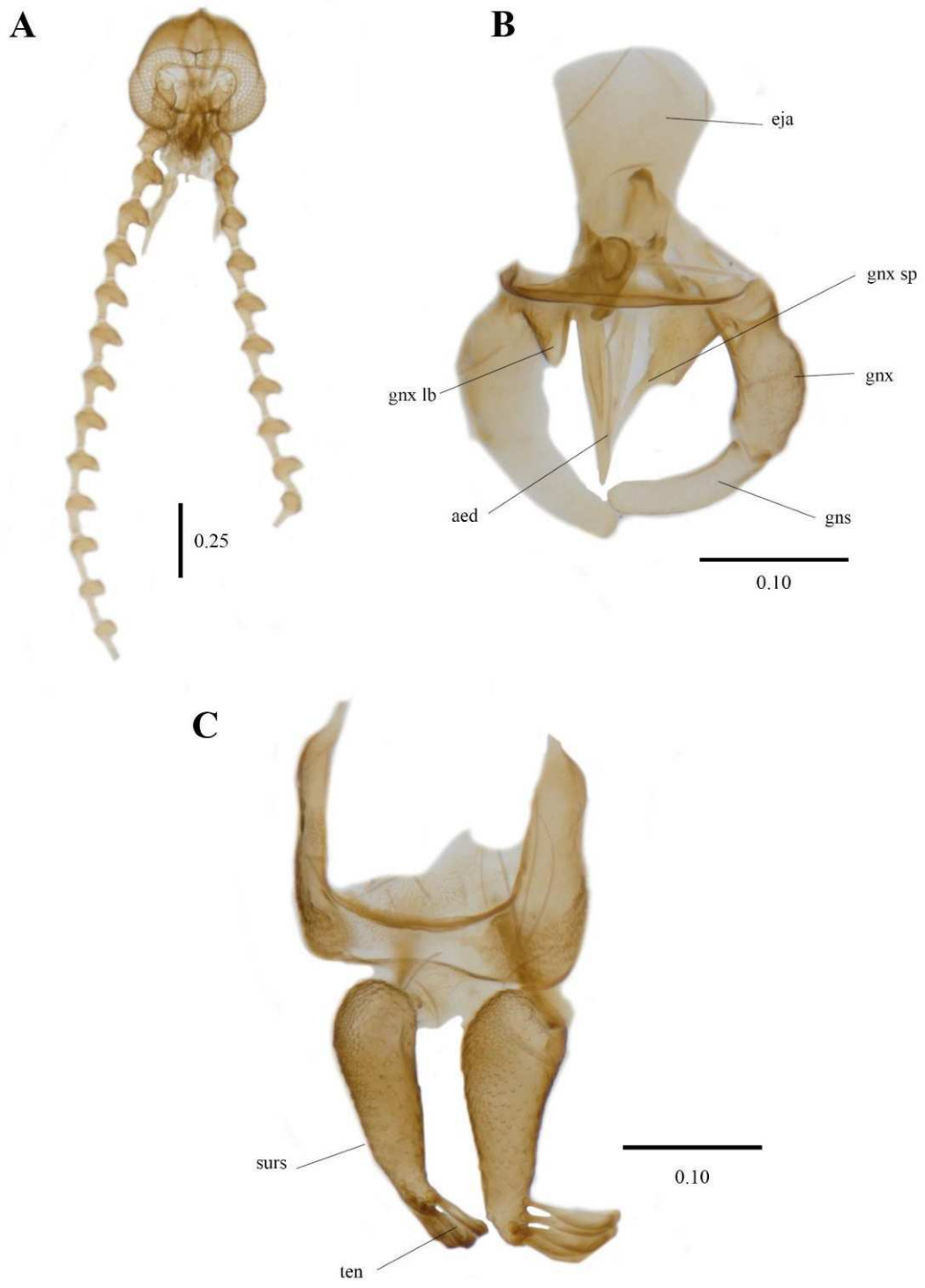
532 Terminalia (Figs. 10 B–C; 11 A–B). Hypandrium is a distinct band that connects the
533 gonocoxites, plate-like; gonocoxites are cylindrical, about two-thirds the length of the
534 gonostyli, gonostyli digitiform; gonocoxal apodeme fused; gonocoxal lobe with 5-6 setae
535 on each side, spine of the gonocoxal lobes present; aedeagus digitiform , with rounded
536 apex, ending at about the same level of the paramere, paramere digitiform, tapering
537 towards apex, with pointed apex; ejaculatory apodeme with anterior margin rounded,
538 about the same length as the aedeagus; epandrium U-shaped,; hypoproct u-shaped, and
539 covered in small setulae, epiproct broader and shorter than hypoproct; surstyli conical
540 narrowing towards the apex, with six apical tenacula, tenacula with rounded apex.

541 **Distribution**

542 Only known from the type locality in Peru (Fig. 1).

543 **Genetics**

544 No specimens were available for DNA extraction.



545

546 **Fig. 10** *Bryopharsos chuspi* Jaume-Schinkel sp. nov., holotype ♂. **A.** Head **B.** Genitalia
547 **C.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus;
548 gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; surs
549 = surstylus; ten = tenacula. All scale bars are in millimeters (mm).

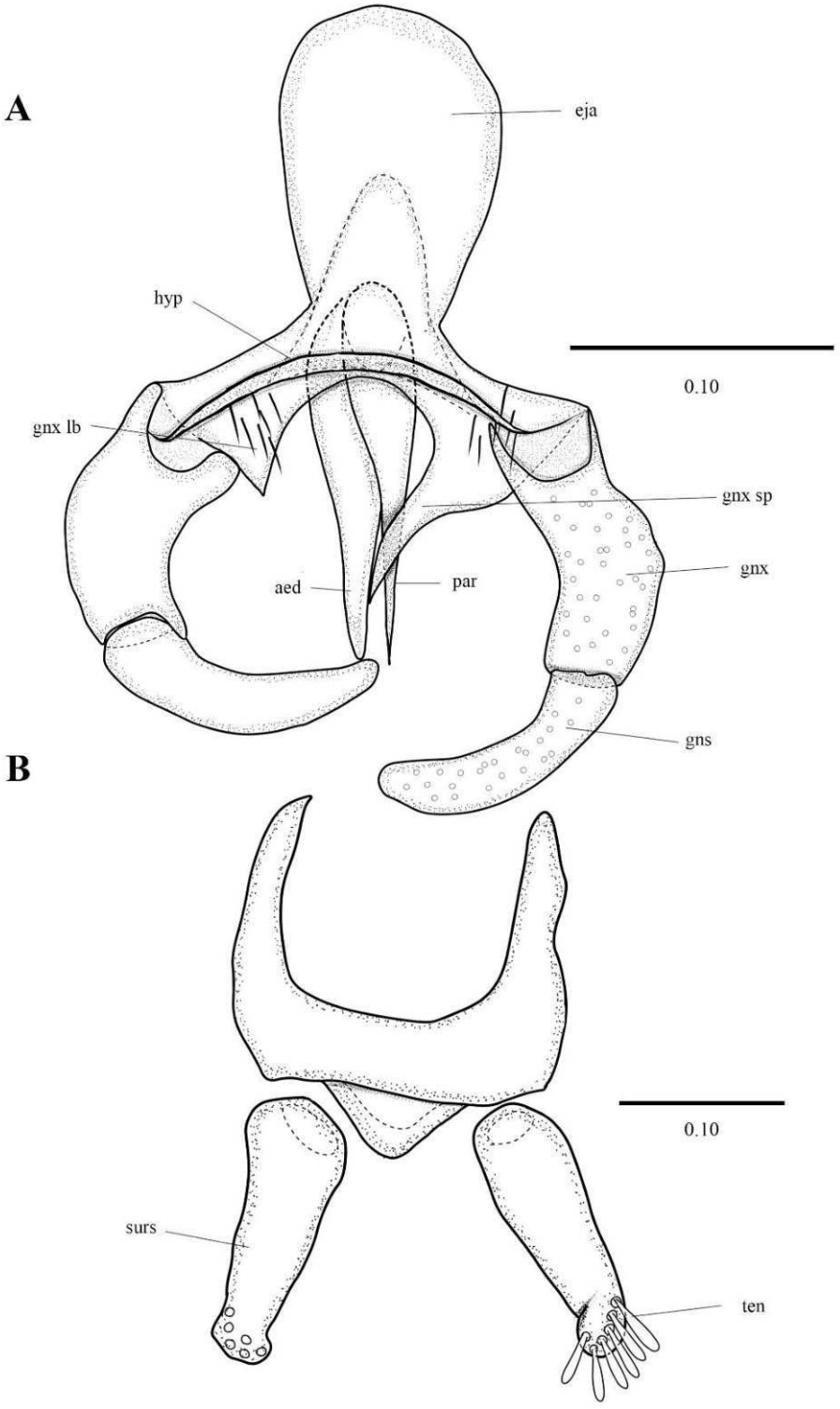


Fig. 11 *Bryopharsos chuspi* Jaume-Schinkel sp. nov., holotype ♂. **A.** Genitalia **B.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of the gonocoxal lobes; hyp = hypandrium; par = paramere; surs = surstylus; ten = tenacula. All scale bars are in millimeters (mm).

556

557 ***Bryopharsos claviformosum*** **Quate, 1996**

558 (Figs 1, 3 C–D)

559 *Bryopharsos claviformosum* Quate, 1996: 41. Type locality: Costa Rica, Heredia,
560 Estación Biológica La Selva [INBio].

561 *Bropharsos claviformosus*: Bravo & Araújo (2019): 370: *Lapsus calami* in identification
562 key.

563 **Diagnosis**

564 Male

565 Eye bridge with four facet rows; wing 2.5 times longer than its width; ejaculatory
566 apodeme rectangular in dorsal view; gonocoxal apodeme with a spine; surstyli with five
567 tenacula. This species is similar to *B. amazonensis* and *B. clavigum* but it can be easily
568 differentiated by the number of tenacula (four in *B. clavigum;* five in *B. amazonensis* and
569 *B. claviformosum*) the length of the tenacula equal length in *B. amazonensis* and *B.*
570 *clavigum*; for long and one shorter in *B. claviformosum*).

571 **Material examined**

572 ECUADOR – **Pichincha** • 2 ♂♂; Pedro Vicente Maldonado, near San Pancracio,
573 roadway to Pachijal, 0.1156° N, -78.9580° E; alt. 750 m; 01–09 Feb. 2022; Kilian, Isabel
574 leg.; ZFMK [ZFMK-DIP-00102088 = ZFMK-TIS-2637066, ZFMK-DIP-00102091 =
575 ZFMK-TIS-2637079]. • 1 ♂ same data as for preceding; 0.115611° N, -78.95805° E; 1–
576 9 Feb. 2022; MECN [ZFMK-TIS-2637137].

577 **Distribution**

578 Costa Rica (Bravo & Araújo, 2019; Quate, 1996) and Ecuador (this publication, new
579 record) (Fig. 1).

580 **Genetics**

581 Three specimens were successfully sequenced: ZFMK-TIS-2637066, ZFMK-TIS-
582 2637079, and ZFMK-TIS-2637137. The maximum intraspecific uncorrected pairwise
583 distance for COI sequences was 0.31 % or 2 bp. GeneBank accession numbers are: To be
584 added.

585 **Remarks**

586 The original description of the male by Quate (1996) is rather short and incomplete, with
587 some important characters missing. Nonetheless, the drawings and the general description
588 are enough to distinguish the males of this species. In the SEM pictures (Fig. 2 C–D), the
589 aedeagus seems curved, with a rounded apex and the paramere seems digitiform and
590 slender, while in the original description, the aedeagus looks straight and tapering towards
591 the apex, while the paramere seems broad. We must point out that for our specimens
592 prepared on permanent slides, the perspective affects the perceived shape of the aedeagus
593 and paramere, making the aedeagus look straight and tapering towards the apex, and the
594 paramere looks digitiform.

595

596 ***Bryopharsos clavigum*** **Quate, 1996**

597 (Figs 1, 12)

Bryopharsos clavigum Quate, 1996: 41. Type locality: Costa Rica, Heredia, Estación Biológica La Selva [INBio].

Diagnosis

Male

Eye bridge with four facet rows; wing 2.5 times longer than its width; ejaculatory apodeme sub-circular in dorsal view; gonocoxal apodeme with spine; surstyli with four tenacula. This species is morphologically similar to *B. amazonensis* and *B. claviformosum* but they can be differentiated by the number of tenacula present in the surstyli (four in *B. clavigum*; five in *B. amazonensis* and *B. claviformosum*).

Female

Unknown.

Material examined

COSTA RICA – **Heredia** • 4 ♂♂; Puerto Viejo de Sarapaqui, Estación Biológica La Selva; 0.11862° N, -78.95802° E; alt. 50-100 m; 15 Dec. 1993; leg.; LACM [LACM-ENT-279401, LACM-ENT-279402, LACM-ENT-279403, INBIO-CRI001470316].

ECUADOR – **Pichincha** • 1 ♂; Pedro Vicente Maldonado, near San Pancracio, roadway to Pachijal, 0.11862° N, -78.95805° E; alt. 770 m; 25–28 Jan. 2020; Kilian, Isabel leg.; MECN [ZFMK-DIP-00082177 = ZFMK-TIS-2628309].

Distribution

Costa Rica (Bravo & Araújo 2019; Quate 1996) and Ecuador (this publication, new record) (Fig. 1).

Genetics

Barcoding for the examined material was unsuccessful.

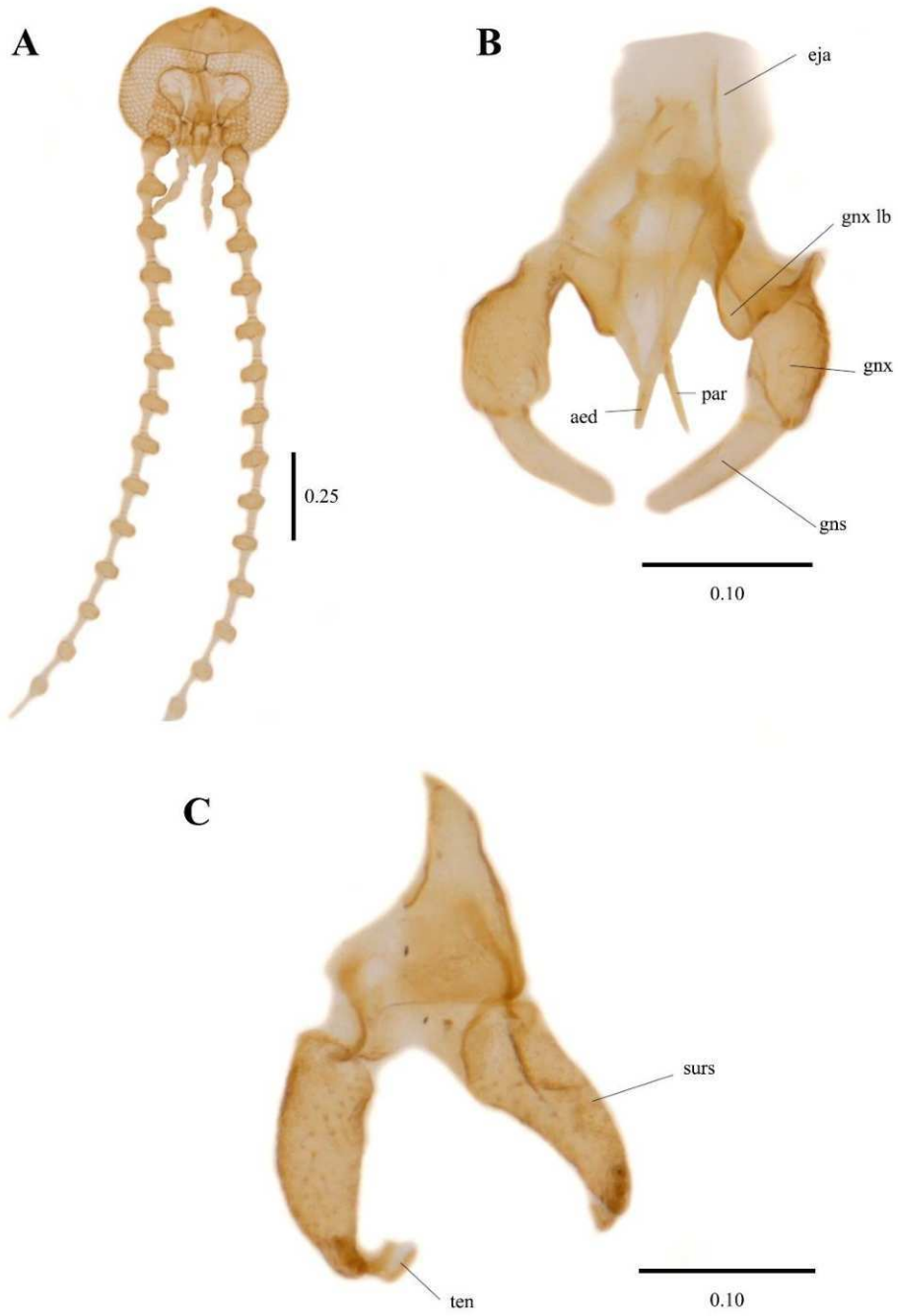


Fig. 12 *Bryopharsos clavigum* Quate, 1996, male. **A.** Genitalia **B.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; par = paramere; surs = surstylus; ten = tenacula. All scale bars are in millimeters (mm).

Bryopharsos curvum **Jaume-Schinkel sp. nov.**

(Figs 1, 13)

Diagnosis

Male

Eye bridge with three facet rows; wing 1.9 times longer than its width; ejaculatory apodeme cylindrical and hour-glass-shaped, about the same length as aedeagus; gonocoxal apodeme fused; surstyli with one tenaculum; aedeagus digitiform, out curved and evenly tapering towards apex. This species shares the same number of apical tenaculum in the surstyli with *B. uncinatum* and *B. paulistensis*. Still, it can be easily differentiated by the number of rows of facets in the eye bridge (three in ***B. curvum*** **sp. nov.**, four in *B. unicnatum,* and five in *B. paulistensis*).

Female

Unknown.

Etymology

From latin *curvus* (neuter curvum). Making reference to the out-curved gonocoxites, as well as the curved aedeagus and paramere. Species epithet to be treated as a noun in apposition.

Material examined

Holotype

COLOMBIA – **Magdalena** • 1 ♂; Sierra Nevada de Santa Marta. El Ramo, alt. 2400 m. Malaise Trap. 10-24.VI.2000. I. Uribe. LACM [LACM-ENT-279396].

Description

Holotype male. Measurements in mm (n=1) Wing length 2.47, wing width 1.28; head length 0.50, head width 0.60; antennal segments: scape: 0.10, pedicel: 0.06, flagellomeres 1-4: 0.11 (0.11-0.12); palpal segment 1: 0.04, palpal segment 2: 0.8, palpal segment 3: 0.07, palpal segment 4: 0.05.

Head. A little wider than its length; eye bridge contiguous, with three rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, triangular with lower margin straight. Antennal scape about two times the length of the pedicel, slightly broader than its width; pedicel spherical, smaller than scape; flagellomeres asymmetrical and nodiform, with scattered setae on the basal half surface, apical flagellomeres absent in examined material, the maximum number of flagellomeres present is four; ascoids absent in examined material. Palpal segments cylindrical, palpal segment 4 with pointed apex, palpal proportions: 1.0:2.0:1.7:1.2; labium without strong sclerites; labella not bulbous with 3–4 setae on outer margin.

Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli. Wing length about 1.9 times its width; wing membrane brown-hyaline; alveoli distributed uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork of R_{2+3} at the same level of M_{1+2} and joining R_4; fork of M_{1+2} weak; R_5 ending at the wing apex; CuA_2 ending at wing margin.

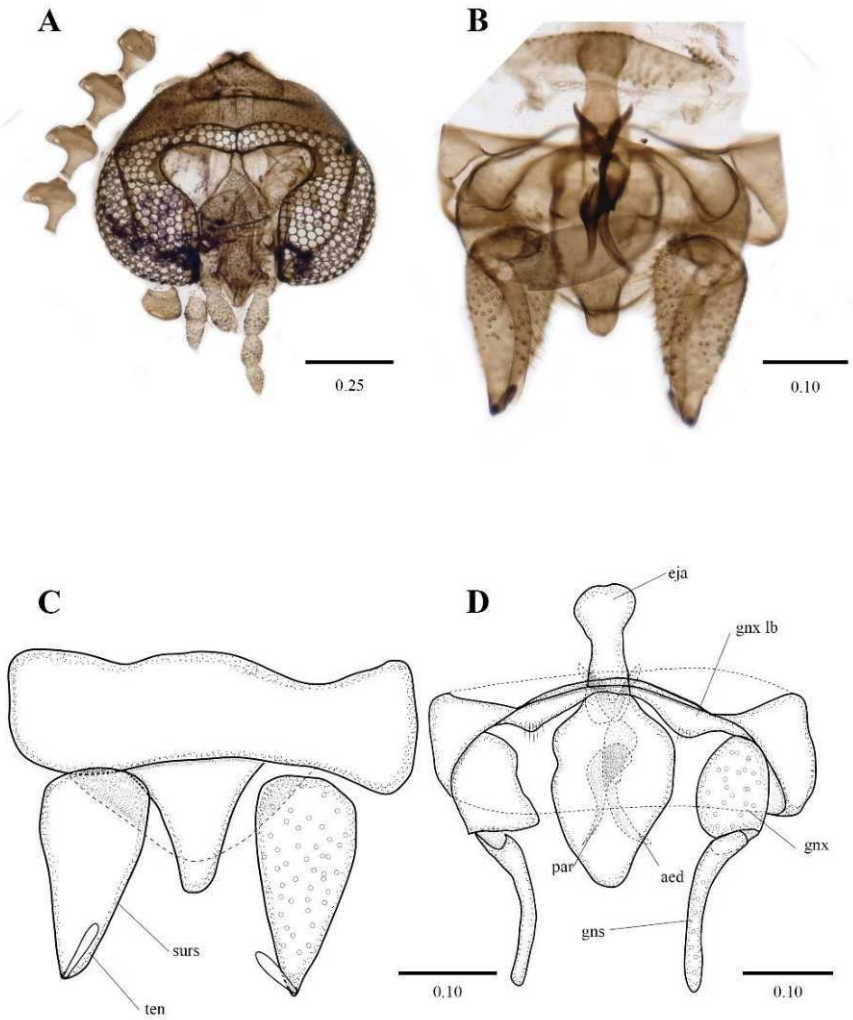
Terminalia (Fig. 13 B–D). Hypandrium is a distinct band that connects the gonocoxites, plate-like; gonocoxites cylindrical, shorter than gonostyli, gonostyli digitiform and out-curved, with rounded-blunt apex; aedeagus digitiform and out curved, evenly narrowing towards the apex, apex pointed, ending at the level of the apex of the paramere, paramere digitiform and out curved, paramere evenly narrowing towards the apex, apex pointed; ejaculatory apodeme almost cylindrical, waisted and resembling a broad hour-glass shape, about the same length of the aedeagus; gonocoxal apodemes fused; epandrium like a narrow rectangle, about four times wider than its length; hypoproct tongue-shaped, longer than epandrium and covered in small setulae, epiproct shorter than hypoproct; surstyli conical, evenly tapering towards the apex, with one apical tenaculum, tenaculum with rounded apex.

Distribution

Only known from the type locality in Colombia (Fig. 1).

Genetics

No specimens were available for DNA extraction.



683

684 **Fig. 13** *Bryopharsos curvum* Jaume-Schinkel sp. nov., male holotype. **A.** Head **B.**
685 Genitalia **C.** Surstyli **D.** Genitalia. Abbreviations: aed = aedeagus; eja = ejaculatory
686 apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; par = paramere;
687 surs = surstylus; ten = tenacula. All scale bars are in millimeters (mm).

688

689 ***Bryopharsos gorgona*** **Jaume-Schinkel sp. nov.**

690 (Figs 1, 14)

691 **Diagnosis**

Male

Eye bridge with four facet rows; wing 2.5 times longer than its width; ejaculatory apodeme short, with anterior margin straight, shorter than the aedeagus; gonocoxal apodeme projecting anteriorly; surstyli with six apical tenacula; aedeagus digitiform, with rounded apex, paramere digitiform and longer than aedeagus. This species shares the same number of apical tenacula in the surstyli as *B. gorgona* **sp. nov.** but it can be easily differentiated by the length of the hypandrium (hypandrium lengh is longer than aedeagal width in *B. gorgona* **sp. nov.**, hypandrium lengh is shorter than aedeagal width in *B. chuspi* **sp. nov.**), and the shape of the epandrium (rectangular in *B. gorgona* **sp. nov.**, U-shaped in *B. chuspi* **sp. nov.**).

Female

Unknown.

Etymology

The species epithet *gorgona* derives from the name of the type locality (Gorgona Island). To be treated as a name in apposition

Material examined

Holotype

COLOMBIA – **Cauca** • 1 ♂; Guapí, Gorgona Island, alta El Mirador; alt. 180 m; 4 to 24 Mar. 2000; R. Doque leg.; 2.9689° N, -78.1856° E; LACM [LACM-ENT-279394].

Paratypes

712 COLOMBIA – **Cauca** • 2 ♂♂; same data as for holotype; LACM [LACM–ENT-
713 279395, LACM-ENT-279388].

714 **Description**

715 Holotype male. Measurements in mm (n=3) Wing length 2.27 (2.204–2.55), wing width
716 0.90 (0.85–0.96); head length 0.40 (0.40–0.40), head width 0.45 (0.45–0.45); antennal
717 segments: scape: 0.10 (0.10–0.10), pedicel: 0.06 (0.06–0.06), flagellomere 1 0.11 (0.11–
718 0.12), flagellomeres 2-9: 0.12 (0.12–0.12), palpal segment 1: 0.05 (0.05–0.06), palpal
719 segment 2: 0.07 (0.07–0.07), palpal segment 3: 0.07 (0.07–0.07), palpal segment 4: 0.08
720 (0.08–0.08).

721 Head. A little wider than its length; eye bridge contiguous, with four rows of facets,
722 interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from
723 the remaining alveoli on the head; the frontal patch of alveoli not divided, rectangular
724 with the lower and upper margins straight. The antennal scape is about two times longer
725 the length of the pedicel, cylindrical; the pedicel is spherical, shorter than the scape;
726 flagellomeres are asymmetrical and nodiform, with scattered setae on the basal half
727 surface, apical flagellomeres absent, the maximum number of flagellomeres present in
728 examined material: 9; ascoids absent in examined material. Palpal segments cylindrical,
729 palpal segment 4 apically pointed, palpal proportions: 1.0:1.3:1.3:1.5; labium without any
730 strong sclerite; labella not bulbous with 3–4 setae on the outer margin.

731 Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli.
732 Wing length about two times its width; wing membrane brown-hyaline; alveoli
733 distributed uniformly on wing membrane; subcostal vein short ending beyond the origin

734 of R_4; fork of R_{2+3} basal to the level of M_{1+2} and joining R_4; fork of M_{1+2} weakly
735 sclerotized; R_5 ending at the wing apex; CuA_2 ending at wing margin.

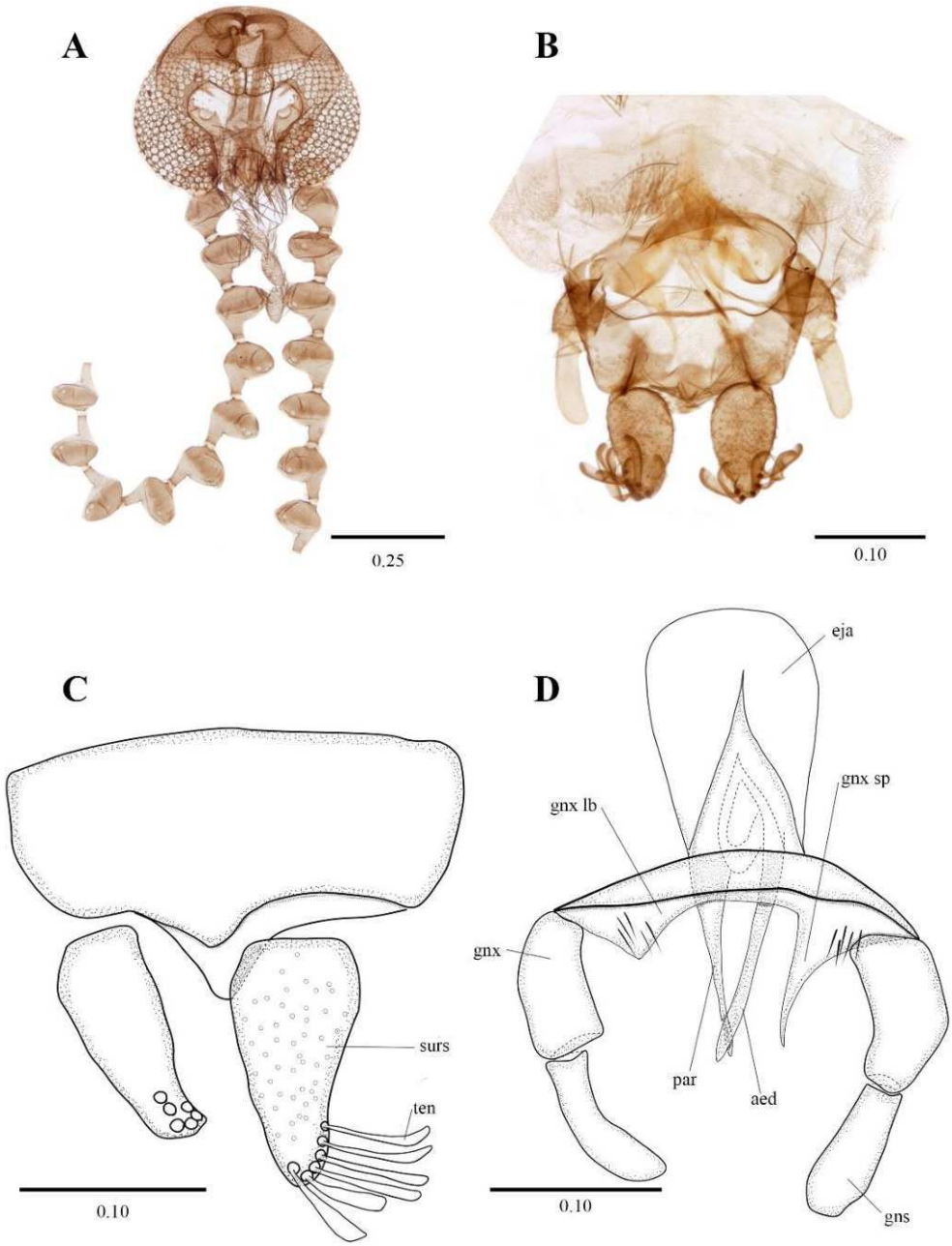
736 Terminalia (Fig. 14 B–D). Hypandrium is a distinct band that connects the gonocoxites,
737 plate-like; gonocoxites are cylindrical, about two-thirds the length of the gonostyli,
738 gonostyli digitiform; gonocoxal lobes without anterior projection, fused, and with 4-5
739 setae on each side; aedeagus digitiform with rounded apex, ending slightly after the apex
740 of the paramere, paramere digitiform, evenly tapering towards apex, with pointed apex ;
741 ejaculatory apodeme with anterior margin straight, about the same length of the aedeagus;
742 epandrium rectangular; hypoproct v-shaped, and covered in small setulae, epiproct
743 broader and shorter than hypoproct; surstyli conical narrowing towards the apex, with six
744 apical tenacula, tenacula with rounded apex.

745 **Distribution**

746 Only known from the type locality in Colombia (Fig. 1).

747 **Genetics**

748 No specimens were available for DNA extraction.



749

750 **Fig. 14** *Bryopharsos gorgona* Jaume-Schinkel sp. nov., male holotype. **A.** Head **B.**
751 Genitalia **C.** Surstyli **D.** Genitalia. Abbreviations: aed = aedeagus; eja = ejaculatory
752 apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine
753 of the gonocoxal lobes; par = paramere; surs = surstyli; ten = tenacula. All scale bars are
754 in millimeters (mm).

755

***Bryopharsos insperatus* Jaume-Schinkel sp. nov.**

(Figs 1, 15–16)

Diagnosis

Male

Eye bridge with four facet rows; wing 2.2 times longer than its width; ejaculatory apodeme cylindrical and hour-glass-shaped, about the same length as aedeagus; gonocoxal apodeme fused; surstyli with one tenaculum; aedeagus digitiform, out curved and evenly tapering towards apex. This species shares the same number of apical tenaculum in the surstyli (one apical tenaculum) with *B. uncinatum* and *B. paulistensis*. Still, it can be easily differentiated by the number of rows of facets in the eye bridge (three in *B. insperatus* sp nov.*,* four in *B. uncinatum,* and five in *B. paulistensis*).

Female

Unknown.

Etymology

The species epithet *insperatus* derives from the Latin word *insperatus* meaning unexpected, referring to unexpectedly finding a new species while looking in the collections. Name to be treated as an adjective.

Material examined

Holotype

775 COSTA RICA – **Heredia** • 1 ♂; Limon Rs. Biol. Hitoy Cerere. 17-26.II.1999. Rio
776 Cerrere, sidestream 100-200 m. Malaise Trap. L.W. Quate. 9.806667° N, -83.0175° E
777 LACM [no specimen number].

778 **Description**

779 Holotype male. Measurements in mm (n=1) Wing length 2.87, wing width 1.29; head
780 length 0.52, head width 0.61; antennal segments: scape: 0.14, pedicel: 0.09,
781 flagellomeres1: 0.18, flagellomeres 2-12: 0.20; palpal segment 1: 0.07, palpal segment 2:
782 0.10, palpal segment 3: 0.10, palpal segment 4: 0.20.

783 Head. A little wider than its length; eye bridge contiguous, with four rows of facets,
784 interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from
785 the remaining alveoli on the head; the frontal patch of alveoli not divided, triangular with
786 lower margin straight. Antennal scape about two times the length of the pedicel,
787 cylindrical; pedicel spherical, smaller than scape; flagellomeres asymmetrical and
788 nodiform, with scattered setae on the basal half surface, apical flagellomeres absent in
789 examined material, the maximum number of flagellomeres present is 12; ascoids
790 rectangular and broad, about the same length, and about two times the width of the
791 flagellomere carrying them. Palpal segments cylindrical, palpal segment 4 apically
792 pointed, with bilobed apex, palpal proportions: 1.0:1.4:1.4:2.8; labium without any strong
793 sclerite; labella not bulbous with 3–4 setae on outer margin.

794 Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli.
795 Wing length about 2.2 times its width; wing membrane brown-hyaline; alveoli distributed
796 uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork
797 of R_{2+3} at the basal to the level of M_{1+2} and joining R_4; fork of M_{1+2} weakly sclerotized,

798 almost appears as it is not joining; R_5 ending at the wing apex; CuA_2 ending at wing
799 margin.

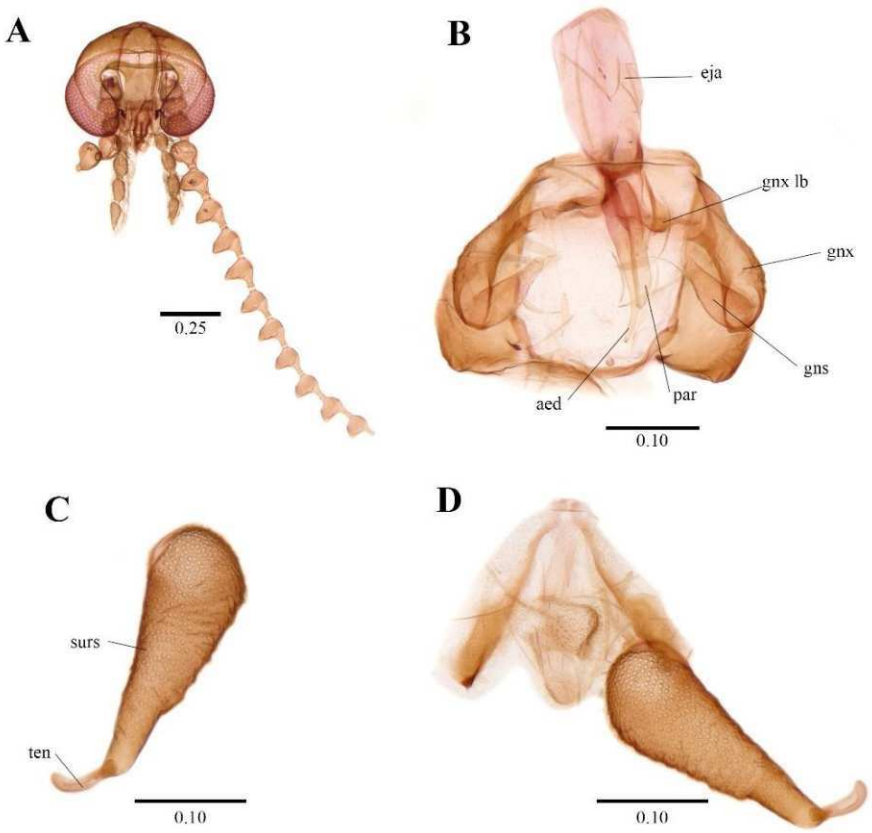
800 Terminalia (Figs. 15 B–D; 16 A–B). Hypandrium is a distinct band that connects the
801 gonocoxites, plate-like; gonocoxites cylindrical, longer than gonostyli, gonostyli conical,
802 with rounded-blunt apex; gonocoxal apodeme not anteriorly projected; gonocoxal lobes
803 posteriorly projected on each side, each lobe with five setae; aedeagus digitiform and out
804 curved, evenly narrowing towards the apex, apex rounded, and ending beyond the apex
805 of the paramere, paramere digitiform, broader than aedeagus, with apex rounded;
806 ejaculatory apodeme with anterior margin round, about the same length of the aedeagus;
807 epandrium not discernable in examined material, but appears U-shaped; hypoproct
808 tongue-shaped, and covered in small setulae, epiproct shorter than hypoproct; surstyli
809 conical, slightly tapering towards the apex and curved dorsally, with one apical
810 tenaculum, tenaculum with rounded apex.

811 **Distribution**

812 Only known from the type locality in Costa Rica (Fig. 1).

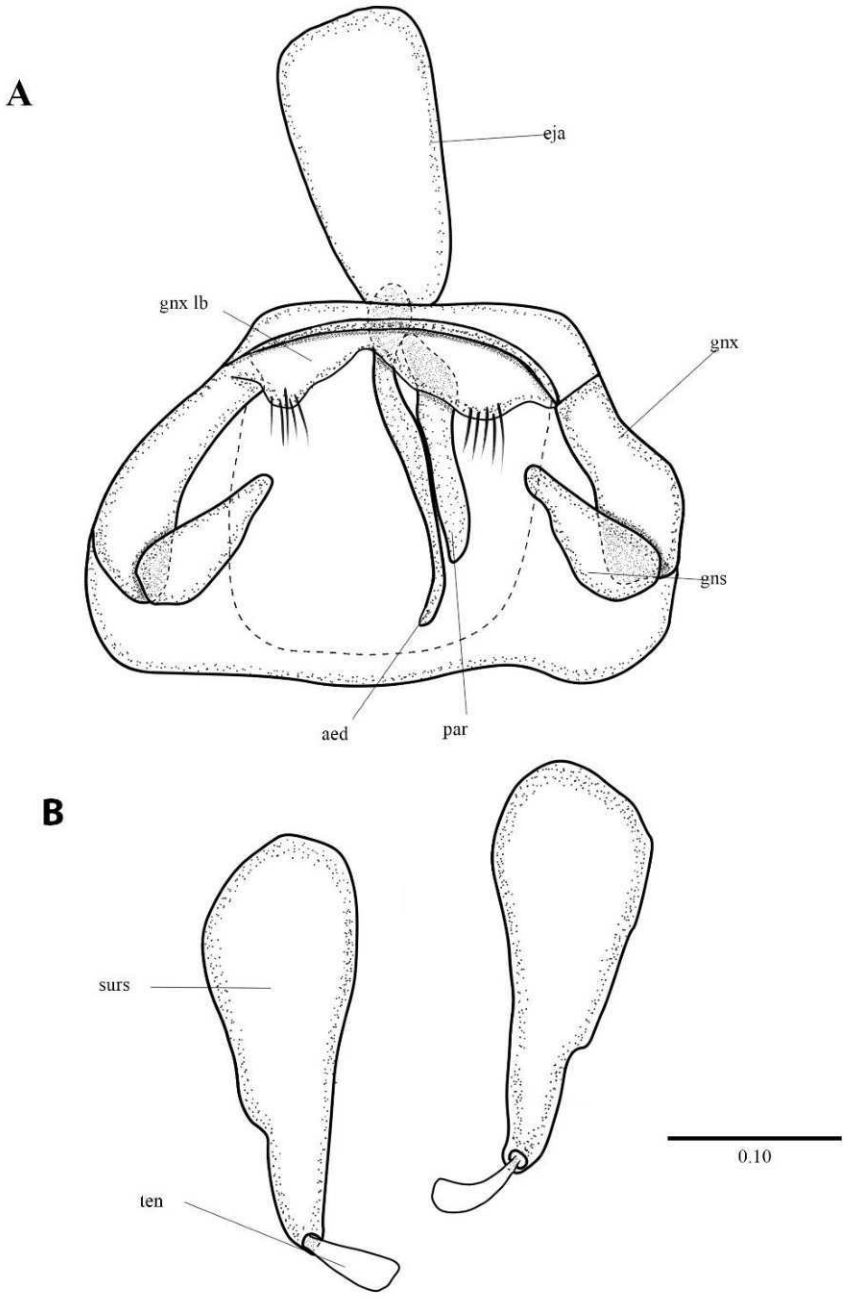
813 **Genetics**

814 No specimens were available for DNA extraction.



815

816 **Fig. 15** *Bryopharsos inesperatus* Jaume-Schinkel sp. nov., male holotype. **A.** Head **B.**
817 Genitalia **C.** Surstylus **D.** Surstylus. Abbreviations: aed = aedeagus; eja = ejaculatory
818 apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; par = paramere,
819 surs = surstyli; ten = tenacula. All scale bars are in millimeters (mm).



820

821 **Fig. 16** *Bryopharsos inesperatus* Jaume-Schinkel sp. nov., male holotype. **A.** Genitalia
822 **B.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus;
823 gnx = gonocoxite; gnx lb = gonocoxal lobes; par = paramere; surs = surstyli; ten =
824 tenacula. All scale bars are in millimeters (mm).

825

Bryopharsos palpiculum Quate, 1996

(Figs 1, 17–18)

Bryopharsos palpiculum Quate, 1996: 41. Type locality: Costa Rica, Heredia, Estación Biológica La Selva [INBio].

Diagnosis

Male

Eye bridge with five facet rows; wing 2.4 times longer than its width; ejaculatory apodeme sub-circular in dorsal view; gonocoxal apodeme with a spine-shaped projection; surstyli with four tenacula; aedeagus long and straight, narrowing at the apex. This species shares five face rows with *B. paulistensis* but they can be easily differentiated by the number of tenacula on the surstyli (four in *B. palpiculum*; one in *B. paulistensis*).

Female

[Adapted from Quate (1999)] Subgenital plate with slender constriction before small apical lobes, triangular in shape before constriction; membranous plate indistinct; genital chamber faint, without distinct structure, appears as a spherical structure partially divided apically [see Quate (1999: 434–435, Fig. 7C)].

Redescription

Female. Head about the same length as its width, eye bridge contiguous with four facet rows; interocular suture absent; five enlarged post-ocular alveoli on each lateral margin; the frontal patch of alveoli undivided, triangular with rounded vertices. Antennal scape about 1.5 times the length of the pedicel, cylindrical; pedicel spherical, shorter than scape;

847 flagellomeres smaller than male flagellomeres, asymmetrical-podiform, apical
848 flagellomeres missing in examined material; palpal segments cylindrical, apical palpal
849 segment with pointed apex; palpal proportions: 1.0:1.2:1.2:1.5.

850 Thorax without allurement organs. Wing. 2.3 times longer than its width, wing membrane
851 brown-hyaline, alveoli distributed uniformly on wing membrane; subcostal vein short
852 ending beyond the origin of R_4; fork of R_{2+3} at the same level of M_{1+2} and joining R_4; fork
853 of M_{1+2} weak; R_5 ending at the wing apex; CuA_2 ending at wing margin.

854 Tergite nine with apical lobes (Fig. 18 A), broad and digitiform, about the same length as
855 tergite nine; subgenital plate membranous except for apical lobes which are sclerotized,
856 basal margin round, with a constriction before apical lobes; genital chamber membranous,
857 barely visible; cerci short, about the same length as the subgenital plate.

858 Egg (Fig. 18 B). Length 0.336 ± 0.020 mm. Width 0.14 ± 0.032 mm (n = 8). No
859 exochorion sculptures where observed along the long axis of the egg. No aeropiles were
860 observed in the posterior pole. The anterior pole presents a cylindricall projection of about
861 0.02 mm, with a truncated apex, without exochorion sculptures.

862 **Material examined**

863 COSTA RICA – **Heredia** • 6 ♂♂; Puerto Viejo de Sarapaqui, Estación Biológica La
864 Selva; 0.11862° N, -78.95802° E; alt. 50-100 m; 15 Dec. 1993; leg.; INBIO
865 CRI001470484, INBIO CRI001470634, INBIO CRI001470595, INBIO CRI001470134,
866 INBIO CRI001470343, INBIO CRI001470240 [LACM].

867 ECUADOR – **Pichincha** • 1 ♂; Pedro Vicente Maldonado, near San Pancracio, roadway
868 to Pachijal, 0.11862° N, -78.95805° E; alt. 770 m; 25-28 Jan. 2020; Kilian, Isabel leg.;

MECN [ZFMK-DIP-00082179 = ZFMK-TIS-2628311] • 1 ♀; same data as for preceding; ZFMK [ZFMK-DIP-00081666 = ZFMK-TIS-2629904]. • 2 ♂♂; same data as for preceding; 0.1156° N, -78.9580° E; alt. 750 m; 01–09 Feb. 2022; ZFMK [ZFMK-DIP-00102090 = ZFMK-TIS-2637073, ZFMK-DIP-00102092 = ZFMK-TIS-2637080] • 1 ♂; MECN [ZFMK-DIP-00102089 = ZFMK-TIS-2637071].

NICARAGUA – **Rio San Juan** • 5 ♂♂ South East of San Carlos; 10.9666° N, -84.3333° E; alt. 30 m; 6-10 Feb. 2000; Leg. L.W. Quate; Malaise Trap; Lowland rain forest; LACM [LACM-ENT-279365, LACM-ENT-279366, LACM-ENT-279367, LACM-ENT-279368, LACM-ENT-279369].

PANAMA – **Canal Zone** • 1 ♂ Barro Colorado Island; 9.1500° N, -79.8500° E; 11–18 Aug. 1993; J. Pickering leg.; LACM[LACM-ENT-279380]; • 1 ♂ same collection data as for preceding; 28 Jul. - 04 Aug. 1993; LACM[no specimen number] • 1 ♂ same collection data as for preceding; 31 Jan. – 07 Feb. 1996; LACM[LACM-ENT-279374]; • 1 ♂ same collection data as for preceding; 06 – 13 Oct. 1996; LACM[LACM-ENT-279373]; • 1 ♂ same collection data as for preceding; 23–30 Oct. 1996; LACM[LACM-ENT-279372]; • 1 ♂ same collection data as for preceding; 18–27 Dec. 1996; LACM[LACM-ENT-279370]; • 1 ♂ same collection data as for preceding; 10–17 NA 1996; LACM[LACM-ENT-279378]. – **Guna Yala** (previously known as San Blas) • 1 ♂ Nusagandi Reserve; 12–19 Feb. 1994; 9.3333° N, -79.0000° E; J. Pickering leg; LACM[LACM-ENT-279377]; • 3 ♂♂ same collection data as for preceding; 24 Jun.– 4 Jul. 1993; LACM [LACM-ENT-279371; LACM-ENT-279379; LACM-ENT-279376].

Genetics

Five specimens were successfully sequenced: ZFMK-TIS-2628311, ZFMK-TIS-2629904, ZFMK-TIS-2637071, ZFMK-TIS-2637073, ZFMK-TIS-2637080. The maximum intraspecific uncorrected pairwise distance for COI sequences was 1.07 % or 7 bp. GeneBank accession numbers are: To be added.

Distribution

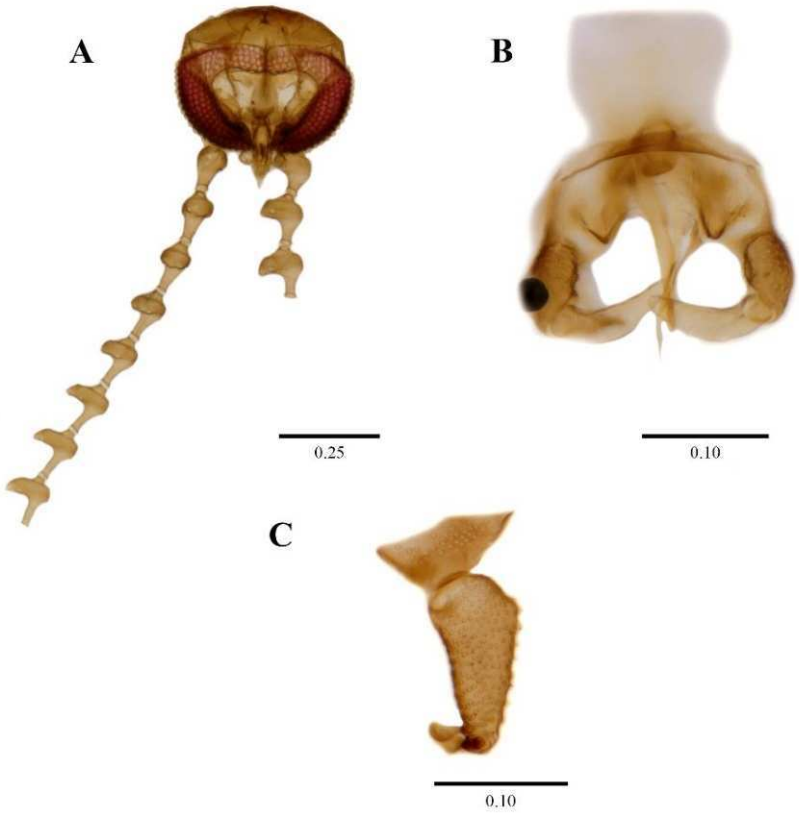
Costa Rica, Panama (Bravo & Araújo 2019; Quate 1996), and Ecuador (this publication, new record) (Fig. 1).

Remarks

Quate (1996) originally described *B. palpiculum* based on male specimens. Later, he (Quate, 1999) described the females collected in Nusagandi Reserve (Panama) appealing to the co-occurrence of these female specimens with males of *B. palpiculum*. These females were the first known and reported female specimens of *Bryopharsos*. The female description is relatively brief and the illustration only shows the genital chamber and the subgenital plate.

In this study, the association between the examined male and female specimens was done using the DNA barcodes (see section Genetics above). Our specimen differs from the drawings provided by Quate (1999), i.e. in specimen ZFMK-DIP-00102090 the paramere looks shorter than Quate's drawing. While, in specimen ZFMK-DIP-00102092 the paramere is strongly curved (although the shape/position likely changed during the mounting process. In specimen ZFMK-DIP-00102089, the base of the paramere looks broader than Quate's drawing, but the general morphology is similar. The variation can be explained by the angle in which the genitalia was prepared on the microscope slides,

913 but when comparing with other specimens deposited in other collections there is no doubt
914 they all belong to the same species.



915

916 **Fig. 17** *Bryopharsos palpiculum* Quate, 1996, ♂ . **A.** Head **B.** Genitalia **C.** Surstyli. All
917 scale bars are in millimeters (mm).

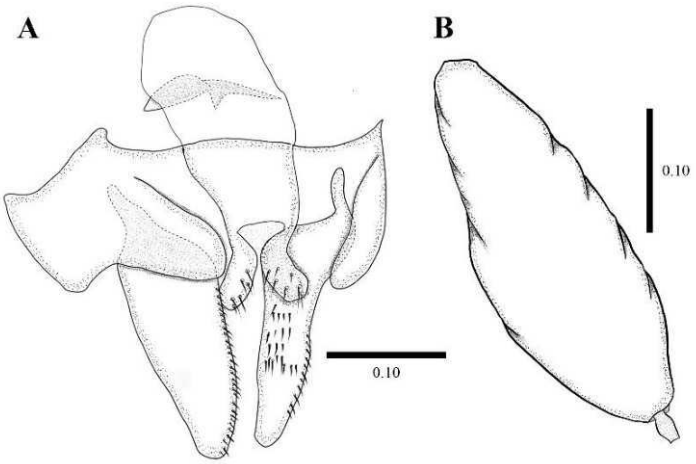


Fig. 18 *Bryopharsos palpiculum* Quate, 1996, ♀ **A.** Genitalia **B.** Egg. All scale bars are in millimeters (mm).

***Bryopharsos paulistensis* Bravo & Araújo, 2019**

(Figs 1, 19)

Bryopharsos paulistensis Bravo & Araújo, 2019: 365. Type locality: Brazil, São Paulo, Sete Barras [MZFS].

Diagnosis

Male

Eye bridge of five facet rows; wing two times longer than its width; ejaculatory apodeme sub-rectangular in dorsal view; gonocoxal apodeme with anterior projection as a lobe; surstyli with one tenaculum; aedeagus long and expanded, hemispheric basally and narrowly pointed in the apex. This species is similar to *B. uncinatum*, both can be easily

differentiated by the number of facet rows in the eye bridge (four in *B. uncinatum*; five in *B. paulistensis*).

Female

Unknown.

Material examined

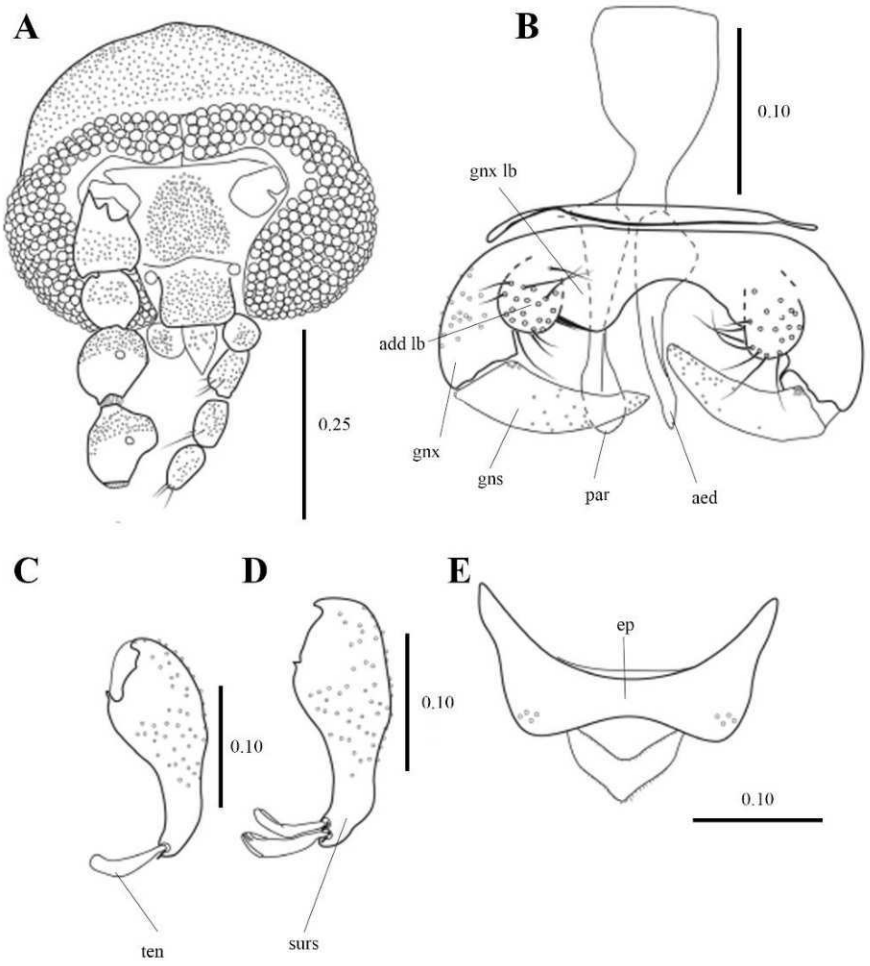
None.

Distribution

Brazil (Bravo & Araújo 2019) (Fig. 1).

Genetics

No specimens were available for DNA extraction.



942

943 **Fig. 19** *Bryopharsos paulistensis* Bravo & Araujo, 2019, ♂. **A.** Head **B.** Genitalia **C.**
944 Surstyli **D.** Surstyli **E.** Epandrium Abbreviations: add lb = additional lobe of conocoxal
945 lobe; aed = aedeagus; ep = epandrium; gns = gonostylus; gnx = gonocoxite; gnx lb =
946 gonocoxal lobes; par = paramere; surs = surstyli; ten = tenacula. All scale bars are in
947 millimeters (mm). Figures adapted from Bravo & Araujo (2019) with permission of the
948 copyright holder © Magnolia Press.

949

***Bryopharsos septenacula* Jaume-Schinkel sp. nov.**

(Figs 1, 20–21)

Diagnosis

Male

Eye bridge with four facet rows; wing 2.1 times longer than its width; ejaculatory apodeme oval, about the same length as aedeagus; gonocoxal apodeme with spine-like anterior projection; surstyli with seven tenacula; aedeagus digitiform and evenly tapering towards apex, and ending before the apex of paramere, paramere as an inverted-J shaped. This species shares the same number of facet rows in the eye bridge as *B. uncinatum, B. triatelum, B. clavigum, B. amazonensis,* and *B. claviformosum*, but it can be easily differentiated by the number of tenacula on the surstyli (seven in *B. septenacula* sp. nov., five in *B. amazonensis* and *B. claviformosum*, one in *B. uncinatum,* and, four in *B. triatelum*).

Female

Unknown.

Etymology

The species epithet derives from the Latin word *septem* meaning seven, and *tenaculum* (plural *tenacula*), the so-called stiff flattened setae on the inner apical surface of the surstyli of many Psychodidae. The name makes reference to the number of tenacula present in the species. Species epithet to be treated as name in apposition.

Material examined

Holotype

ECUADOR – **Pichincha** • 1 ♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonado, near San Pancracio, roadway to Pachijal; 0.11561° N, -78.95805° E; alt. 750m; 1–9 Feb. 2022; Kilian, Isabel leg.; ZFMK-TIS-2637106[MECN].

Paratypes

ECUADOR – **Pichincha** • 5 ♂♂; same data as holotype; ZFMK[ZFMK-DIP-00102099 = ZFMK-TIS-2637088, ZFMK-DIP-00102100 = ZFMK-TIS-2637153, ZFMK-DIP-00102101 = ZFMK-TIS-2637157, ZFMK-DIP-00102102 = ZFMK-TIS-2637174, ZFMK-DIP-00102103 = ZFMK-TIS-2637170]; • 1 ♂; same data as for preceding; MECN [ZFMK-TIS-2637178] • 1 ♂; same data as preceding; 0.11862° N, -78.95805° E; alt. 770 m; 30 Dec. 2021 – 05 Jan. 2022; ZFMK[ZFMK-DIP-00081942 = ZFMK-TIS-2636933].– **Esmeraldas** • 1 ♂; Parroquia San Francisco del Cabo, canton Bunche; 0.64562° N, -80.0253° E; alt. 46 m; 30 Dec. 2021 – 05 Jan. 2022; ZFMK[ZFMK-DIP-00081851 = ZFMK-TIS-2636988].

Description

Holotype male. Measurements in mm (n=5) Wing length 1.70 (1.80–1.50), wing width 0.79 (0.80–0.72); head length 0.40 (0.40), head width 0.35 (0.35); antennal segments: scape: 0.10 (0.11–0.08), pedicel: 0.06 (0.06–0.05), flagellomeres 1–7: 0.1 (0.11–0.10); palpal segment 1: 0.05 (0.5), palpal segment 2: 0.07 (0.07), palpal segment 3: 0.07 (0.07), palpal segment 4: 0.07 (0.07).

Head. A little wider than its length; eye bridge contiguous, with four rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from

993 the remaining alveoli on the head; the frontal patch of alveoli not divided, trapezoidal
994 with upper and lower margins straight. Antennal scape about two times the length of the
995 pedicel, almost cylindrical; pedicel spherical, smaller than scape; flagellomeres
996 asymmetrical and nodiform, with scattered setae on the basal half surface, apical
997 flagellomeres absent in examined material, the maximum number of flagellomeres
998 present seven; ascoids rectangular and broad, about the same length, and about two times
999 the width of the flagellomere carrying them. Palpal segments cylindrical, palpal segment
1000 4 with pointed apex, palpal proportions: 1.0:1.5:1.5:1.5; labium without any strong
1001 sclerite; labella not bulbous with 3–4 setae on outer margin.

1002 Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli.
1003 Wing length about 2.1 times its width; wing membrane brown-hyaline; alveoli distributed
1004 uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork
1005 of R_{2+3} at the same level of M_{1+2} and joining R_4; fork of M_{1+2} weak; R_5 ending at the wing
1006 apex; CuA_2 ending at wing margin.

1007 Terminalia (Figs. 20 B; 21). Hypandrium is a distinct band that connects the gonocoxites,
1008 plate-like; gonocoxites about the same length as gonostyli, gonostyli slightly incurved,
1009 with rounded-blunt apex; aedeagus digitiform, evenly narrowing towards the apex, apex
1010 rounded, ending beyond the level of paramere, , paramere resembling an inverted “J”,
1011 apex pointed ; ejaculatory apodeme oval, about the same length than aedeagus, anterior
1012 margin rounded; gonocoxal apodemes projected anteriorly and fused, resembling an
1013 inverted and wide “u”, spine of the gonocoxal lobes present; epandrium narrow,
1014 rectangular, about three times wider than its length; hypoproct digitiform, shorter than
1015 epandrium and covered in small setulae, epiproct shorter than hypoproct; surstyli conical,

slightly tapering towards the apex and curved dorsally, with seven apical tenacula, tenacula with rounded apex.

Distribution

Only known from the type locality in Ecuador (Fig. 1).

Genetics

13 specimens were successfully sequenced ZFMK-TIS-2629871, ZFMK-TIS-2629873, ZFMK-TIS-2636933, ZFMK-TIS-2636988, ZFMK-TIS-2637088, ZFMK-TIS-2637106, ZFMK-TIS-2637153, ZFMK-TIS-2637157, ZFMK-TIS-2637170, ZFMK-TIS-2637174, and ZFMK-TIS-2637178. The maximum intraspecific uncorrected pairwise distance for COI sequences was 0.46% or 2 bp. GeneBank accession numbers are: To be added.

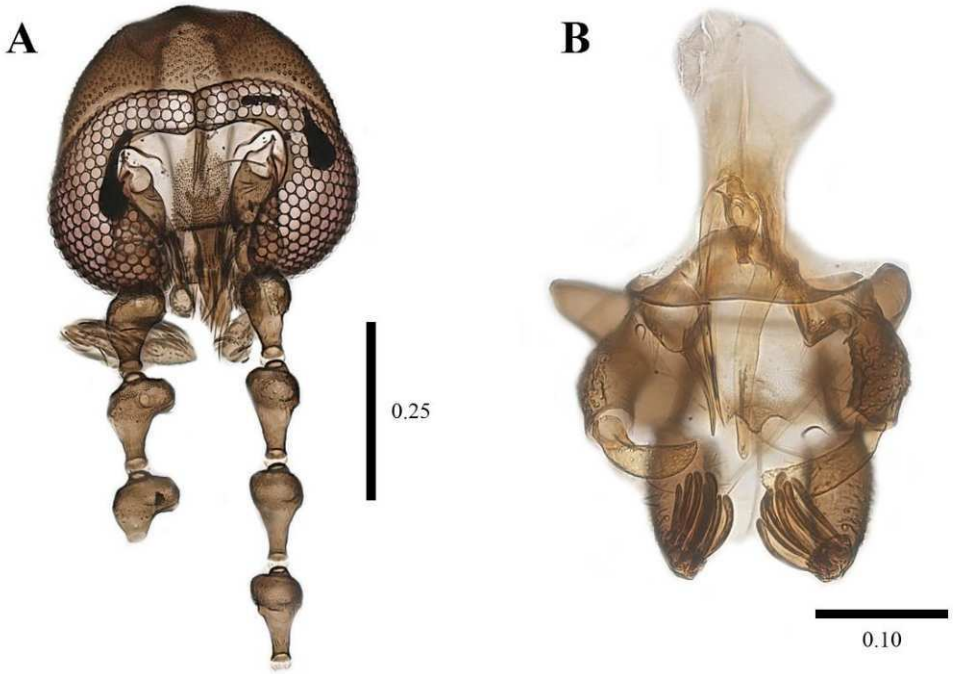
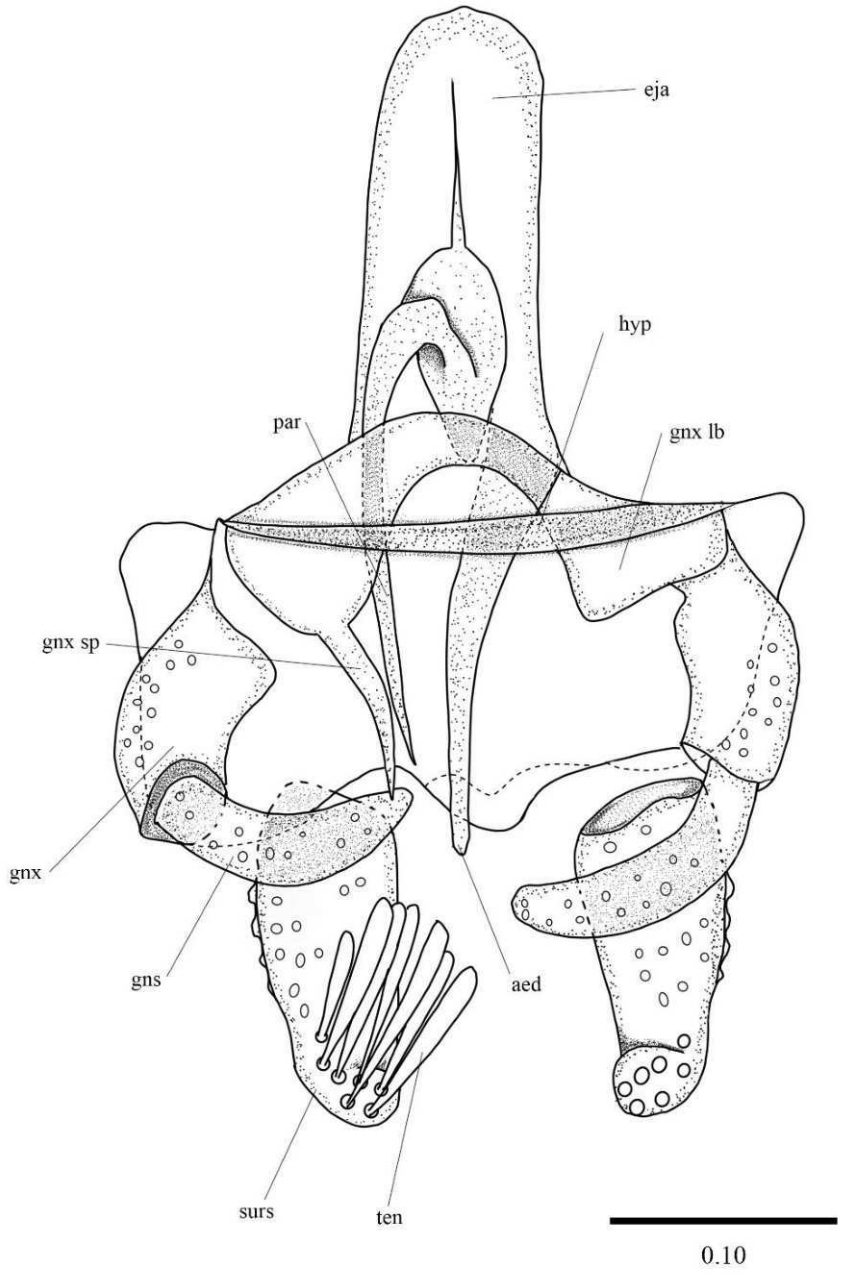


Fig. 20 *Bryopharsos septenacula* Jaume-Schinkel sp. nov., holotype ♂. **A.** Head **B.** Genitalia. All scale bars are in millimeters (mm).



1029

1030 **Fig. 21** *Bryopharsos septenacula* Jaume-Schinkel sp. nov., male holotype. Genitalia.
1031 Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx =
1032 gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of gonocoxal lobe; par =
1033 paramere; surs = surstyli; ten = tenacula. Scale bar in millimeters (mm).

Bryopharsos tetracanthus Jaume-Schinkel sp. nov.

(Figs 1, 22)

Diagnosis

Male

Eye bridge with four facet rows; wing 2.4 times longer than its width; ejaculatory apodeme cylindrical and hour-glass-shaped, about the same length as aedeagus; gonocoxal apodeme fused; surstyli with three apical tenacula; aedeagus digitiform, curved and evenly tapering towards apex. This species shares the same number of apical tenacula in the surstyli (three tenacula) with *B. tritaleum* but it can be easily differentiated by the distribution of the apical tenaculum in the surstyli (close-together at the apex in *B. tetracanthus* sp. nov., 2 apical tenacula separated from 1 basal tenaculum in *B. tritaleum*), and the shape of the aedeagus (digitiform and curved in *B. tetracanthus* sp. nov., digitiform and straight in *B. tritaleum*).

Female

Unknown.

Etymology

The species epithet *tetracanthus* derives from the Greek word *τετρα-* (tetra-) as a prefix, meaning four, and the Greek word *ἄκανθος* (ákanthos), meaning spine. The epithet makes reference to the four spines located in between the apical tenacula in the surstyli.

Material examined

Holotype

COLOMBIA – **Magdalena** • 1 ♂; Sierra Nevada de Santa Maria, El Ramo, alt. 2400 m.; 10–24 May. 2000; I. Uribe leg.; Malaise Trap' LACM [LACM-ENT-279397].

Description

Holotype male.Measurements in mm (n=1) Wing length 1.92, wing width 0.80; head length 0.39, head width 0.46; antennal segments: scape: 0.08, pedicel: 0.06, flagellomeres 1-4: 0.11; palpal segment 1: 0.05, palpal segment 2: 0.06, palpal segment 3: 0.06, palpal segment 4: 0.07.

Head. A little wider than its length; eye bridge contiguous, with four rows of facets, interocular suture absent; post-ocular alveoli not enlarged and non-distinguishable from the remaining alveoli on the head; the frontal patch of alveoli not divided, trapezoidal with the lower margin with a concavity in the middle. Antennal scape about the same the length of the pedicel, cylindrical; pedicel spherical, about the same length of scape; flagellomeres asymmetrical and nodiform, with scattered setae on the basal half surface, apical flagellomeres absent in examined material, the maximum number of flagellomeres present is seven; ascoids absent in examined material. Palpal segments cylindrical, palpal segment 4 apically pointed, palpal proportions: 1.0:1.2:1.2:1.4; labium without any strong sclerite; labella not bulbous with 3–4 setae on outer margin.

Thorax without allurement organs; all coxae with a stripe of three to five rows of alveoli. Wing length about 2.4 times its width; wing membrane brown-hyaline; alveoli distributed uniformly on wing membrane; subcostal vein short ending beyond the origin of R_4; fork of R_{2+3} at the same level of M_{1+2} and joining R_4; fork of M_{1+2} normally sclerotized; R_5 ending at the wing apex; CuA_2 ending at wing margin.

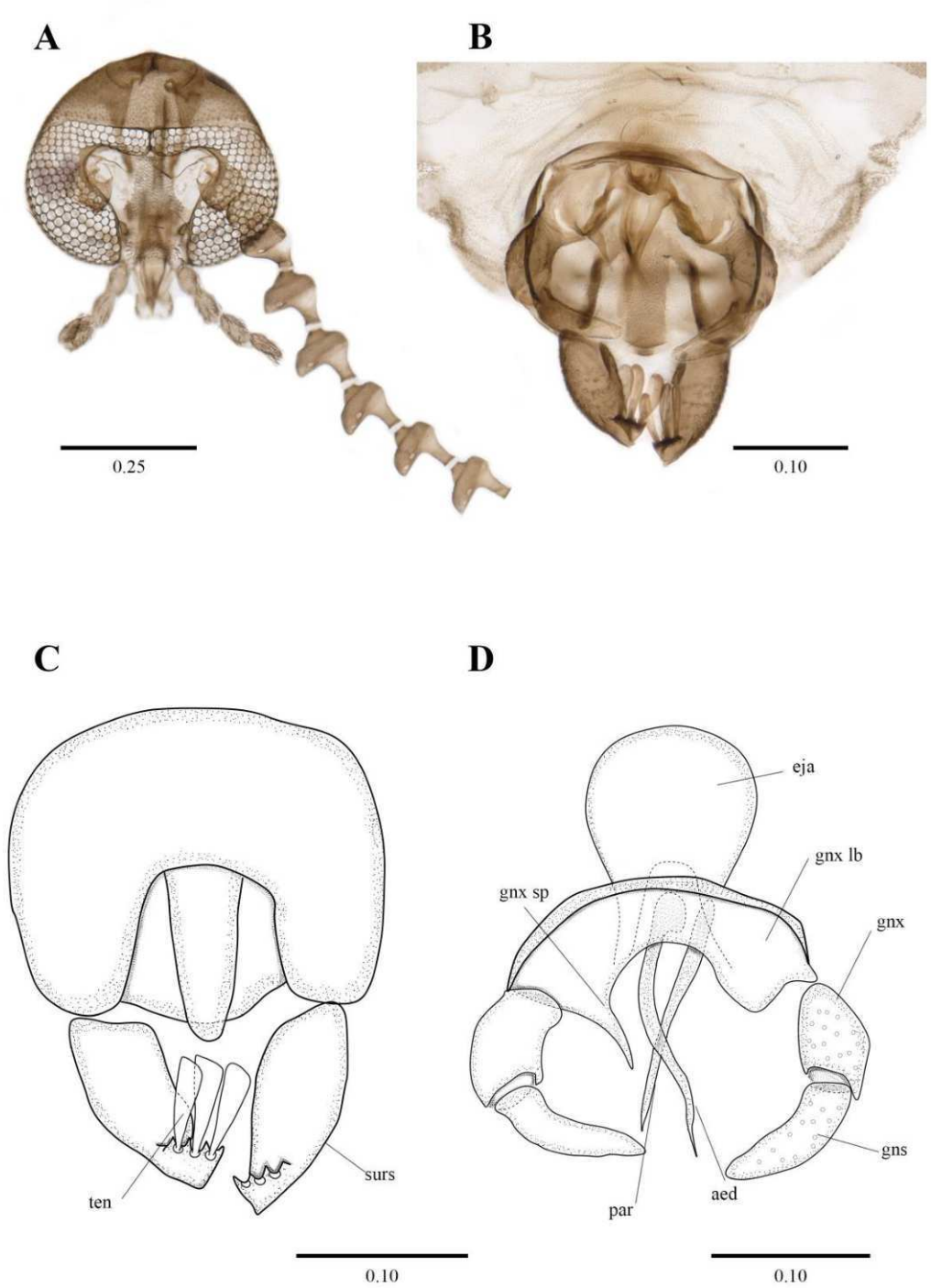
Terminalia (Figs. 22 B–D). Hypandrium is a distinct band that connects the gonocoxites, stripe-like; gonocoxites cylindrical, shorter than gonostyli, gonostyli digitiform, with rounded apex; gonocoxal apodeme anteriorly projected with rectangular anterior margin; gonocoxal lobes with four setae on each side, spine of the gonocoxal lobes present; aedeagus digitiform and curved, evenly narrowing towards the apex, apex pointed, ending beyond the apex of the paramere, paramere digitiform, rounded at apex; ejaculatory apodeme with anterior margin rounded, shorter than the length of the aedeagus; epandrium plate-like, square and resembling an inverted U(Fig. 22 C); hypoproct elongated, almost thumb-like, about three times longer than its width, and covered in small setulae, epiproct shorter than hypoproct, about the same width than its length; surstyli conical, tapering towards the apex and curved dorsally, with three close-together apical tenacula, tenacula with rounded apex, with additional 4 spiniform projections in-between the tenacula (Fig. 22 B–D).

Distribution

Only known from the type locality in Colombia (Fig. 1).

Genetics

No specimens were available for DNA extraction.



1095

1096 **Fig. 22** *Bryopharsos tetracanthus* Jaume-Schinkel sp. nov., male holotype. **A.** Head **B.**
1097 Genitalia **C.** Epandrium and surstyli **D.** Genitalia. Abbreviations: aed = aedeagus; eja =
1098 ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx
1099 sp = spine of gonocoxal lobe; par = paramere; surs = surstyli; ten = tenacula. All scale
1100 bars are in millimeters (mm).

Bryopharsos tritaleum Quate, 1996

(Figs 1, 23)

Bryopharsos tritaleum Quate, 1996: 41. Type locality: Costa Rica, Heredia, Estación Biológica La Selva [INBio].

Diagnosis

Male

Eye bridge with four facet rows; wing 2.2 times longer than its width; ejaculatory apodeme ovoid in dorsal view; gonocoxal apodeme with a spine-shaped projection; surstyli with three tenacula; aedeagus straight with a pointed apex. This species is similar to *B. asymmetricum* **sp. nov.** they can be easily differentiated by the number of facet rows in the eye bridge (four in *B. tritaleum*; five in *B. asymmetricum* **sp. nov.**), and the length of the ejaculatory apodeme (about the same length as the length of the aedeagus in *B. tritaleum*; shorter than the aedeagus in *B. asymmetricum* **sp. nov.**).

Female

Unknown.

Material examined

None.

Distribution

Costa Rica (Bravo & Araújo 2019; Quate 1996) (Fig. 1).

Genetics

No specimens were available for DNA extraction.

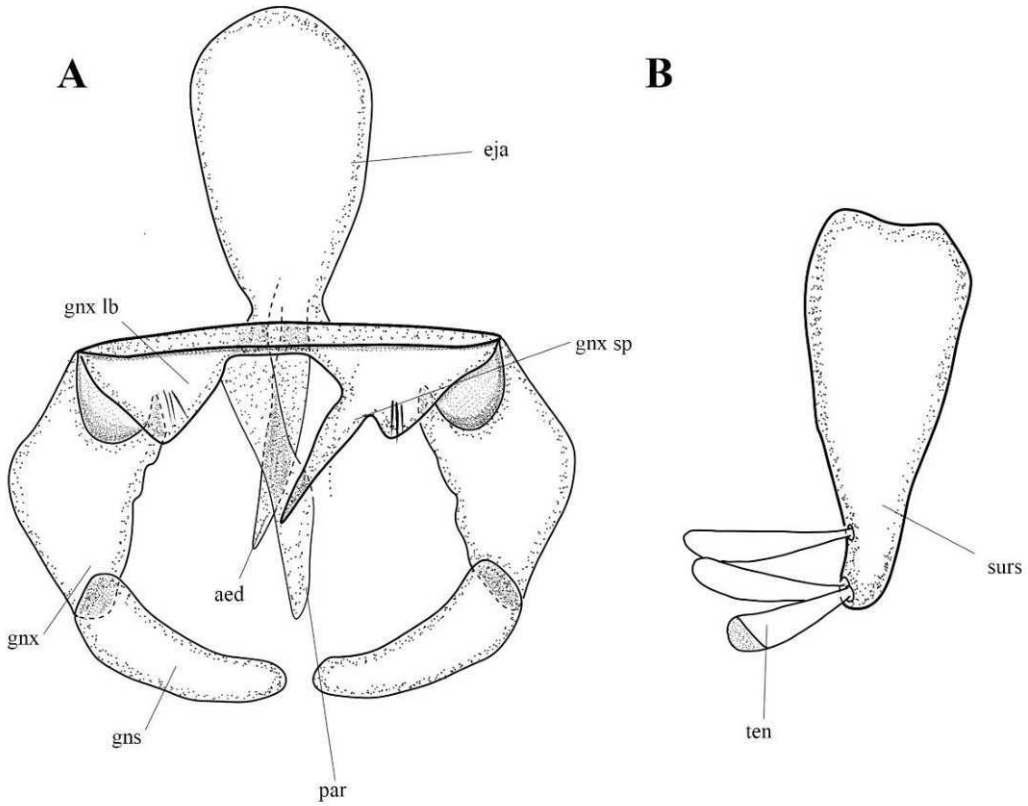


Fig. 23 *Bryopharsos tritaleum* Quate, 1996, male. **A.** Genitalia **B.** Surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; gns = gonostylus; gnx = gonocoxite; gnx lb = gonocoxal lobes; gnx sp = spine of gonocoxal lobe; par = paramere; surs = surstyli; ten = tenacula. No scales available. Figures adapted from Quate (1996).

Bryopharsos uncinatum **Bravo & Araújo, 2019**

(Figs 1, 24)

Bryopharsos uncinatum Bravo & Araújo, 2019: 365. Type locality: Brazil, São Paulo, Sete Barras [MZFS].

Diagnosis

Male

Eye bridge with four facet rows; wing two times longer than its width; ejaculatory apodeme sub-circular in dorsal view; gonocoxal apodeme without anterior projection; surstyli with one tenaculum; aedeagus hook-shaped. This species is similar to *B. paulistensis* with both species presenting only one tenaculum in the surstyli (the other species present 3–7 tenacula), but they can be differentiated by the number of facet rows in the eye bridge (four in *B. uncinatum*; five in *B. paulistensis*) and the spine of the gonocoxal apodeme (absent in *B. uncinatum*; present in *B. paulistensis* as rounded projections (referred to as lobes in Bravo & Araújo, 2019)).

Female

Unknown.

Material examined

None.

Distribution

Brazil (Bravo & Araújo 2019) (Fig. 1).

Genetics

No specimens were available for DNA extraction.

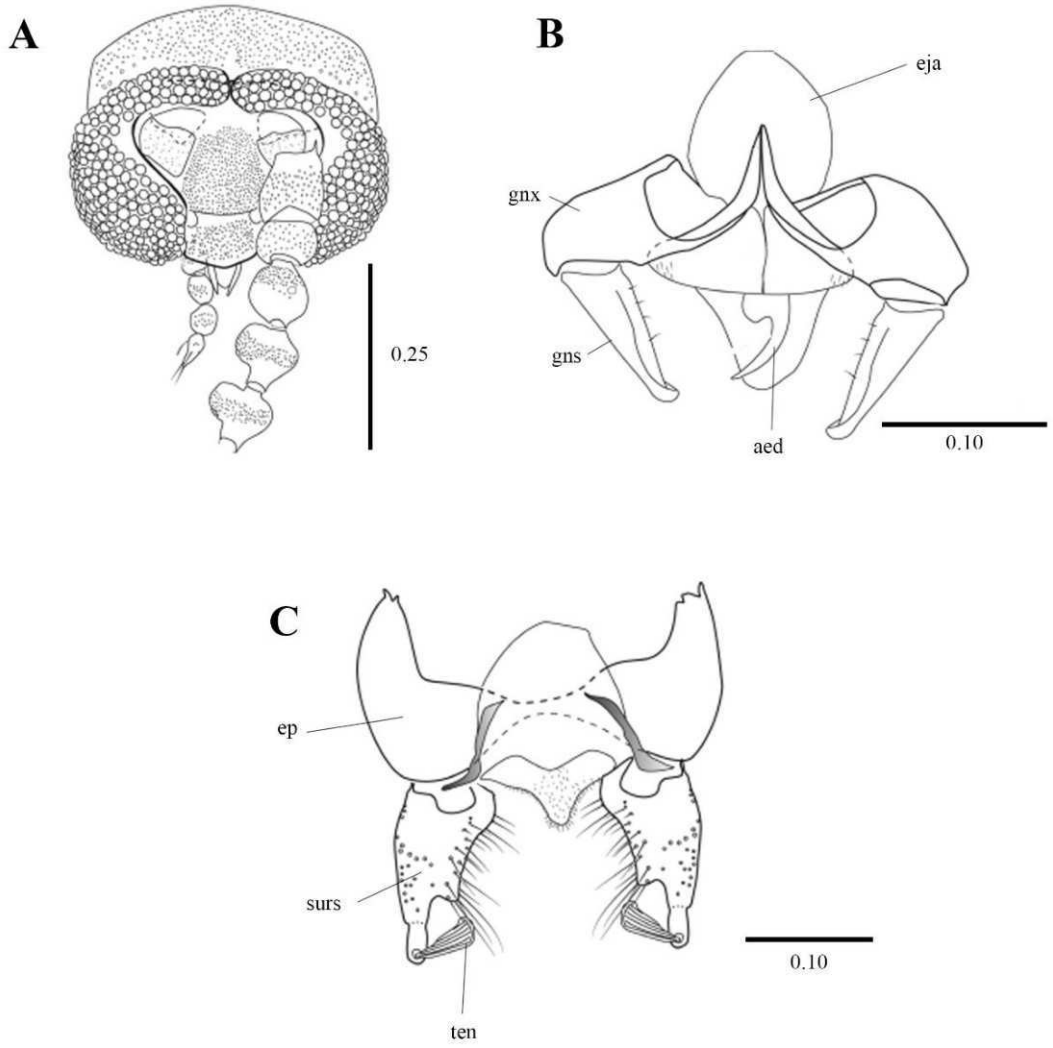


Fig. 24 *Bryopharsos uncinatum* Bravo & Araujo, 2019, male. **A.** Head **B.** Genitalia **C.** Epandrium and surstyli. Abbreviations: aed = aedeagus; eja = ejaculatory apodeme; ep = epandrium; gns = gonostylus; gnx = gonocoxite; surs = surstyli; ten = tenacula. All scale bars are in millimeters (mm). Figures adapted from Bravo & Araujo (2019) with permission of the copyright holder © Magnolia Press.

Identification key to *Bryopharsos* males

1. Gonostyli bifurcate…***B. bifidum* Jaume-Schinkel sp.nov.**

-. Gonostyli not bifurcate, digitiform…**2**

2. Surstyli with one tenaculum (Fig. 13 B, C)…**3**

-. Surstyli with two to seven tenacula (Figs 2 C; 3 A–D)…**6**

3. Eye bridge with five facet rows (as in Figs 2 A, 4 A); gonocoxal lobes with additional enlarged lobes (Fig. 19 B); aedeagus blade-like (Fig. 19 B); parameres clavate (Fig. 19 B)… ***B. paulistensis*** **Bravo & Araújo, 2019**

-. Eye bridge with three or four facet rows (as in Fig. 6 A); other characters variable…**4**

4. Eye bridge with three facet rows; gonostyli curved outwards (as in Fig. 13 B, D); aedeagus digitiform and curved outwards (as in Fig. 13 B, D)…***B. curvum*** **Jaume-Schinkel sp.nov.**

-. Eye bridge with four facet rows (Fig. 15 A); gonostyli curved inwards; aedeagus variable …**5**

5. Aaedeagus hook-shaped (Fig. 24 B); paramere wide and triangular…***B. uncinatum*** **Bravo & Araújo, 2019**

-. Aedeagus digitiform (as in Figs 15 B; 16 A); paramere digifitorm (as in Figs 15 B; 16 A)...***B. inesperatus*** **Jaume-Schinkel sp. nov.**

6. Surstyli with three to seven apical tenacula (Fig. 2 C)…**8**

-. Surstyli with only two apical tenacula (Figs 5; 8 C,9 B)…**7**

7. Aedeagus digitiform, (Figs 4 C; 5 C); paramere digitiform, longer than the aedeagus (Figs 4 C; 5); some specimens present two tenacula on one surstyli and three on the other (as in Figs 4 C, 5)…***B. asymmetricum*** **Jaume-Schinkel sp. nov.**

-. Aedeagus digitiform with a rounded apex (Figs 8 B; 9 A); paramere digitiform, shorter than the aedeagus (Figs 8 B; 9 A); specimens always with two tenacula on each surstyli (Figs 8 B; 9A)…***B. bitenacula*** **Jaume-Schinkel sp. nov.**

8. Surstyli with four to seven apical tenacula (Fig. 20 B)…**10**

-. Surstyli with only three apical tenacula (Fig. 22 C)…**9**

9. Aedeagus digitiform, curved (Fig. 22 B, D), longer than paramere; surstyli with apical tenacula close together (Fig. 22 C)…***B. tetracanthus*** **Jaume-Schinkel sp. nov.**

-. Aedeagus digitiform (Fig. 23 A), straight, shorter than paramere; surstyli with apical tenacula separated, two apically and one basally placed (Fig. 23 B)…***B. tritaleum*** **Quate, 1996**

10. Eye bridge with four facet rows; aedeagus digitiform, straight, about the same length of paramere (Fig. 12 B); surstyli with four tenacula (Fig. 12 C); tenacula of equal length …***B. clavigum*** **Quate, 1996**

-. Eye bridge with four or five facet rows; aedeagus shape variable; length of aedeagus and paramere variable; surstyli with five to seven tenacula (Fig. 20 B); tenacula length variable. … **11**

11. Aedeagus digitiform, straight; aedeagus shorter than paramere; eye bridge with five face rows; surstyli with three or four apical tenacula (as in Fig. 17 C); tenacula of equal length …***B. palpiculum*** **Quate, 1996**

-. Aedeagus shape variable; length of aedeagus and paramere variable; eye bridge with four facet rows; surstyli with five, six, or seven apical tenacula; tenacula length variable…**12**

12. Aedeagus digitiform (Fig. 21); paramere strongly curved resembling an inverted "J" (Fig. 21); surstyli with seven tenacula (Fig. 20 B); tenacula of the same length…***B. septenacula*** **Jaume-Schinkel sp. nov.**

-. Aedeagus digitiform, straight; surstyli with five or six tenacula; tenacula length variable…**13**

13. Aedeagus digitiform, broader than the base of the paramere (Fig. 2C, see also Bravo & Araujo (2019): fig. 29); surstyli with five tenacula…**15**

-. Aedeagus digitiform, narrower than the base of the paramere (Figs 11 A; 14 D); surstyli with six tenacula…**14**

14. Hypandrium length is shorther than aedeagal width; epandrium C-shaped…***B. chuspi*** **Jaume-Schinkel sp. nov.**

-. Hypandrium length longer than aedeagal width; epandrium rectangular and not C-shaped…***B. gorgona*** **Jaume-Schinkel sp. nov.**

15. All five tenacula of the same length (Fig. 2 C); ejaculatory apodeme shorter than aedeagus…***B. amazonensis*** **Bravo y Araújo, 2019**

-. Tenacula of different lengths, four tenacula of equal length and one tenaculum shorter than others (Fig. 3 C); ejaculatory apodeme about the same length as aedeagus…***B. claviformosum*** **Quate, 1996**

Genetics

All sequenced specimens form well supportaed clades (BS = 100) in the NJ tree (Fig. 25). Molecular clades match morphological identification. The maximum intraspecific uncorrected pairwise distance for COI sequences for *B. amazonensis* is 0.32%, similarly, specimens of *B. claviformosum* have a maximum uncorrected pairwise distance of 0.16%, specimens of *B. palpiculum* have a maximum uncorrected pairwise distance 0.92%, and specimens of *B. septenacula* have a maximum uncorrected pairwise distance 0.45%. The interspecific uncorrected distance ranges from 16.62 to 19.51% between all the sequenced taxa.

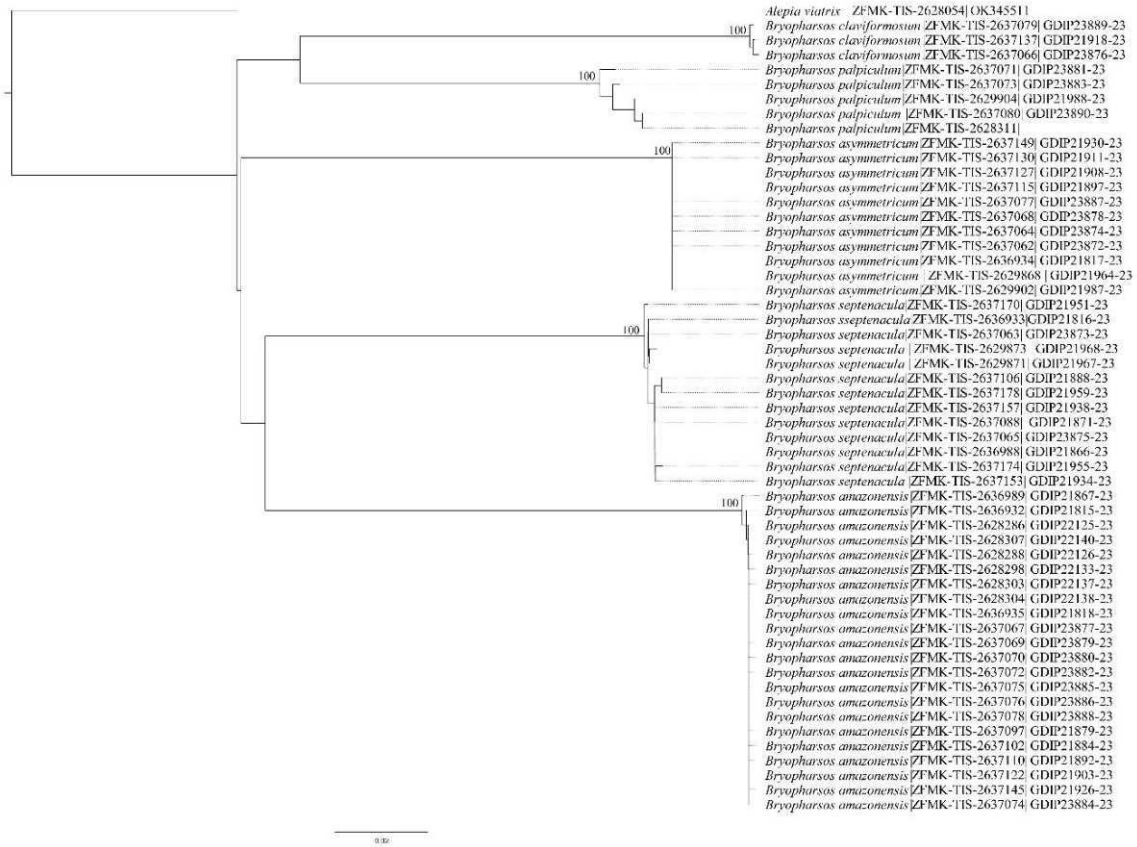


Fig. 25 Neighbor-Joining tree using Jukes-Cantor model based on the COI sequences of the examined material. The name for each specimen is composed by: the name of the species | sample ID | Process ID or GenBank accession number. Bootstrap support values are given at the nodes.

Genus distribution model.

Species distribution models calculated by MaxEnt show good model quality according to evaluation measures. MaxEnt shows comprehensible results according to our knowledge of species inside the genus *Bryopharsos* preferences regarding climatic conditions. Of the 19 WorldClim variables, 13 were correlated, while only 6 were significant for the model construction. The variable that contributed most to the construction of this model was bio10 "Mean Temperature of Warmest Quarter" (73.1%), the second most important variable was bio11 "Mean Temperature of Coldest

Quarter" (8.9%), meaning that temperature significantly conditions the distribution of this genus (Fig. 26).

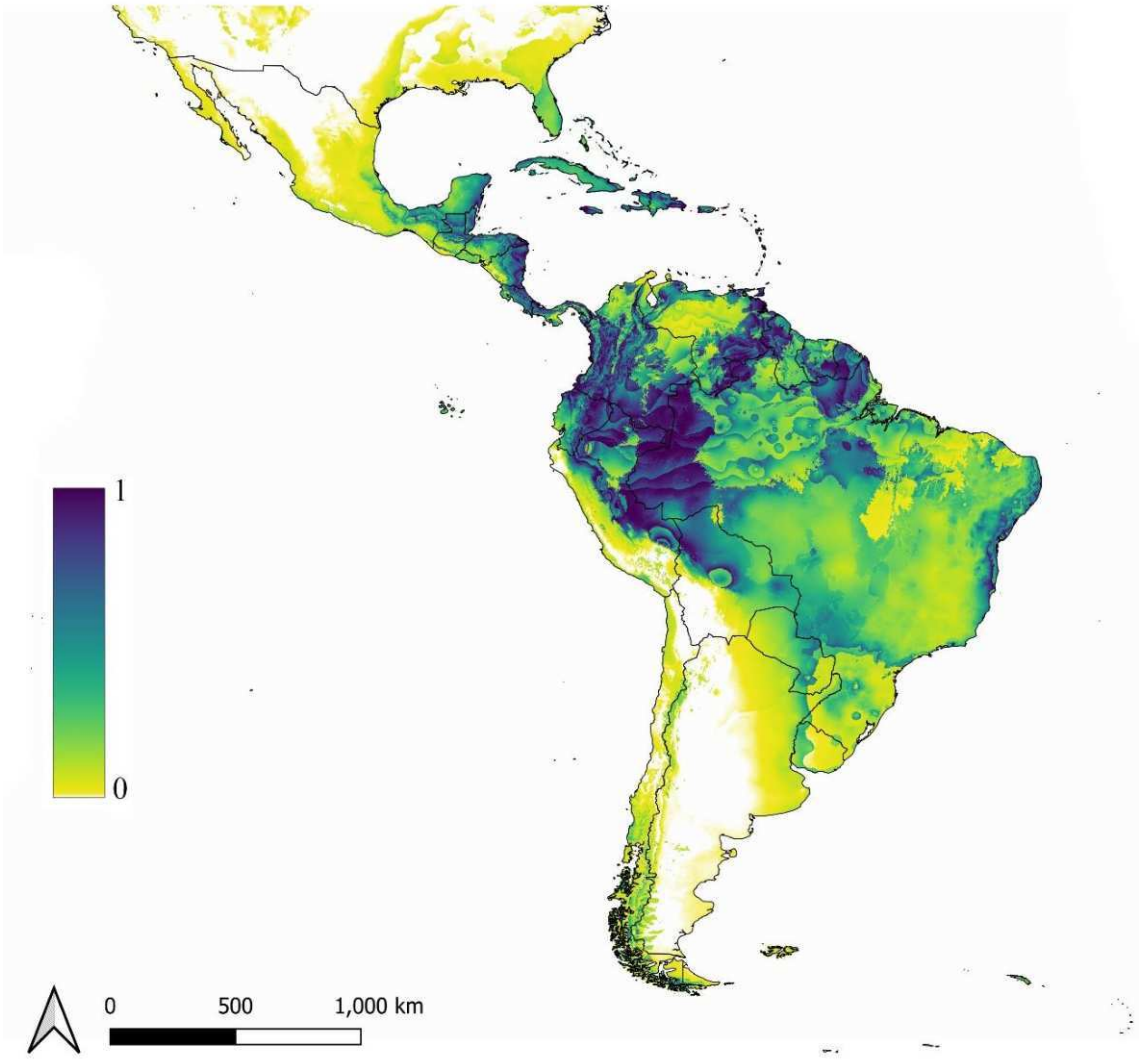


Fig. 26 Species distribution modeling map using MaxEnt 3.4.4.

According to the predictions, under current climatic conditions, the moth fly genus *Bryopharsos* is potentially established in much of the sub-humid to humid tropics and subtropics of America (Figs 1, 26). The modelled suitability of the climate for *Bryopharsos* fits well with the known occurrences of its species in tropical America

between 23° N and 25° S. The results indicate that all Caribbean islands, most of Central America from the state of Tabasco in Mexico southward, extending throughout the Chocó-Darién biodiversity hotspot in Panama, western Colombia and Ecuador. On the other hand, the model predicts a continuous distribution through the Andes mountain range in Ecuador and Colombia, to the Amazonian lowland forest region, from western Amazonia to central and northern Amazonia through Colombia, Ecuador, Venezuela, Peru, Amazonas, Acre and Rondonia states (Brazil) and northern Bolivia. A discontinuous distribution is observed in Amazonian forest areas such as Guyanas and Pará. In addition, through Atlantic forests patches along the coasts and southern Brazil.

Discussion

Our results support DNA barcodes (*COI* sequences) as a good complementary tool for species identification in *Bryopharsos*, even for similar-looking species such as *B. amazonensis* and *B. claviformosum*. Our sequenced taxa form well supported clades as seen in the NJ tree (Fig. 25). Our DNA barcodes have a maximum intraspecific uncorrected pairwise distance of 0.92% (in *B. palpiculum*), a value similar to other known intraspecific distances within psychodine species, such as *Alepia viatrix* Jaume-Schinkel, Kvifte, Weele & Mengual, 2022 (5.75%), *Tonnoira chuki* Jaume-Schinkel, 2023 (1.87 %), or *Platyplastinx ibanezbernali* Jaume-Schinkel & Kvifte, 2022 (1.35%) (Jaume-Schinkel *et al*. 2022, Jaume-Schinkel & Kvifte 2022, Jaume-Schinkel 2023). We, thus, believe that an integrative taxonomical approach, combining adult morphology and DNA barcoding, becomes a key component when discovering the number of species present in entomological samples.

The usage of the term surstyli (surstylus in singular) has been a long source of discussion in Psychodidae as different terms have been used to refer to the same caudal appendages (Curler & Moulton 2012). Furthermore, these appendages are non-homologous with those of the Eremoneura (Cumming & Wood 2017); hence, some authors have referred to them as "epandrial lobes" (Sinclair *et al*. 2013), "epandrial claspers" (Santos & Curler 2014), "epandrial appendages" (Kvifte & Wagner 2017a), or "hypopods" (Kvifte & Wagner 2017b). According to Cumming & Wood (2017), the surstyli are apically clasping lobes derived from epandrium, and true surstyli are only present in the Eremoneura, whilst similar-looking appendages occur in a few nematocerous families (including Psychodidae) and some Psychodidae species appear to have appendages originating from both the epandrium and proctiger (Kvifte & Wagner 2017b). Nevertheless, as shown in Figure 3, the surstyli in the *Bryopharsos* species seem to originate from the epandrium as articulate appendages, thus we deem it appropriate to refer to the epandrial appendages with the term surstyli in the genus *Bryopharsos*. Nonetheless, further morphological data is desirable to understand the origin and functionality of these genital structures with the hope of finding common ground of the homology with other dipteran groups.

We have noticed that when specimens are slide-mounted (i.e., Figs 2 C; 4 C), the aedeagus and paramere seem to originate from within the abdomen close to the ejaculatory apodeme. This perception changes with specimens that are not slide-mounted and observed through a SEM microscope (as in Fig. 3): the aedeagus seems to originate from within the abdomen piercing through the membrane of the gonocoxal apodeme (Fig. 3 B, D [red color]) and the paramere seems to be part of the gonocoxal lobes membrane (Fig. 3 B, D [blue color]). The male genitalia are composed of three-dimensional structures that are flattened during the slide preparation process, making them seem almost flat and

two-dimensional. This distortion may lead to misinterpretation of characters and careful interpretation is advised.

The spine present in the gonocoxal lobes can vary in size and shape, even among individuals of the same species, and can be found on either side (left or right), or be completely absent. For instance, the majority of the examined material of *B. amazonensis* does not present the spine, but three individuals display it on the left side. Similarly, examined material of *B. septenacula* Jaume-Schinkel sp. nov. has a spine present either on the left or on the right side. Although the position of the spine is different in these species, the structure is similar and it seems that there is no broken spine on the other empty side of the gonocoxal lobes. If there was a spine on each side originally, we suppose that we could see the rest of the broken spine, and this is not the case. The function of the spine, as well as the variation of left/right placement, is unknown, and further study is required to clarify the possible reproductive function or evolutionary implications.

To date, *Bryopharsos* remains a genus present in the Neotropical Region only (Fig. 1). With the herein newly described species, the genus has 16 species. The herein-reported distribution of the species of *Bryopharosos* is restricted to seven countries: Brazil, Colombia, Costa Rica, Ecuador, Panama, Peru, and, Venezuela (Fig. 1). Nonetheless, our species distribution model (Fig. 26) predicts that the genus could be present in other areas of the American continent, including countries in South, Central, and North America. Moreover, our model also predicts the potential presence of *Bryopharsos* in the Caribbean countries. The presence of known and undescribed species in more Neotropical countries is highly probable, and the lack of records can be derived from different socio-political scenarios, but we speculate it is mainly due to a lack of systematic collection and the lack

of taxonomical expertise in those countries. Consequently, we encourage to carry on sampling through South, Central, and North America to gain a better understanding of the distribution of the species of *Bryopharsos*.

Acknowledgments.

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1409

Appendix 13. (Publication chapter 15)

Chapter 15 – Publication

Jaume-Schinkel S, Kvifte GM (2024) Revision of the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae) with the description of three new species from the Neotropical Region. Journal of European Taxonomy, 935(1), 81–100. https://doi.org/10.5852/ejt.2024.935.2547

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Research article

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Revision of the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae), with the description of three new species

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Abstract. We review the diagnosis of the genus *Eugenys* Quate, 1996 (Diptera: Psychodidae) which occurs in the Neotropical Region. Initially known from Costa Rica, Nicaragua, and Panama, we describe one additional species from Costa Rica, *Eugenys singularis* sp. nov., and two species from Ecuador, namely, *Eugenys micra* sp. nov. and *E. upsilon* sp. nov., bringing the total known species to six. This study provides detailed descriptions of the new species based on male and female specimens, along with the first DNA barcodes for the genus and some of the newly described species. We also provide an identification key for identifying male specimens of the genus worldwide. Finally, we discuss the morphological characteristics of *Eugenys* and compare the genus with other taxa, tentatively suggesting a placement within the tribe Pericomaini.

Keywords. Psychodinae, taxonomy, moth flies, integrative taxonomy.

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Introduction

Eugenys Quate, 1996 is a Neotropical genus of Psychodidae Newman, 1834 (moth flies) with previously only three described species, namely, *Eugenys clavellata* Quate, 1996 from Costa Rica and Nicaragua, *Eugenys cymosa* Quate, 1999 from Panama, and *Eugenys panamensis* Quate, 1999 from Panama (Collantes & Martinez-Ortega 1999; Quate 1996, 1999). *Eugenys* was originally placed under the tribe Mormiini Enderlein, 1937, due to the wing venation with a pectinate radial sector and the basal position of radial and medial forks (Quate 1996, 1999). This tribal placement was followed by Wagner & Ibañez-Bernal (2009). On the other hand, modern studies using molecular characters suggested that this character system is insufficient to establish Mormiini in this sense as a monophyletic group (Espíndola *et al.* 2012;

Curler & Moulton 2012; Kvifte 2018). Recently, Kvifte (2018) placed *Eugenys* as an unplaced genus in his tentative tribal classification within Psychodidae.

In the present manuscript, we describe three new species of *Eugenys* based on male and female specimens. Moreover, we report the genus in Ecuador for the first time. In addition, the herein newly described species are compared with the previously known fauna of *Eugenys* and contextualized within Psychodinae through new interpretations of male genital homologies. Furthermore, we provide the first DNA barcodes (cytochrome c oxidase subunit 1 or COI) for the genus and for some of the species described herein. Finally, we provide an identification key for the males of the currently six described species of *Eugenys* worldwide

Material and methods

Terminology

We follow the general terminology proposed by Cumming & Wood (2017) except for epandrial appendages which are here called hypopods following Kvifte & Wagner (2017)

Collection and preparation of specimens

Specimens from Ecuador were collected using a Malaise trap, euthanized and preserved in 96° ethanol, and stored at -20°C. The preparation of permanent slides follows the procedure outlined by Jaume-Schinkel *et al.* (in press), the DNA extraction methodology is non-destructive allowing us the recovery of the entire specimen for slide mounting. Specimens coming from Costa Rica were collected using a Malaise trap and mounted on slides by L.W. Quate.

The remnants of DNA extracts are stored at ZFMK's Biobank facilities (https://bonn.leibniz-lib.de/en/biobank), accessible through the tissue codes ZFMK-TIS as listed in the Material examined section.

Institutional abbreviations

CAS = The California Academy of Sciences, San Francisco, California, USA
INABIO = Instituto Nacional de Biodiversidad, Quito, Ecuador
NHM = National History Museum, London, UK
USNM = Smithsonian Institution, National Museum of Natural History, Washington, USA
ZFMK = Museum Koenig, Leibniz-Institut zur Analyse des Biodiversitätswandels (LIB) (previously known as Zoologisches Forschungsmuseum Alexander Koenig), Bonn, Germany

Abbreviations for morphological terms

aed = aedeagus
eja = ejaculatory apodeme
ep = epandrium
gns = gonostyli
gnx = gonocoxite
hyp = hypandrium
par = paramere
par scl = parameral sclerite
par sh = parameral sheath

Genetics

Extraction and PCR were performed at ZFMK, and PCR products were shipped to Beijing Genomic Institute (BGI) (China, Hong Kong) for bidirectional sequencing. DNA sequences were assembled, aligned, and cleaned using Geneious ver. R7 (Biomatters, Auckland, New Zealand). The sequence length was set to 658 bp.

Results

Taxonomy

Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Suborder Psychodomorpha Hennig, 1968
Family Psychodidae Newman, 1834
Subfamily Psychodinae Newman, 1834

Genus ***Eugenys*** Quate, 1996

Eugenys Quate, 1996: 43.

Eugenys – Quate 1999: 432 (description of new species). — Kvifte 2018: 603 (tribal classification).

Type species

Eugenys clavellata Quate, 1996: 432 (by original designation).

Differential diagnosis

Eugenys is a very distinctive genus and it can be easily differentiated from other genera of Psychodinae by the following characters: eyes contiguous, eye bridge with three facet rows; antenna with 14 symmetric fusiform flagellomeres, except flagellomere 14 which is elongated and longer than the previous flagellomere, with a terminal apiculus; ascoids very long (the length of 3–4 flagellomeres), wavy-zigzag and rod-shaped (unknown in *E. cymosa*); mouthparts atrophied or very reduced; palpi short, not extending beyond flagellomere 3–4; thorax without allurement organs, with alveoli on the central part of anepisternum, posteriorly to anterior spiracle; broad wings, vein R_{2+3} very short, arising from R_4 (Rs pectinate), R_5 ending in wing apex or slightly after apex, CuA_2 ending beyond medial fork; male genitalia with asymmetrical aedeagal complex, hypopods with one tenaculum or multiple tenacula.

Remarks

Quate's (1996) original description of the genus was based only on the type species; however, when he later described two additional species in this genus (Quate 1999: *E. cymosa* and *E. panamensis*), not all the characters and structures were available for comparison, e.g., in type specimens of *E. panamensis* the antennae are incomplete; therefore, the last flagellomere is unknown. In type specimens of *E. cymosa* the ascoids are missing; consequently, there is no comparison of this character between the species. Furthermore, the last flagellomere in *E. clavellata* is considerably longer than the one present in *E. cymosa*. Lastly, the number of tenacula present on the hypopods varies from one in *E. clavellata* and *E. panamensis* to 14–16 in *E. cymosa* which makes *E. cymosa* hard to fit in the original diagnosis of the genus by Quate (1996) based solely on the number of tenacula and terminal flagellomeres. The updated diagnosis proposed above fits all of the previously described species, as well as the herein described species.

Biology

To date, nothing is known about the immature stages and the biology of the species of *Eugenys*. Adults have atrophied (or very reduced) mouthparts, and it is believed that adults do not feed (see Discussion).

Species included

Eugenys clavellata Quate, 1996, *E. cymosa* Quate, 1999, *E. micra* sp. nov., *E. panamensis* Quate, 1999, *E. singularis* sp. nov. and *E. upsilon* sp. nov. Species distribution is shown in Fig. 1.

Eugenys clavellata Quate, 1996

Figs 1, 2A, 3C

Eugenys clavellata Quate, 1996: 43. Type locality: Costa Rica, Heredia, Estacion Biologica la Selva. Additional reference: Collantes & Martinez-Ortega (1999).

Differential diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the following characters: hypopods with singular apical tenaculum (10–14 in *E. singularis* sp. nov. and *E. cymosa*, one in *E. micra* sp. nov., *E. panamensis* and *E. upsilon* sp. nov.); antennal flagellomeres without patch of darkened sensilla (present in *E. upsilon* sp. nov. and *E. panamensis*, absent in remaining species); aedeagus long, more than half length of ejaculatory apodeme (aedeagus less than half length of ejaculatory apodeme in *E. micra*).

Material examined

Paratypes

COSTA RICA – **Heredia** • 1 ♂; Puerto viejo de Sarapaqui, Estacion Biologica LaSelva; alt. 50–100 m.; 1 Jul. 1993; Malaise trap; USNMENT00857092; USNM • 1 ♂; same collection data as for preceding; USNMENT00857112; USNM • 1 ♂; same collection data as for preceding; CAS • 1 ♂; same collection

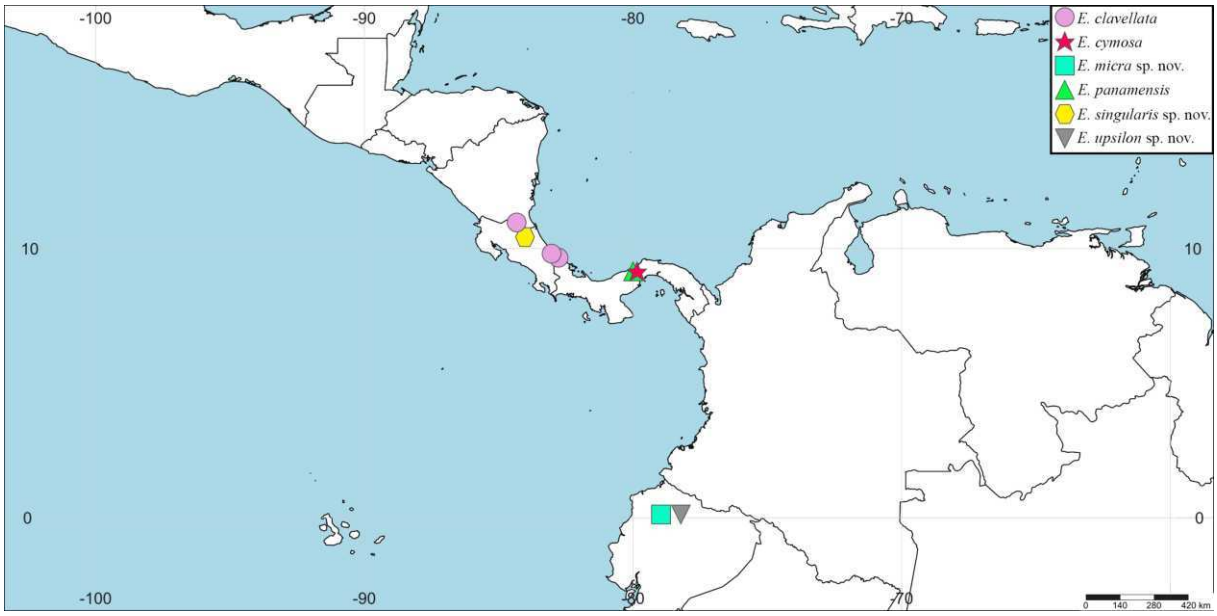

Fig. 1. Distribution map of the species of *Eugenys* Quate, 1996.

data as for preceding; INBIO-CRI0Q1-470625; NHM • 1 ♂; same collection data as for preceding; INBIO-CRI0Q1-470323; NHM.

Distribution

Costa Rica, Nicaragua (Quate 1996; Collantes & Martinez-Ortega 1999).

Genetics

No specimens were available for DNA sequencing.

Eugenys cymosa Quate, 1999
Figs 1, 2B, 3A

Eugenys cymosa Quate, 1999: 432. Type locality: Panama, Canal Zone, Barro Colorado Island.

Differential diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the following characters: hypopods with 10–14 apical tenacula (12–14 present in *E. singularis* sp. nov., one apical tenaculum in remaining species).

Material examined

Paratypes

PANAMA – **Canal Zone** • 1 ♀; Barro Colorado Island; 9.1500° N -79.8500° W; 11–18 Aug. 1993; J Pickering leg.; BMNH(E)2001-8; NHM • 1 ♀; same collection data as for preceding; 1–8 Nov. 1993; NHM • 1 ♂; same collection data as for preceding; 23–4 Aug. 1993; NHM • 1 ♂; same collection data as for preceding; 4–11 Aug. 1993; NHM.

Distribution

Panama (Only known from the type locality, Quate, 1999).

Genetics

No specimens were available for DNA sequencing.

Eugenys micra sp. nov.
urn:lsid:zoobank.org:act:DDCD52D2-BE5D-4E0F-B3DC-BABEF28BEFDB
Figs 1, 3E, 4, 5A

Differential diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the following characters: hypopods with singular apical tenaculum (10–14 present in *E. singularis* sp. nov. and *E. cymosa*, one in the remaining species); antennal flagellomeres without patch of darkened sensilla (present in *E. upsilon* sp. nov. and *E. panamensis*, absent in remaining species); aedeagus less than half length of ejaculatory apodeme (aedeagus long, more than half length of ejaculatory apodeme in *E. clavellata*).

Etymology

The speciphic epithet '*micra*' is derived from the Greek '*mīkrós*' (feminine '*mikrí'*), referring to the small size of the aedeagus of the species.

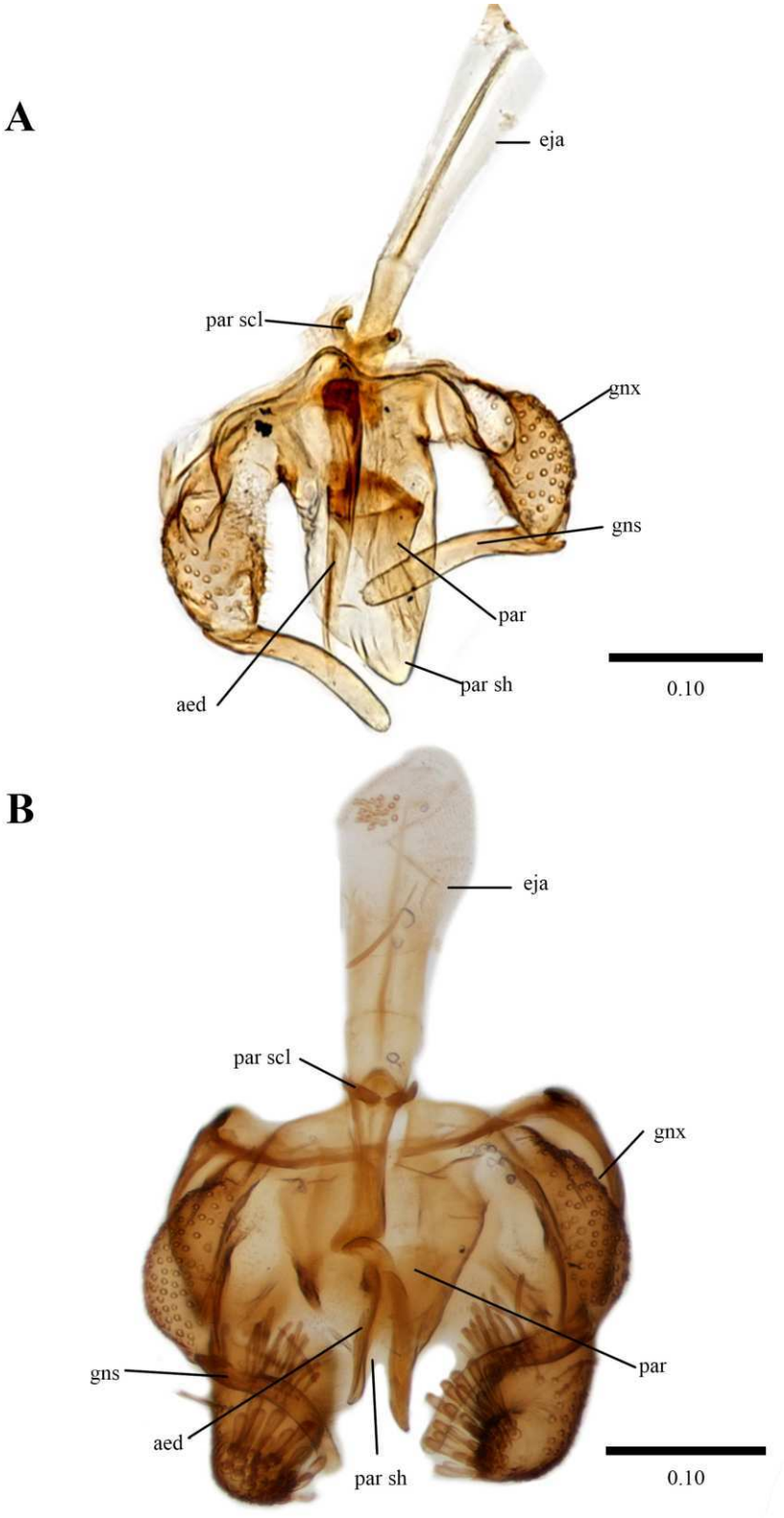

Fig. 2. A. *Eugenys clavellata* Quate, 1996, ♂, paratype (USNMENT00857092), genitalia (ventral view). **B**. *Eugenys cymosa* Quate, 1999, ♂, paratype (BMNH(E)2001-8), genitalia (ventral view). Photos A by David Pecor, Smithsonian Institute Museum, B by Santiago Jaume-Schinkel. Scales in millimeters (mm). Abbreviations: see Material and methods.

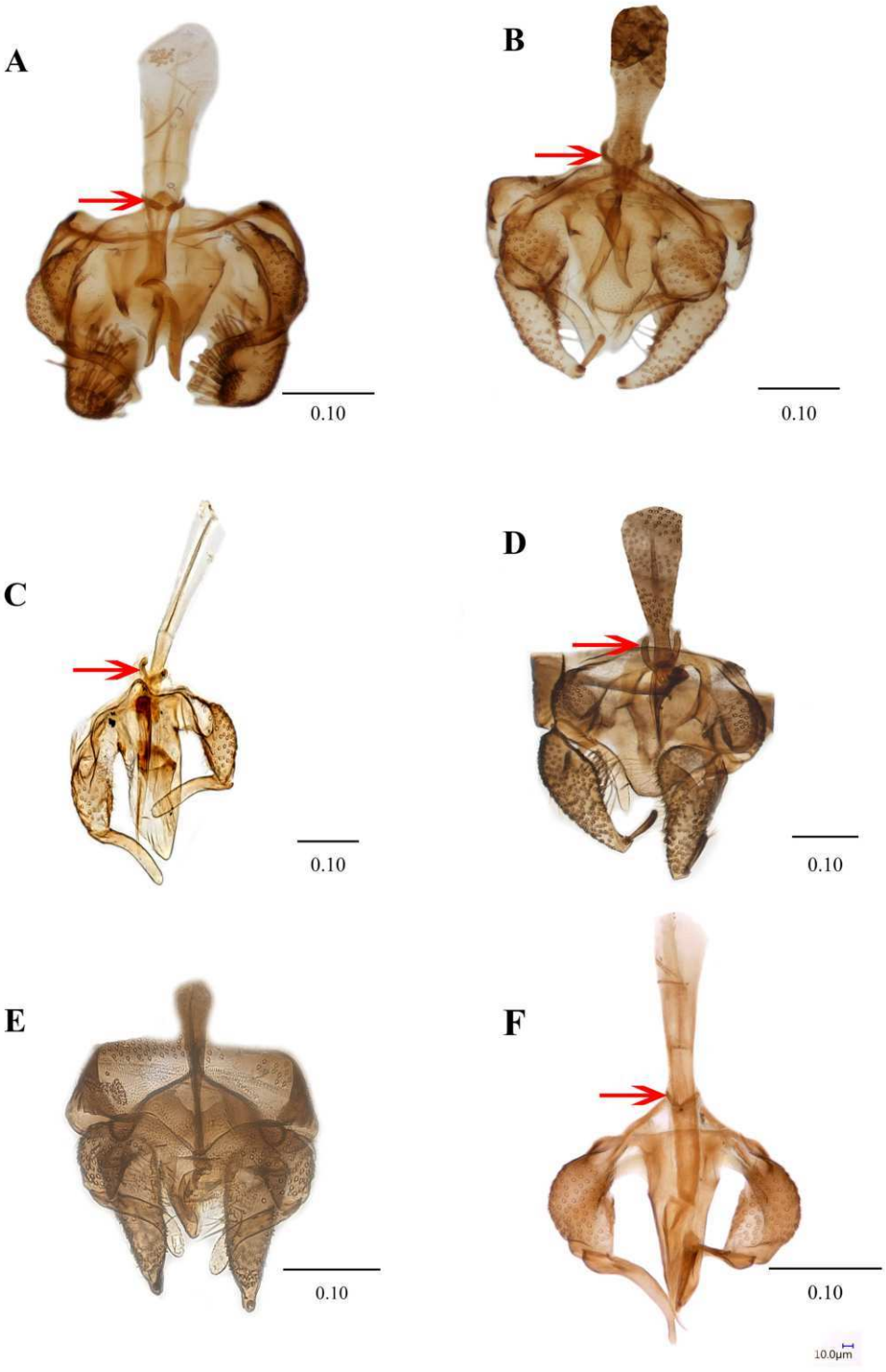

Fig. 3. Male genitalia, ventral view. **A**. *Eugenys cymosa* Quate, 1999, paratype (BMNH(E)2001-8). **B**. *Eugenys panamensis* Quate, 1999, paratype (BMNH(E)-2001-8). **C**. *Eugenys clavellata* Quate, 1996, paratype (USNMENT00857092). **D**. *Eugenys upsilon* sp. nov., holotype (ZFMK-DIP-00097918). **E**. *Eugenys micra* sp. nov., holotype (ZFMK-DIP-00097917). **F**. *Eugenis singularis* sp. nov., holotype (INBIO-CRI001470242). Red arrows point to the parameral sclerites. Photos: A–B, D–F by Santiago Jaume-Schinkel, C by David Pecor, Smithsonian Institute Museum. Scales in millimeters (mm).

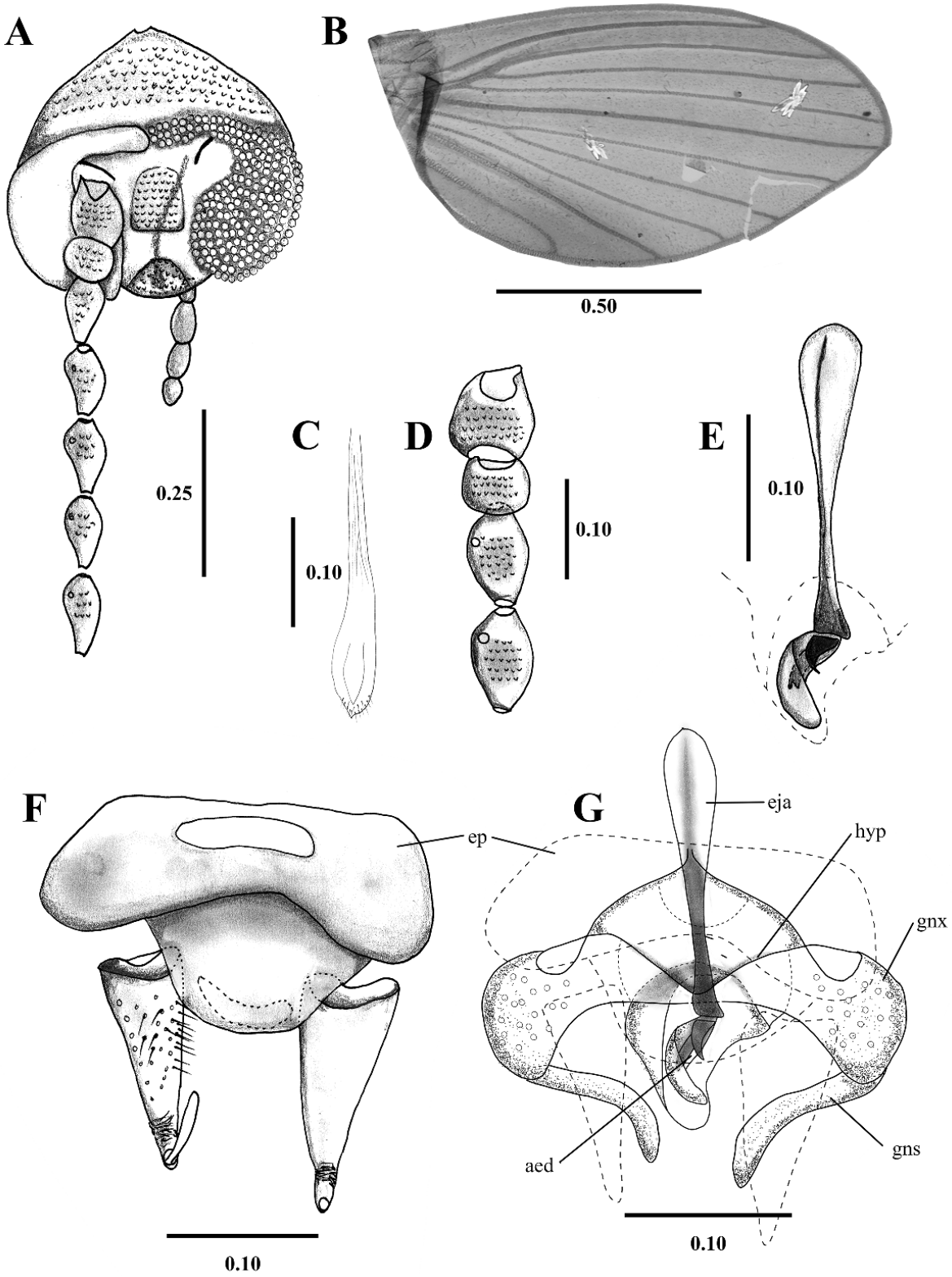

Fig. 4. *Eugenys micra* sp. nov., ♂, holotype (ZFMK-DIP-00097917). **A**. Head. **B**. Wing. **C**. Mouth parts. **D**. Scape, pedicel and first two flagellomeres. **E**. Aedeagus (ventral view). **F**. Epandrium and hypopods (ventral view). **G**. Aedeagus, gonocoxites, and gonostyli (ventral view). Scales in millimeters (mm). Abbreviations: see Material and methods.

Material examined

Holotype

ECUADOR – **Pichincha** • ♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonato, Roadway to Pachijal; 0.11882° N, 78.95802° W; alt. 750 m; 1–9 Feb. 2022; I. Kilian leg.; ZFMK-DIP-00097917; INABIO.

Paratypes

COSTA RICA – **Limon** • 1 ♂; Hitoy-Cerere Biological Reserve; 9.80666° N, -83.02500° E; alt. 100–200 m; 17–26 Feb. 1999; L.W. Quate leg.; INBIO-CRI001472882; LACM • 1 ♂; same collection data as for preceding; INBIO-CRI001472884; INBIO • 1 ♂; same collection data as for preceding; INBIO-CRI001472896; INBIO.

Description

Male (holotype)

Measurements (in mm). Wing length 1.08, width 0.90; head length 0.17, width 0.45; antennal segments, scape 0.09, pedicel 0.06, flagellomeres 1–3: 0.10; palpal segments 1: 0.03, 2: 0.06, 3: 0.05, 4: 0.03.

Head. About 2 × as wide as long; eye bridge contiguous, eye bridge with three facet rows; no enlarged alveoli on postocular area (Fig. 4A); interocular suture appears absent. Antenna with scape about same width as its length, asymmetrical; pedicel spherical, about same width as its length; flagellomeres posterior to 4 lost in all examined specimens, 1–4 fusiform, flagellomeres without darkened patch of sensilla; ascoids absent in examined material; frontal alveoli patch quadrate; mouthparts reduced, not extending beyond margin of head. Palpal segments short and sclerotized, apical segment not corrugated; not extending more than half of flagellomere 2, palpal proportions 1.0:2.0:1.6:1.0.

Thorax. Without allurement organs; wing length about 2 × its width, hyaline, with alveoli on entire surface (Fig. 4B); Sc vein almost straight, ending at origin of R_5, fork of R_{2+3} slightly basal to M_{1+2}, R_{2+3} joining R_4, wing apex rounded, R_5 ending slightly below apex.

Terminalia (Fig. 4E–G). Hypandrium very narrow, plat-like, and V-shaped; gonocoxites about same length as gonostyli, longer than wide, gonocoxites joining in middle, and together giving origin to semi-sclerotized parameral sheath protruding beyond aedeagus, covering aedeagus only on dorsal surface (see Figs 3E, 4E, G); gonostyli narrow, outwardly curved; ejaculatory apodeme about 3 × as long as aedeagus, narrow but broadening towards anterior end, aedeagus curved, with claw-shaped and heavily sclerotized paramere; aedeagal sclerites apparently absent; epandrium about 4 × as wide as long, concave on posterior margin; hypopods elongate, longer than epandrium, curved and tapering towards apex, apex of hypopods with single tenaculum with crowned apex. Epiproct very wide, tongue-shaped with rounded margin, whole surface covered in small setulae.

Female

Unknown.

Distribution

Costa Rica and Ecuador.

Genetics

The holotype was successfully sequenced for COI, GenBank accession number OQ706370.

Eugenys panamensis Quate, 1999
Figs 1, 3B, 5B

Eugenys panamensis Quate, 1999: 430. Type locality: Panama, Canal Zone, Barro Colorado Island.

Diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the following characters: hypopods with a singular apical tenaculum (10–14 present in *E. singularis* sp. nov. and *E. cymosa*, one in *E. micra* sp. nov., *E. panamensis* and *E. upsilon* sp. nov.); antennal flagellomeres with patch of darkened sensilla (present in *E. upsilon*, absent in remaining species); aedeagus straight, with distinguishable paramere (aedeagus curved, without distinguishable paramere in *E. upsilon*).

Material examined

Paratypes

PANAMA – **Canal Zone** • 1 ♂; Barro Colorado Island; 11–18 Aug. 1993; 9.1500° N, -79.8500° W; J. Pickering leg.; #1662; BMNH(E)-2001-8; NHM • 1 ♂ same collection data as for preceding; 4–11 Aug. 1993; #1709; NHM.

Genetics

No specimens were available for DNA sequencing.

Eugenys singularis sp. nov.
urn:lsid:zoobank.org:act:AA14FE8F-5A54-430F-9D08-56AC089FFB68
Figs 1, 3F, 6

Differential diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the hypopods with a lateral digitiform projection (hypopods without lateral projection in remaining species).

Etymology

The species epithet is derived from the Latin '*singulāris*', referring to the unusual shape of the lateral projection of the hypopods.

Material examined

Holotype

COSTA RICA – **Heredia** • ♂; Puerto Viejo de Sarapaquí, Est. Biol. LaSelva; alt. 50–100 m; 1 Dec. 1993; Malaise trap M/00/009; INBIO-CRI001470242; LACM.

Paratypes

COSTA RICA • 1 ♂; same collection data as for holotype; INBIO-CRI001470896; LACM.

Description

Male

Measurements (in mm; n = 2). Wing length 1.8 (1.8–1.8), width 0.89 (0.89–0.90); head length 0.35 (0.32–0.38), width 0.40 (0.40–0.40); antennal segments, scape 0.09 (0.08–0.10), pedicel 0.05 (0.05–0.06), flagellomeres 1: 0.09 (0.09–0.10), 2–13: 0.08 (0.08–0.09), flagellomere 14: 0.09; palpomeres 1: 0.04 (0.04–0.05), 2: 0.06 (0.06–0.06), 3: 0.05 (0.05–0.05), 4: 0.04 (0.04–0.04).

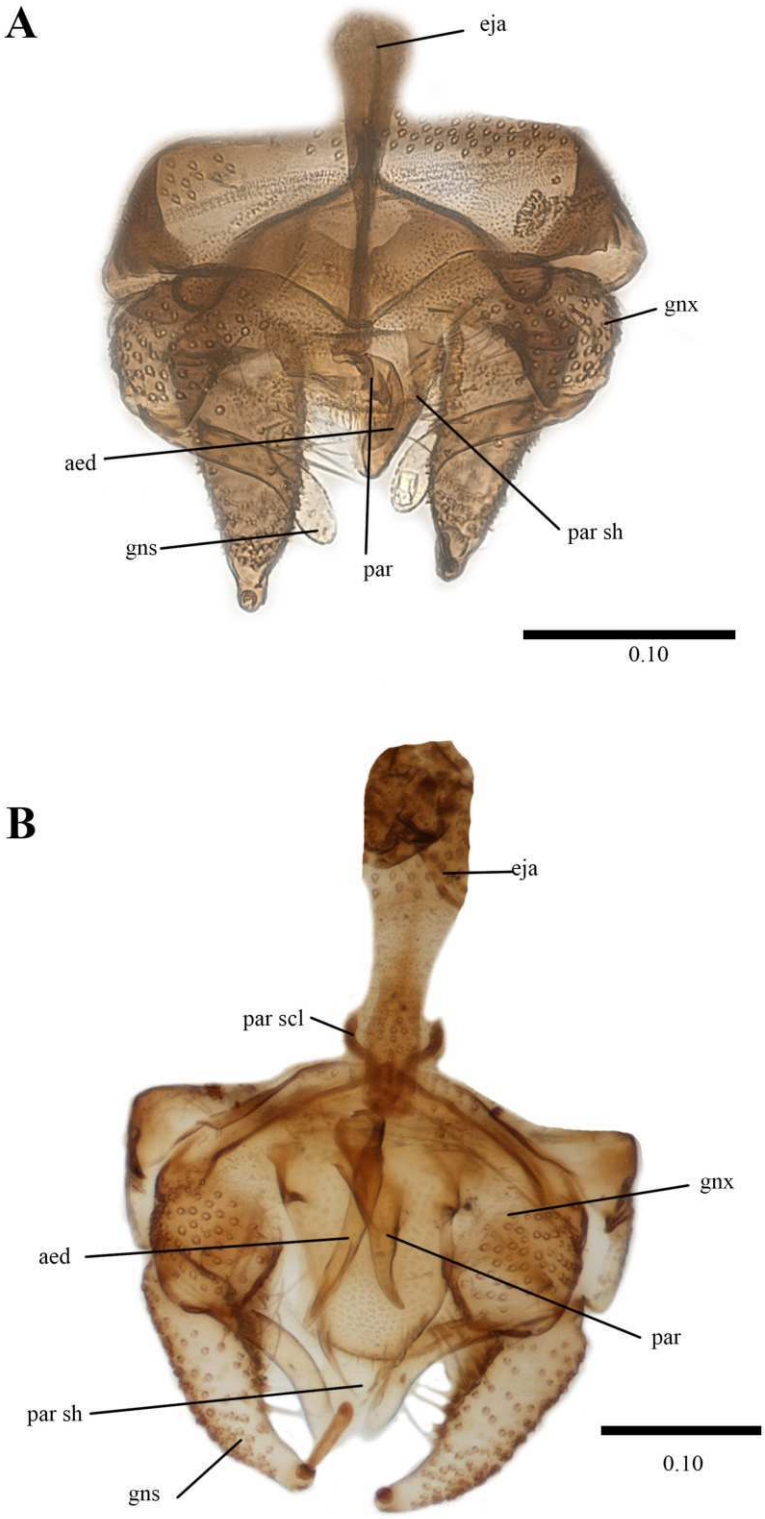

Fig. 5. Male genitalia, ventral view. **A**. *Eugenys micra* sp. nov., holotype (ZFMK-DIP-00097917). **B**. *Eugenys panamensis* Quate, 1999, paratype (BMNH(E)-2001-8). Scales in millimeters (mm). Abbreviations: see Material and methods.

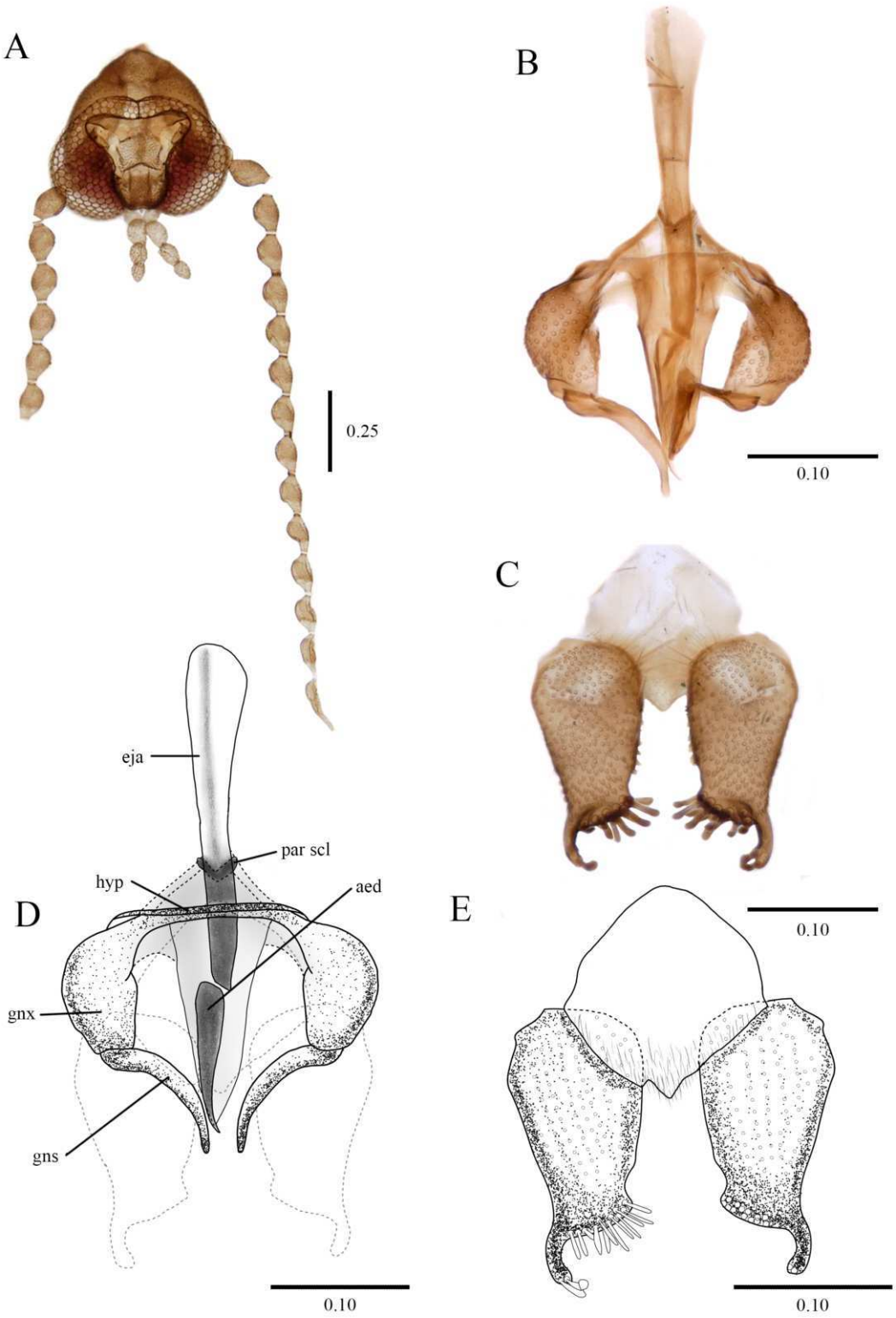

Fig. 6. *Eugenys singularis* sp. nov., ♂, holotype (INBIO-CRI001470242). **A**. Head. **B**. Aedeagus, gonocoxites and gonostyli (ventral view). **C**. Hypopods (ventral view). **D**. Aedeagus, gonocoxites and gonostyli (ventral view). **E**. Hypopods. Scales in millimeters (mm). Abbreviations: see Material and methods.

Head. Slightly wider than long; eye bridge contiguous, eye bridge with three facet rows; no enlarged alveoli on postocular area; interocular suture non-discernible in examined material (Fig. 6A). Antenna with scape cylindrical, about 2 × as long as pedicel; pedicel spherical; 14 flagellomeres, 1–13 fusiform, flagellomere 14 elongated, slightly longer than flagellomere 13, ending in digitiform apiculus, flagellomeres without patch of darkened sensilla; ascoids lanceolate, about 2 × as long as flagellomere carrying them; frontal alveoli patch square, lower margin with concavity in middle; mouthparts reduced, not extending beyond margin of head. Palpal segments short and sclerotized, apical segment not corrugated; not extending more than half of flagellomere 4, palpal proportions 1.0:1.4:1.1:1.0.

Thorax. Without allurement organs; wing length about 2 × its width, hyaline, with alveoli on entire surface; Sc vein almost straight, ending at about origin of R_5, fork of R_{2+3} basal to M_{1+2}, R_{2+3} joining R_4, wing apex rounded, R5 ending at wing apex.

Terminalia. Hypandrium narrow, plate-like; gonocoxites cylindrical, about two-thirds length of gonostyli; gonocoxites joining in middle, and together giving origin to semi-sclerotized parameral sheath protruding beyond aedeagus, covering aedeagus only on sternal surface (see Fig. 6B, D); gonostyli digitiform, tapering towards apex, with rounded apex, outwardly curved; ejaculatory apodeme about 2 × as long as aedeagus, narrow but broadening towards basal margin; aedeagus digitiform and tapering towards the apex, apex pointed; parameral sclerites fused, forming single Y-shaped sclerotized segment, linked to aedeagal sheath and aedeagus; epandrium not discernible in examined material; hypopods cylindrical, with outer laterally curved projection (Fig. 6C, E), lateral projection carrying two apical tenacula, while margin prior to projection in apex of hypopods carries 12–14 tenacula, all tenacula with rounded apex; epiproct v-shaped, covered in small setulae.

Female

Unknown.

Distribution

Only known from the type locality in Costa Rica.

Genetics

No specimens were available for sequencing.

Eugenys upsilon sp. nov.

urn:lsid:zoobank.org:act:56AB3A46-1F40-471F-B126-5C667FA33B48

Figs 1, 3D, 7–8

Differential diagnosis

This species can be easily differentiated from all other species of *Eugenys* by the following characters: hypopods with singular apical tenaculum (10–14 present in *E. singularis* sp. nov. and *E. cymosa*, one apical tenaculum in *E. micra* sp. nov., *E. panamensis* and *E. upsilon* sp. nov.); antennal flagellomeres with patch of darkened sensilla (present in *E. panamensis*, absent in remaining species); aedeagus curved, without distinguishable paramere (aedeagus straight, with distinguishable paramere in *E. panamensis*).

Etymology

The species epithet is derived from the Greek letter 'ύψιλον' ('ýpsilon'), referring to the Y- to U-shaped parameral sclerite.

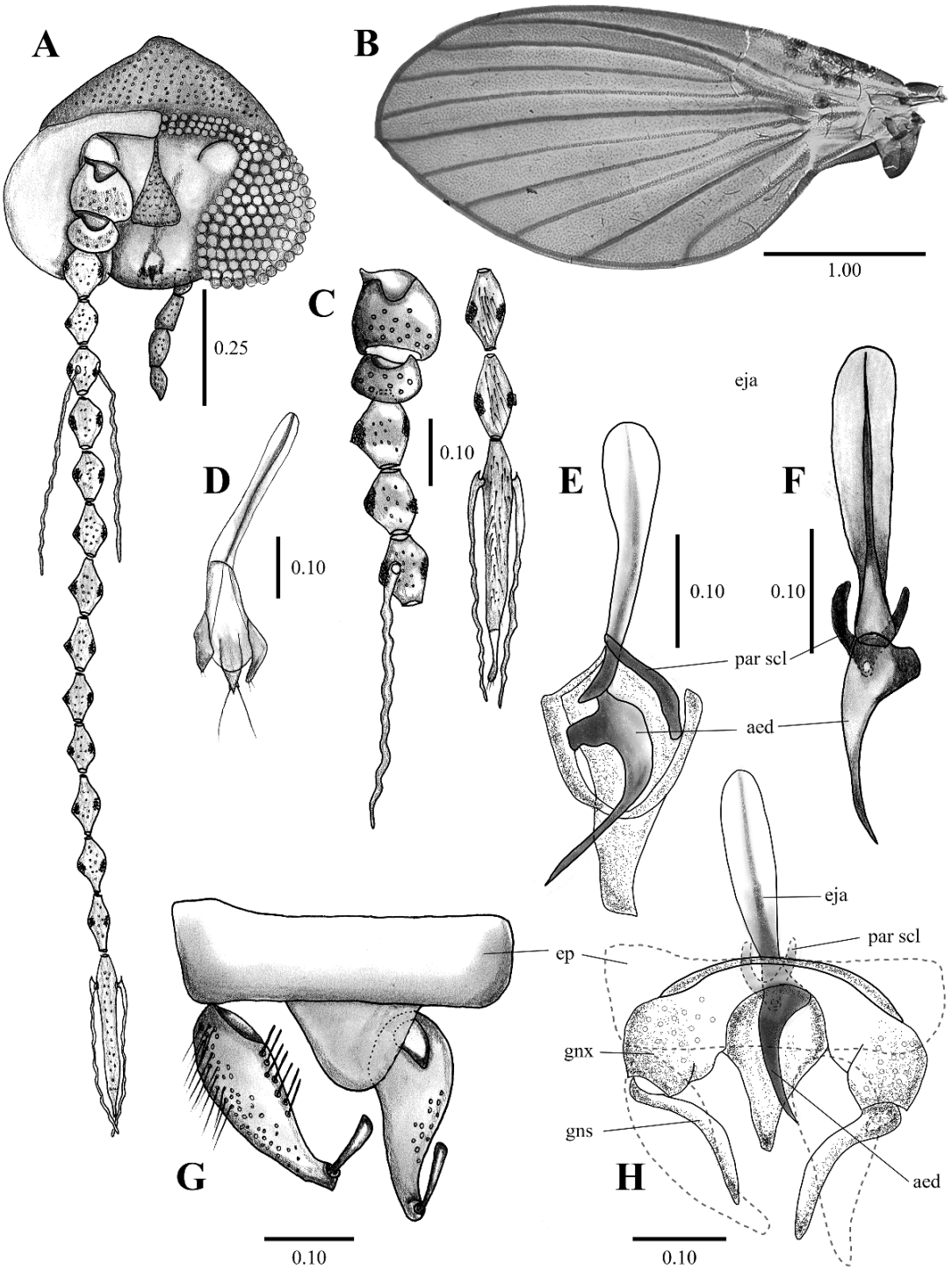

Fig. 7. *Eugenys upsilon* sp. nov. **A**. ♂, paratype **(**ZFMK-DIP-00097921), head. **B**. ♂, paratype **(**ZFMK-DIP-00097914), wing. **C**. ♂, paratype **(**ZFMK-DIP-00097921), mouth parts. **D**. ♂, paratype **(**ZFMK-DIP-00097921), first antennal segments. **E–H**. ♂, holotype (ZFMK-DIP-00097918). **E**. Aedeagus (lateral view). **F**. Aedeagus (ventral view). **G**. Epandrium and hypopods (ventral view). **H**. Aedeagus, gonocoxites, and gonostyli (ventral view). Scales in millimeters (mm). Abbreviations: see Material and methods.

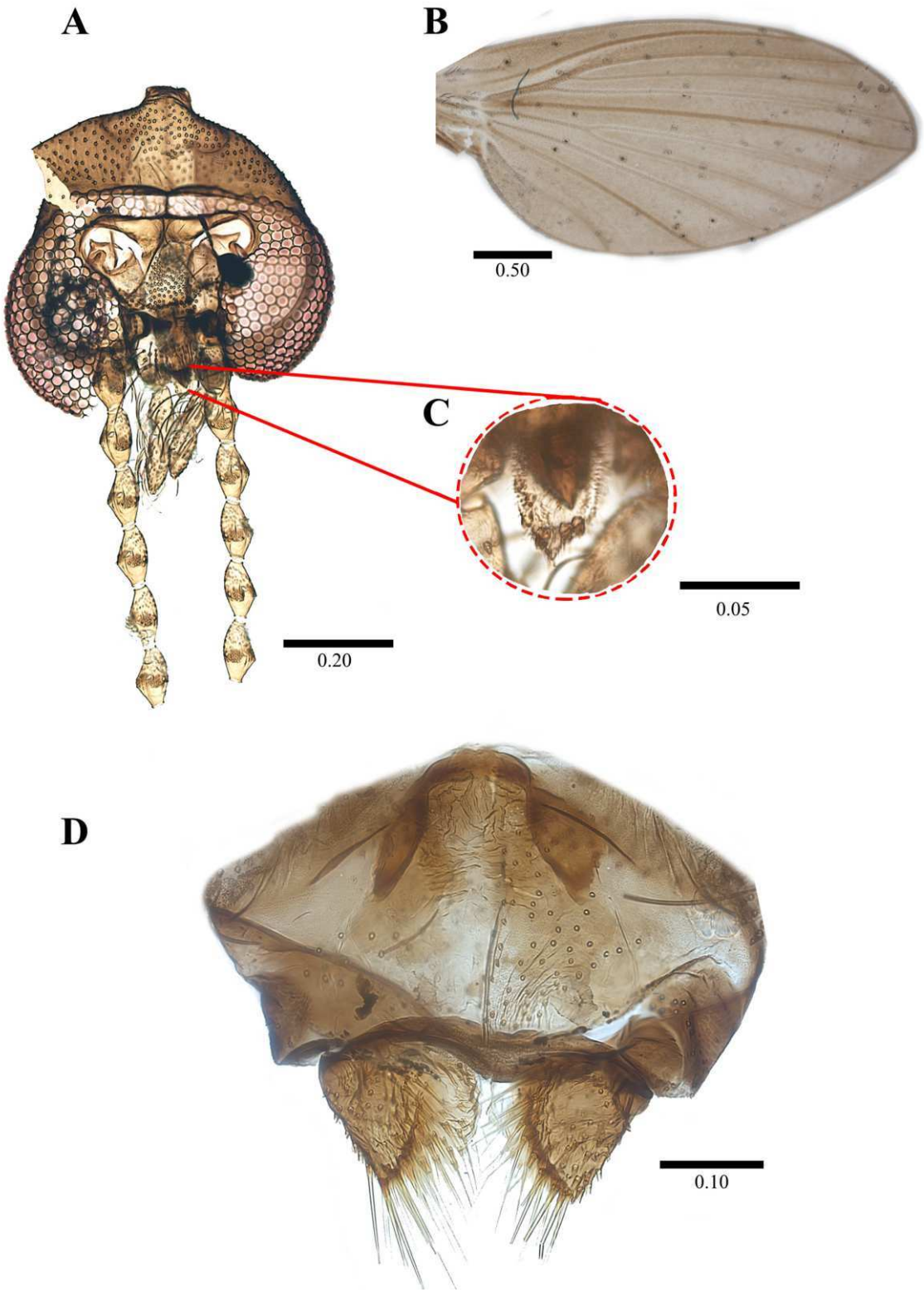

Fig. 8. *Eugenys upsilon* sp. nov., ♀, paratype (ZFMK-DIP-00097916). **A**. Head. **B**. Wing. **C**. Mouth parts. **D**. Genitalia (ventral view). Scales in millimeters (mm).

Material examined

Holotype

ECUADOR – **Pichincha** • ♂; Pedro Vicente Maldonado, Parroquia Pedro Vicente Maldonato, Roadway to Pachijal; 0.11882° N, 78.95802° W; alt. 750 m; 1–9 Feb. 2022; I. Kilian leg.; ZFMK-TIS-2637135; ZFMK-DIP-00097918; INABIO.

Paratypes

ECUADOR • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637099; ZFMK-DIP-00097913; INABIO • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637105; ZFMK-DIP-00097914; ZFMK • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637111; ZFMK-DIP-00097915; ZFMK • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637140; ZFMK-DIP-00097919; ZFMK • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637141; ZFMK-DIP-00097920; ZFMK • 1 ♂; same collection data as for holotype; ZFMK-TIS-2637144; ZFMK-DIP-00097921; ZFMK • 1 ♀; same collection data as for holotype; ZFMK-TIS-2637123; ZFMK-DIP-00097916; ZFMK.

Description

Male

Measurements (in mm; n = 7). Wing length 3.04 (2.8–3.4), width 1.53 (1.3–1.63); head length 0.53 (0.56–0.50), width 0.63 (0.66–0.60); antennal segments, scape 0.13, pedicel 0.08, flagellomeres 1: 0.11, 2–12: 0.10, 13:0.11, 14: 0.36; palpal segments 1: 0.05, 2: 0.01, 3: 0.08, 4: 0.05.

Head (holotype). Wider than long; eye bridge contiguous, eye bridge with two facet rows on narrowest part and three facet rows on remaining part of bridge; no enlarged alveoli on postocular area; interocular suture appears absent (Fig. 7A). Antenna with scape wider than long, asymmetrical; pedicel spherical, about 2 × as wide as long; 14 flagellomeres, 1–13 fusiform, flagellomere 14 elongated, 3 × as long as previous flagellomere, ending in digitiform apiculus, flagellomeres with patch of darkened sensilla; ascoids very long, at least length of three flagellomeres, rod-like and with zigzag shape; frontal alveoli patch egg-shaped; mouthparts reduced, not extending beyond margin of head. Palpal segments short and sclerotized, apical segment not corrugated; not extending more than half of flagellomere 4, palpal proportions 1.0:2.0:1.6:1.0.

Thorax. Without allurement organs; wing length about 2 × its width, hyaline, with alveoli in whole surface (Fig. 7B); Sc vein almost straight, ending at origin of R_5, fork of R_{2+3} basal to M_{1+2}, R_{2+3} joining R_4, wing apex rounded, R5 ending slightly below apex.

Terminalia. Hypandrium narrow, plate-like, and rounded, Gonocoxites about half length of gonostyli, about as wide as long, gonocoxites joined in middle, and together giving origin to semi-sclerotized parameral sheath protruding beyond aedeagus, covering aedeagus only on sternal surface (Figs 3D, 7F, H); gonostyli narrow, outwardly curved; ejaculatory apodeme slightly longer than aedeagus, narrow but broadening towards basal margin, aedeagus curved in ventral view, and claw-shaped in lateral view, aedeagus with heavily sclerotized lump at base, which in lateral view extends towards dorsal surface (Fig. 7E); parameral sclerite forming single Y-shaped sclerotized structure, linked to aedeagal sheath and aedeagus; epandrium about 4 × as wide as long; hypopods elongate, longer than epandrium, tapering towards apex, apex of hypopods with single tenaculum with crowned apex. Epiproct tongue-shaped with rounded margin, covered in small setulae.

Female

Same as male except for palpi consisting of three segments; terminal flagellomeres absent in material examined; maximum number of flagellomeres present: 6. Mouthparts slightly more developed than in male, mouthparts with teeth-like sclerotized structures (Fig. 8C).

Distribution

Only known from the type locality in Ecuador.

Genetics

All specimens examined were successfully sequenced. All sequences are identical. Genebank's accession numbers are OQ706367, OQ706373, OQ706374, OQ706368, OQ706372, OQ706366, OQ706371, OQ706369.

Key to the males of Eugenys Quate, 1996

1. Hypopods with a lateral digitiform and curved projection (see Fig. 6C, E.) ....***E. singularis*** sp. nov.
– Hypopods without a lateral digitiform projection (as in Fig. 3A–E) 2

2. Hypopods with 14–16 apical tenacula (Figs 2B, 3E) .....***E. cymosa*** Quate, 1999
– Hypopods with a single apical tenaculum (Fig. 3B, D–E) 3

3. Antennal flagellomeres without a patch of darkened sensilla (Fig. 4A) 5
– Antennal flagellomeres with a patch of darkened sensilla (Fig. 8A) 4

4. Aedeagus curved, without distinguishable paramere apart from the parameral sclerite (Figs 3D, 7E–F, H) .....***E. upsilon*** sp. nov.
– Aedeagus straight, with distinguishable paramere in addition to the parameral sclerite(Figs 3B, 5B) ..... ***E. panamensis*** Quate, 1999

5. Aedeagus short, less than half the length of the ejaculatory apodeme; parameral sclerite not Y-shaped, appears absent (Figs 3E, 4E, G, 5A) .....***E. micra*** sp. nov.
– Aedeagus long, more than half the length of the ejaculatory apodeme; parameral sclerite Y-shaped and sclerotized (Figs 2A, 3C) .....***E. clavellata*** Quate, 1996

Discussion

With the present publication, we increase the number of species inside *Eugenys* to six. All of them are restricted to the Neotropical Region, and the currently recorded species have been recorded in four countries (Fig. 1), namely, Costa Rica with three species (*E. clavellata*, *E. micra* sp. nov., and *E. singularis* sp. nov.), Ecuador with two species (*E. micra* sp. nov. and *E. upsilon* sp. nov.), Nicaragua with one species (*E. clavellata*), and Panama with two species (*E. cymosa* and *E. panamensis*). The presence of this genus in other Neotropical countries is very likely, and the distributional gap might be due to the lack of systematic collections, but any further hypothesis might just be speculation.

Little is known about the biology of this genus, there is no information regarding larval stages or development. The reduced and/or atrophied mouthparts suggest that the adults of the genus are likely not to feed. However, as observed in Fig. 8C, the female of *Eugenys upsilon* sp. nov. possesses slightly more developed mouthparts than her male counterpart, and we observed some teeth-like sclerotized structures around the mouthparts that might suggest the mouthparts are not completely atrophied and some feeding might occur; however, there is no strong evidence, and this is a conjecture based on the observed morphological structures.

To date, the females are only known for two species (*E. panamensis* and *E. upsilon* sp. nov.) and both females are morphologically similar. Quate (1999) stated that the female genitalia is unlike that of all other Psychodinae. Male and female association in Quate's work (1999) was based on co-occurrence while describing the species. Male and female association in this study was based on DNA barcodes

(male and female barcodes of *E. upsilon* are identical, differing by 15% with the barcodes of *E. micra* sp. nov.). For the two species found at the same locality in Ecuador, namely, *E. micra* and *E. upsilon*, males can be easily separated through genital characters. On the contrary, the female of *E. micra* remains unknown, making it difficult to compare morphological characters with other known females. In the future, DNA barcodes will be helpful in describing new species and dealing with sex associations.

Most species of Psychodinae with fusiform flagellomeres, asymmetric male genitalia, and hypopods with a single apical tenaculum belong to the tribe Maruinini Enderlein, 1937 as defined by Duckhouse (1987, 1990; see also Quate & Brown 2004 who treated these taxa as Setomimini Vaillant, 1982). Some of the apomorphic characters of *Eugenys* indeed also occur in some Maruinini such as the elongated ascoids (similar to those observed in *Setomima* Enderlein, 1937 (see Duckhouse 1978)). However, whereas the linkage between the parameral-aedeagal complex and the subepandrial sclerite in Maruinini is developed as a distinct "ball-and-socket" configuration as described by Duckhouse (1987), this linkage is developed as a U- to Y-shaped parameral-subepandrial sclerite resembling what Vaillant (1971) described as "furca" in his Telmatoscopini (Fig. 3, indicated with a red arrow).

Kvifte (2014) suggested that the linkage parameral-subepandrial sclerite is mostly paramerally derived and showed its presence across most lineages of his Paramormiini Enderlein, 1937 (part of Pericomaini s. lat. of Kvifte 2018) and not just the ones considered by Vaillant (1971) to possess the "furca". The "Paramormiini" taxa in which the Y- to V-shaped parameral-subepandrial sclerite is most conspicuous (as observed in *Eugenys,* see Fig. 7E), however, mostly have nodiform flagellomeres, vein Rs not pectinate, male genitalia symmetrical (parameres paired or reduced, except in *Lepiseodina* Enderlein, 1937 and *Moruseodina* Bravo & Cordeiro, 2014, which have a single unpaired paramere) and hypopods with multiple aseriate apical tenacula (as in *Eugenys cymosa* and *E. singularis* sp. nov.).

The narrow eyebridge of only three facet rows and the pectinate Rs are characters shared with *Mormia* s. lat., which prompted Quate (1996) to place *Eugenys* in Mormiini originally. However, *Mormia* s. lat. all have nodiform flagellomeres, male genitalia symmetrical, and hypopods with multiple aseriate apical tenacula. Moreover, the narrow eyebridge of three facet rows, the fusiform flagellomeres and the male genitalia with a single paramere are shared characters with species of *Tonnoiriella* Vaillant, 1971 (the paramere was considered a distiphallic sclerite by Wagner & Withers 2020). However, *Tonnoiriella* differs from *Eugenys* by much shorter ascoids and hypopods with multiple aseriate apical tenacula. Nevertheless, we deem it likely that both *Eugenys* and *Tonnoiriella* may warrant a placement within or basal to Pericomaini s. lat. sensu Kvifte (2018) based on morphological characters, in particular the Y-shaped linkage of the aedeagal-parameral complex with the subepandrial sclerite.

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Appendix 14. (Publication chapter 16)

Chapter 16 – Publication

Jaume-Schinkel S, Kvifte GM (2022) First record of *Lepidiella* Enderlein, 1937 from the Oriental Region (Diptera, Psychodidae). *Zookeys,* 1115, 73–79. https://doi.org/10.3897/zookeys.1115.81668

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RESEARCH ARTICLE

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First record of *Lepidiella* Enderlein, 1937 from the Oriental Region (Diptera, Psychodidae)

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Abstract

We provide the first record of the genus *Lepidiella* Enderlein, 1937 from the Oriental Region with the description of *Lepidiella limicornis* **sp. nov.**, based on two male specimens collected in Thailand. Additionally, we provide a list of the world species of *Lepidiella* and discuss the diagnosis and taxonomic placement of the genus.

Keywords

Moth flies, new record, new species, Psychodinae, taxonomy

Introduction

The moth fly fauna of the Oriental Region is highly diverse and understudied, with the family Psychodidae including more than 420 described species (Lewis 1987; Ježek 2010; Curler and Priyadarsanan 2015; Ježek et al. 2015; Kvifte and Andersen 2016; Polseela et al. 2019). Regardless of the recent attention this family has received due to the medical importance of the subfamily Phlebotominae, there is still a large number of species that remain undescribed (Duckhouse and Duckhouse 2000; Curler 2009; Ježek 2010).

The genus *Lepidiella* Enderlein, 1937, formerly known as *Syntomoza* Enderlein, 1937 (see Quate, 1963; Collantes and Hodkinson 2003) has been thought to be restricted to the Neotropical Region. This genus has been recorded in Brazil, Bolivia, Colombia, Costa Rica, Mexico, Nicaragua, Panama, Peru, and the island of Santa Lucia in the Caribbean (Ibáñez-Bernal 2008; Bravo and Santos 2011; Araújo and Bravo 2013, 2019).

Here, we describe a new species of the genus *Lepidiella* and discuss its generic placement. Additionally, we record *Lepidiella* for the first time outside the Neotropical Region, and we update the generic diagnosis of this genus.

Materials and methods

The studied specimens are deposited at the Department of Natural History, University Museum of Bergen, Bergen, Norway (**ZMBN**). Specimens were collected with a hand net, stored in ethanol, and then mounted on permanent slides. In the material examined section, at the end of each record and between square brackets ([ ]), the holding institution is indicated. In the description of type labels, the contents of each label are enclosed in double quotation marks (“ ”), italics denote handwriting, and the individual lines of data are separated by a double forward-slash (//).

Measurements were made with an ocular micrometer in a microscope Leitz model Dialux 20, measures in millimeters (mm). Head width was taken at the widest part, approximately above the insertion of antennal scape, whereas the length was taken from the vertex to the lower margin of clypeus; wing length measured from the base of the wing at the start of the costal node to the apex of the wingtip, while the width was taken approximately at an imaginary vertical line crossing the radial and medial forks; palpal proportions consider the length of the first palpal segment as a unit (1.0).

Terminology

We follow the general terminology proposed by Cumming and Wood (2017). For the male genitalia, we follow the term of hypopods instead of cerci or surstyli proposed by Kvifte and Wagner (2017) as the origin of these caudal appendages seems to have combined origins of the proctiger and epandrium.

Results

Genus *Lepidiella* Enderlein, 1937

Lepidiella Enderlein 1937: 89. Type species: *Lepidiella lanuginosa* Enderlein 1937: 89–90, by monotypy and original designation.

Syntomoza Enderlein 1937: 88–89. Type species: S*yntomoza niveitarsis* Enderlein 1937: 89, by monotypy and original designation.

Kupara Rapp 1945: 310. Type species: *Kupara albipeda* Rapp 1945: 311, by monotypy and original designation (Bravo and Santos 2011; Collantes and Hodkinson 2003).

Diagnosis. Males and females with vertex dorsally expanded; males with or without corniculi, females without corniculi; males and females with 4 rows of facets on eye bridge, antennae with 14 barrel-shaped flagellomeres, flagellomeres 1–11 with a pair of simple digitate ascoids, flagellomeres 12–14 reduced in size and without ascoids; wing vein R_4 ending slightly before or at the wing apex; males with multiple apical tenacula on hypopods.

Species included. *Lepidiella albipeda* (Rapp, 1945), *L. amaliae* (Collantes & Martínez-Ortega, 1997), *L. cervi* (Satchell, 1955), *L. flabellata* Bravo & Santos, 2011, *L. hansoni* (Quate, 1996), *L. lanuginosa* Enderlein, 1937, *L. larryi* Ibáñez-Bernal, 2010, *L. limicornis* sp. nov., *L. maculosa* Araújo & Bravo, 2019, *L. matagalpensis* (Collantes & Martínez-Ortega, 1988), *L. monteveredica* (Quate, 1996), *L. niveitarsis* (Enderlein, 1937), *L. olgae* Bravo & Araújo, 2013, *L. pickeringi* (Quate, 1999), *L. robusta* Bravo & Santos, 2011, *L. spinosa* Bravo, 2005, *L. wagneri* Araújo & Bravo, 2019, *L. zumbadoi* (Quate, 1999).

Lepidiella limicornis sp. nov.

https://zoobank.org/067E7A52-E761-4849-B781-C52EAF35BACE

Figs 1–5

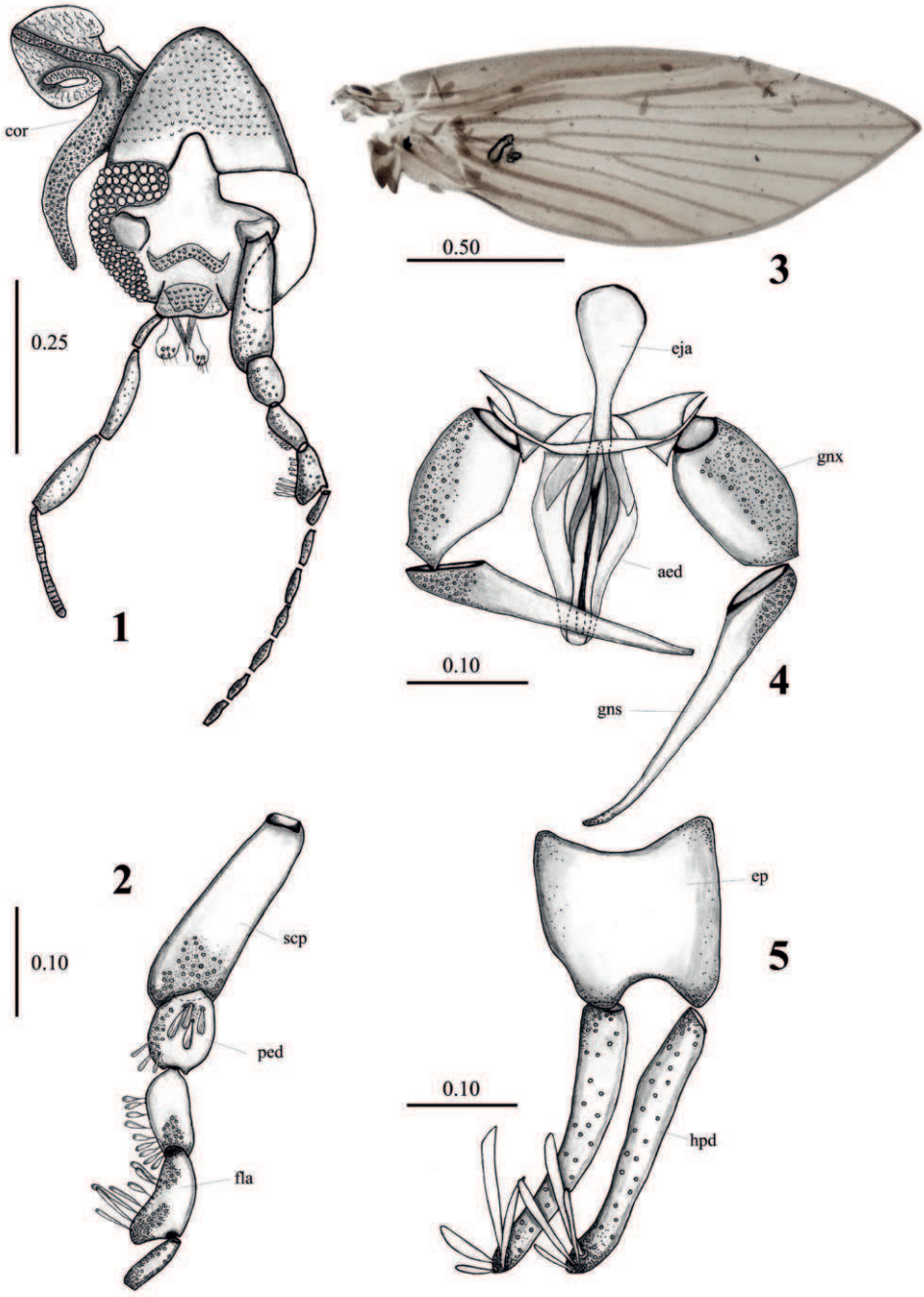
Examined material. ***Holotype***, ♂, slide mounted, . "*Lepidiella limicornis #m* // *HOLOTYPE* // Thailand: Chiang Mai, // Doi Pui Mong village, // waterfall/pond, // 18.8163°N, 98.8831°E // 9.IV.1991, (hand net) // J. Kjaerandsen leg. // **ZMBN #:**", [ZMBN], paratype, ♂, slide mounted, same label information [ZMBN].

Differential diagnosis. This species can be easily differentiated from all the species in *Lepidiella* by the combination of the following characters: eyes separated by 4 facet diameters, interocular suture as inverted U, second flagellomere asymmetrical, and hypopods with four tenacula.

Type locality. Thailand, Chiang Mai, Doi Pui Mong village (18.8163°N, 98.8831°E).

Description. Measurements in mm (n = 2). Wing length 1.81, width 0.68; head length 0.45, width 0.34; Antennal segments, scape: 0.19, pedicel: 0.07, flagellomere 1: 0.08, flagellomere 2: 0.08, flagellomeres 3–9: 0.06; Palpomeres 1: 0.08, 2: 0.12, 3: 0.12, 4: 0.16.

Male. ***Holotype. Head*** 2 × longer than wide, with a pair of 3-branched cornicula, eyes separated by approximately 4 facet diameters; eye bridge with four facet rows; interocular suture as an inverted U, extending towards middle of vertex, a little longer than eye bridge width. Antenna with scape about 4× longer than its width, about 3× length of pedicel, cylindrical, tapered at base, and broadening at apex; first flagellomere cylindrical, symmetrical, about ½ width of scape, second flagellomere asymmetrical with a protuberance on inner margin, subsequent flagellomeres symmetrical, cylindrical, about ½ width of first and second flagellomeres. Total number of flagellomeres unknown as apical flagellomeres are missing in examined specimens; maximum number of flagellomeres = 7. Palps extending to flagellomere 6, palpal proportions, 1.0:1.5:1.5:2.



Figures 1–5. *Lepidiella limicornis* sp. nov., male holotype. **1** head **2** first antennal segments **3** wing **4** hypandrium, gonocoxites, gonostyli, aedeagus **5** epandrium and surstyli. Abbreviations: aed = aedeagus, cor = corniculi, eja = ejaculatory apodeme, ep = epandrium, fla = flagellomere, gns = gonostylus, gnx = gonocoxites, scp = scape, hpd = hypopod. Scale bars in millimeters.

Wing 2.7 × longer than wide, hyaline except costal cell which is brownish; Sc not reaching C but extending to junction of R_{2+3}+R_5; R_4 ending at wing apex, CuA reaching wing margin.

***Terminalia*.** Hypandrium narrow, with rounded margin, seems partially fused with gonocoxites; length of gonocoxites 0.60 length of gonostyli, about 2× longer than wide; gonostyli narrow, tapered towards apex, with alveoli in outer basal ⅓; gonocoxal apodemes triangular, medial extension connected to base of aedeagus; aedeagus symmetrical, bifurcated; paramere narrow, well sclerotized; ejaculatory apodeme dorsoventrally flattened, rounded at anterior margin and tapering towards aedeagus; epandrium about same length and width; basal margin concave around entire length, apical margin strongly concave at middle; hypopods about 1.75× length of gonocoxites, narrow with apical margin rounded; 4 apical tenacula on each; tenacula apex rounded, concave; epiproct triangular with apical margin rounded, covered in micropilosity.

Female. Unknown.

Etymology. From Latin *līmus* = oblique + *cornus* = horns, making references to the oblique shape of the fourth antennal segment (second flagellomere).

Distribution. Only known from the type locality.

Discussion

Quate (1996) recognized three diagnostic characters for the genus (as *Syntomoza*): corniculi present in males; males and females with vertex expanded dorsally; males and females with the apex of vein R_4 ending at the wing apex. Bravo (2005) later described a new species and transferred *Pericoma hansoni* (now *Lepidiella hansoni*) without corniculi. Bravo and Santos (2011) updated the diagnosis of the genus. Finally, Araújo and Bravo (2019) described a new species without the presence of corniculi and recognized six characters for the identification of males and females, specifically: vertex dorsally expanded; antenna with 14 barrel-shaped flagellomeres; flagellomeres 12–14 smaller, without ascoids; R4 ending at the wing apex; males with multiple tenacula on hypopods (as cercus); gonocoxal apodemes fused, forming a narrow and plate-like bridge, not extending anteriorly.

Of these six characters only five fit with the species described here: gonocoxal apodemes are not fused and are extended anteriorly. The diagnosis presented above reflects this.

Corniculi are present in many genera, including *Clytocerus* Eaton, 1904, *Jungiella* Vaillant, 1972, *Panimerus* Eaton, 1913, *Pangeogladiella* Ježek, 2001, *Mystropsychoda* Duckhouse, 1975, and *Neoarisemus* Botoseneanu & Vaillant, 1970. However, this species can be easily separated from *Mystropsychoda* by the presence of an eye bridge (absent in *Mystropsychoda*); from *Neoarisemus*, *Panimerus*, and *Jungiella* by barrel-shaped flagellomeres and wing venation with R_4 ending at the apex of the wing and R_5 endsing beyond apex (flagellomeres fusiform and R_4 before and R_5 at the apex in *Neoarisemus*, *Panimerus*, and *Jungiella*). Finally, *Lepidiella* can be differentiated from *Clytocerus* by

the absence of fusion of flagellomeres 1 and 2 (fused in *Clytocerus*), the absence of tuft of curved setae on basal flagellomeres (present in *Clytocerus*), and the setae alveoli of the frons being in a large continuous patch (*Clytocerus* having two separate patches).

The characters separating *Lepidiella* from *Clytocerus* are unique characters for *Clytocerus* and probably represent apomorphies. It may, therefore, be that *Lepidiella* represents either a plesiomorphic sister group to *Clytocerus* or even is the paraphyletic ancestoral taxon to it. As *Clytocerus* generally have fused gonocoxal apodemes, while *Lepidiella* as shown here is polymorphic for this character, we deem it more likely that *Lepidiella* is paraphyletic to *Clytocerus*. However, we refrain from synonymizing the two until more unambiguous characters, including molecular ones, are available.

Acknowledgements

We are grateful to Jostein Kjærandsen for collecting the type specimen of the new species and to Trond Andersen for making it available for us to study. Per Djursvoll and Steffen Roth kindly facilitated us working together for a month at the University Museum of Bergen. SJS extends his gratitude to Morgane A. Kerdoncuf for opening her flat to him during his stay in Bergen.

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Appendix 15. (Publication chapter 17)

Chapter 17 – Publication

Jaume-Schinkel S, Kvifte GM (in prep). Four new species of Gondwanoscurus Ježek (Diptera, Psychodidae).

Four new species of *Gondwanoscurus* Ježek (Diptera, Psychodidae) from Thailand and Laos

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Running title: Four new species of *Gondwanoscurus* (Diptera, Psychodidae)

Abstract. *Gondwanoscurus kjaerandseni* **sp. nov.**, *G. ostentatus* **sp nov.**, *G. sagittarius* **sp. nov.,** and ***G.*** **sp. nov.** are described based on male specimens collected in 1991 from Thailand and Laos. Additionally, we provide a worldwide list of species, a distribution map, and a taxonomical key for the males of the world.

Keywords. Oriental region; Afrotropical region; moth flies; Taxonomy; Dark Taxa.

Introduction.

The genus *Gondwanoscurus* Ježek, 2001 includes 11 described species (Curler 2009; Curler & Priyadarsanan 2015; Ježek 2001; Ježek & Tkoč 2012; Kvifte & Andersen 2019). The genus was described to include one new species, *G. malaysiensis* Ježek, 2001, and one

previously described species, *G. mcclurei* (Quate 1962) (as *Telmatoscopus* Eaton, 1904), from Malaysia. Later, Curler (2009) published a revision of the genus, including a key, which encompassed seven species, including four previously described as *Telmatoscopus*. Subsequently, Ježek & Tkoč (2012) described a new species, and then Curler & Priyadarsanan (2015) transferred *Telmatoscopus arcuatus* Vallant, 1965 to *Gondwanoscurus* and described a new species. Finally, Kvifte (2019) described a new species and provided an updated key for the males of the world, simultaneously extending their known worldwide distribution.

To date, this genus is present in the Afro Tropical and Oriental Regions, recorded in India, Malaysia, Tanzania, Thailand, and Yemen (Curler 2009; Curler & Priyadarsanan 2015; Ježek 2001; Ježek & Tkoč 2012; Kvifte & Andersen 2019). But little is known about the highly diverse Psychodinae species from the Oriental Region most of which are still undescribed (Curler 2009). In the present manuscript, we describe four new species of the genus *Gondwanoscurus* based on male specimens, updating the total number of species to 15, providing an updated key for the males of the world.

Material and Methods.

The studied specimens are deposited at the Department of Natural History, University Museum of Bergen, Bergen, Norway (ZMBN). Specimens were collected with a hand net or Malaise trap and stored in ethanol, and subsequently mounted on permanent slides.

Measurements rendered with an ocular micrometer in a microscope Leitz model Dialux 20, measures in millimeters (mm). Head width was taken at the widest part, approximately above the insertion of the antennal scape, whereas the length was taken from the vertex to the lower

margin of the clypeus; wing length was measured from the base of the wing at the start of the costal node to the apex of the wingtip, while the width was taken approximately at an imaginary vertical line crossing the radial and medial forks; palpal proportions are given considering the length of the first palpal segment as a unit (1.0).

Terminology. We follow the general terminology proposed by Cumming & Wood (2017) and Kvifte & Wagner (2017).

In the description of type labels, the contents of each label are enclosed in double quotation marks (“ ”), italics denote handwriting, and the individual lines of data are separated by a double forward-slash (//).

Results.

Gondwanoscurus Ježek 2001

Gondwanoscurus Ježek 2001: 6. Type species *Gondwanoscurus mcclurei* (Quate, 1962) [as *Telmatoscopus*], by designation of Ježek (2001).

***Gondwanoscurus kjaerandseni* sp. nov.**

Figure 1 A-E, 2 A.

Type locality: THAILAND, Chiang Mai, Doi Suthep.

Description. Measurements in mm (n=4) Wing length 2.10, width 0.92; head length 0.45, width 0.44; antennal segments, scape 0.12, pedicel 0.05, flagellomeres 1-12, 14 0.08, flagellomere 13 0.06; palpomeres 1: 0.75, 2: 0.13, 3: 0.14, 4: 0.18.

Male. Holotype. Head about as wide as long, vertex one-third head length; eye bridge contiguous, eye bridge with four facet rows, a single row of postocular bristles; interocular suture very small, appears absent. Antenna with scape about two times longer than its width, cylindrical; pedicel spherical, about half the length of scape, 14 flagellomeres asymmetrically

nodiform, except apical flagellomere which has a very small apiculus and a lateral-apical projection; ascoids missing in all revised material, but multiple insertions present in a single ring in flagellomeres, fewer in apical three segments; frontal alveoli patch oval; clypeus delimited by posterior suture, strongly sclerotized; labella bulbous, setose. Palpal segments sclerotized, apical segment corrugated; extending towards flagellomere 8, palpal proportions 1.0:1.5:1.5:2.5.

Wing length 2.5 times its width, hyaline except in costal cell, Sc vein straight, forks of R_{2+3} and M_{1+2} at the same level, with dark spots on apices of all veins, wing apex rounded, vein R4 ending before, and R5 ending below the apex.

Terminalia. Hypandrium narrow, plat-like, rounded, Gonocoxites 0.66 the length of gonostyli, about two times longer than wide, cylindrical; gonostyli narrow, tapered towards the apex, incurved at apical ⅓; Aedeagal apodeme 1.66 the length of gonocoxites, narrow, basally pointed, broadening towards aedeagus, edeagal/parameral complex with basal margin concave, appears to be covering together with the parameral sheath the whole complex, with two projections in the middle with inner margin serrated and a pair of spine-like short setae epandrium about twice wider than long; surstily elongate, longer than epandrium, apex with a distal cluster of 18-20 tenacula with complete apices. Epiproct tongue-shaped with rounded margin, covered in small setulae.

Examined material. *HOLOTYPE*, male, slide mounted, deposited at ZMBN. "*Gondwanoscurus kjaerandseni sp. nov. #m* // *HOLOTYPE* // **THAILAND:** Chiang Mai, // Doi Suthep, // 11-15.IV.1991 (Malaise), // J. Kjaerandsen leg. // **ZMBN #:**", *PARATYPE*, male, slide mounted, deposited at: ZBMN, same label information. paratype, #m, slide mounted, deposited at: ZBMN "*Gondwanoscurus kjaerandseni* #m // **THAILAND**: Chiang Mai, // 1 km above Doi Suthep // temple, // 18.805°N, 98.922°E // 11.IV.1991, (hand net) //

T. Andersen, Leg. // **ZMBN #:**" *PARATYPE*, #m, slide mounted, deposited at ZBMN, same label information as previous paratype except date, "15.IV.1991".

Etymology. The species is dedicated to Jostein Kjaerandsen who collected the specimens.

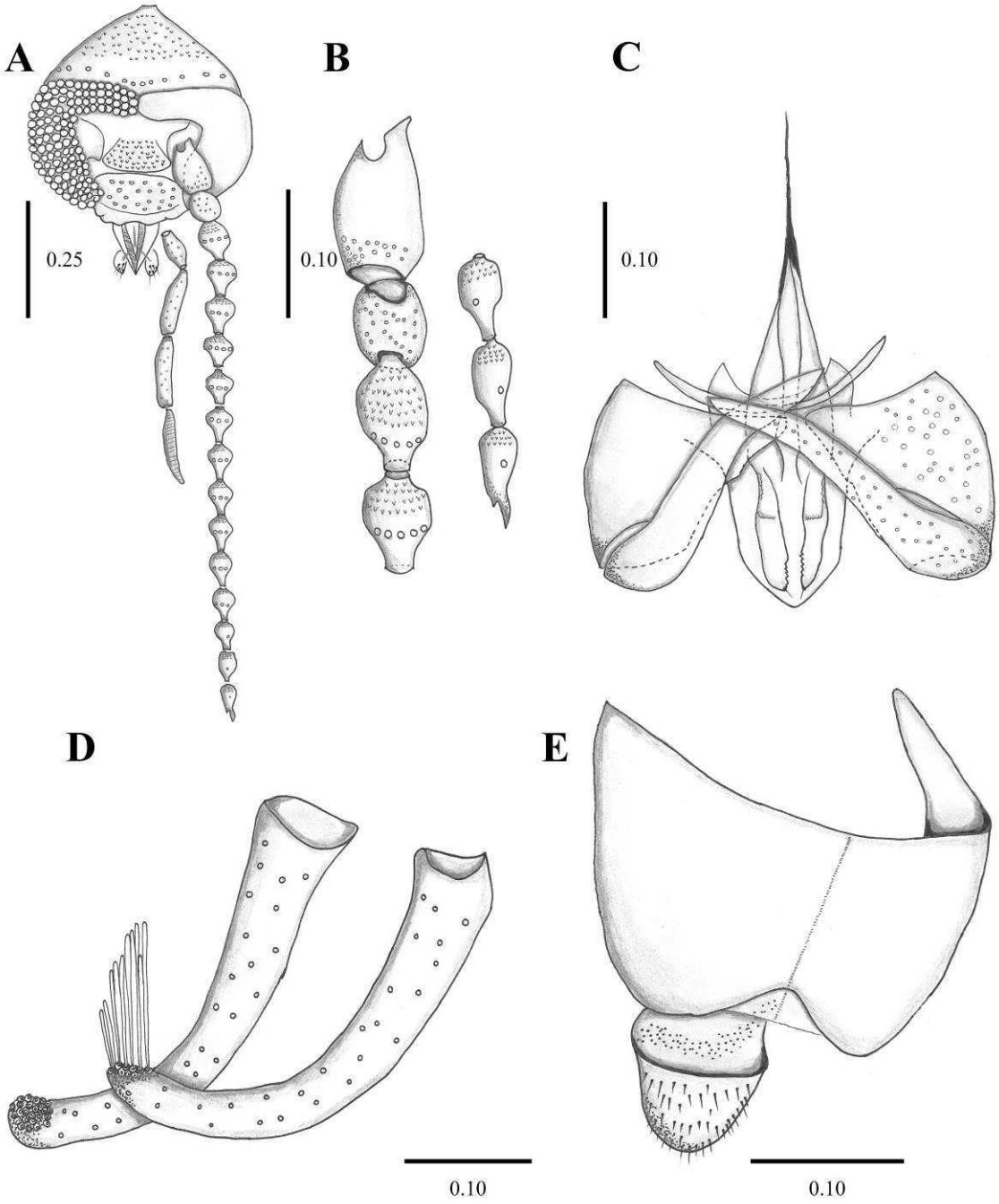

Figure 1. *Gondwanoscurus kjaerandseni* **sp. nov.** Male holotype. A. Head; B. First and last antennal segments; C. Aedeagal complex, gonocoxites and gonostyli; D. Surstyli, E. Epandrium and Epiproct. Scale lines in millimeters (mm).

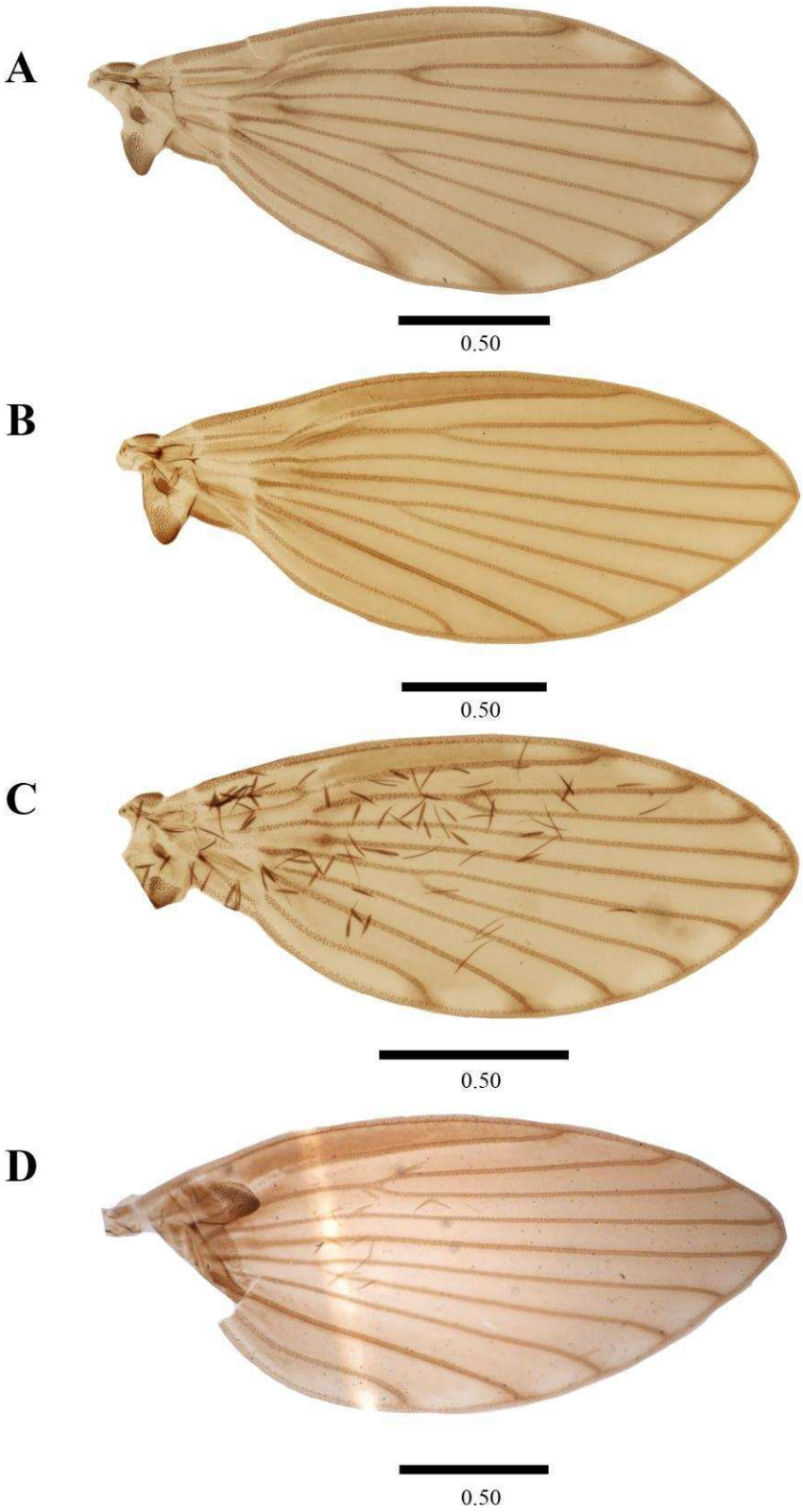

Figure 2. Wing photographs, male holotypes. A. *Gondwanoscurus kjaerandseni*; B. *G. ostentatus;* C. *G. sagittarius*; D. *G. quadrifurcata.* Scale lines in millimeters (mm).

***Gondwanoscurus ostentatus* sp. nov.**

Figures 2 B, 3, 4.

Type locality: THAILAND, Chiang Mai, Doi Suthep.

Description. Measurements in mm (n=2) Wing length 2.11 , width 0.95; Head length 0.0.46, width 0.45; antennal segments, scape 0.14, pedicel 0.06 , flagellomeres 1: 0.09, 2-8: 0.08, 9-13: 0.06, 14: 0.07; palpomeres 1: 0.08, 2: 0.17 , 3: 0.16 , 4: 0.22.

Male. Holotype. Head about the same length as width, vertex about ½ of head length, margin concave, eye bridge contiguous, with four facet rows, interocular suture very faint, small, appears absent; a single row of postocular setae. Antenna scape 2.3 times longer than its width, cylindrical; pedicel spherical, about half the length of scape, 14 flagellomeres, first flagellomere longer than pedicel, elongate-nodiform; following flagellomeres asymmetrical and nodiform, apical flagellomeres twice the length of subapical flagellomere, with a small apiculus and a lateral-apical elongated projection. ascoids small, simple, and spine-shaped, multiple insertions present in all flagellomeres, with a single ring-like line and a second irregular line below the first one; frontal alveoli patch trapezoidal; clypeus delimited by posterior suture, labella bulbous, setose. Palpal segments are sclerotized, and the apical segment is corrugated; palpal proportions 1.0:1.7:1.6:2.4.

Wing length two times its width, hyaline except on costal cell, without dark spots on veins apices, Sc vein straight, wing apex rounded, vein R4 ending before, and R5 ending below the apex.

Terminalia. Hypandrium narrow, plate-like with a median elongation, gonocoxites 0.64 times the length of gonostyli, gonocoxite width about 0.8 times its length, trapezoidal shape, broad; gonostyli bifurcated at basal third, base bulbous with a cluster of around 50 alveoli which appear to have setae as long as half the length of surstyli, mesal ramus about two times the length of the lateral ramus, both rami incurved, lateral ramus with the apex hook-shaped, with

an appendage inserted in basal third, mesal ramus S-shaped in lateral view. Epandrium about two times wider than its length, crescent shape at anterior margin, with lateral tips joining the gonocoxites, and with a rectangular posterior projection, with two foramina; surstyli length about two times the length of the epandrium, conical, almost strait, tapering towards the apex, with an apical cluster of 15-18 tenacula, tenacula; epiproct tongue-shaped, about the same length than its width, covered in small setulae.

Examined material. *HOLOTYPE,* #m, slide mounted, deposited at: ZBMN, "*Gondwanoscurus #m* // *kjaerandseni HOLOTYPE* // **THAILAND:** Chiang Mai, // Doi Suthep, // 11-15.IV.1991 (Malaise), // J. Kjaerandsen leg. // **ZMBN #:**" *PARATYPE,* #m, slide mounted, deposited at: ZBMN "*Gondwanoscurus kjaerandseni* #m // **THAILAND**: Chiang Mai, // 1 km above Doi Suthep // temple, // 18.805°N, 98.922°E // 11.IV.1991, (hand net) // T. Andersen, Leg. // **ZMBN #:**"

Etymology. From Latin *ostentatus* meaning exhibit, show off, making references to the extraordinary length of the aedeagus.

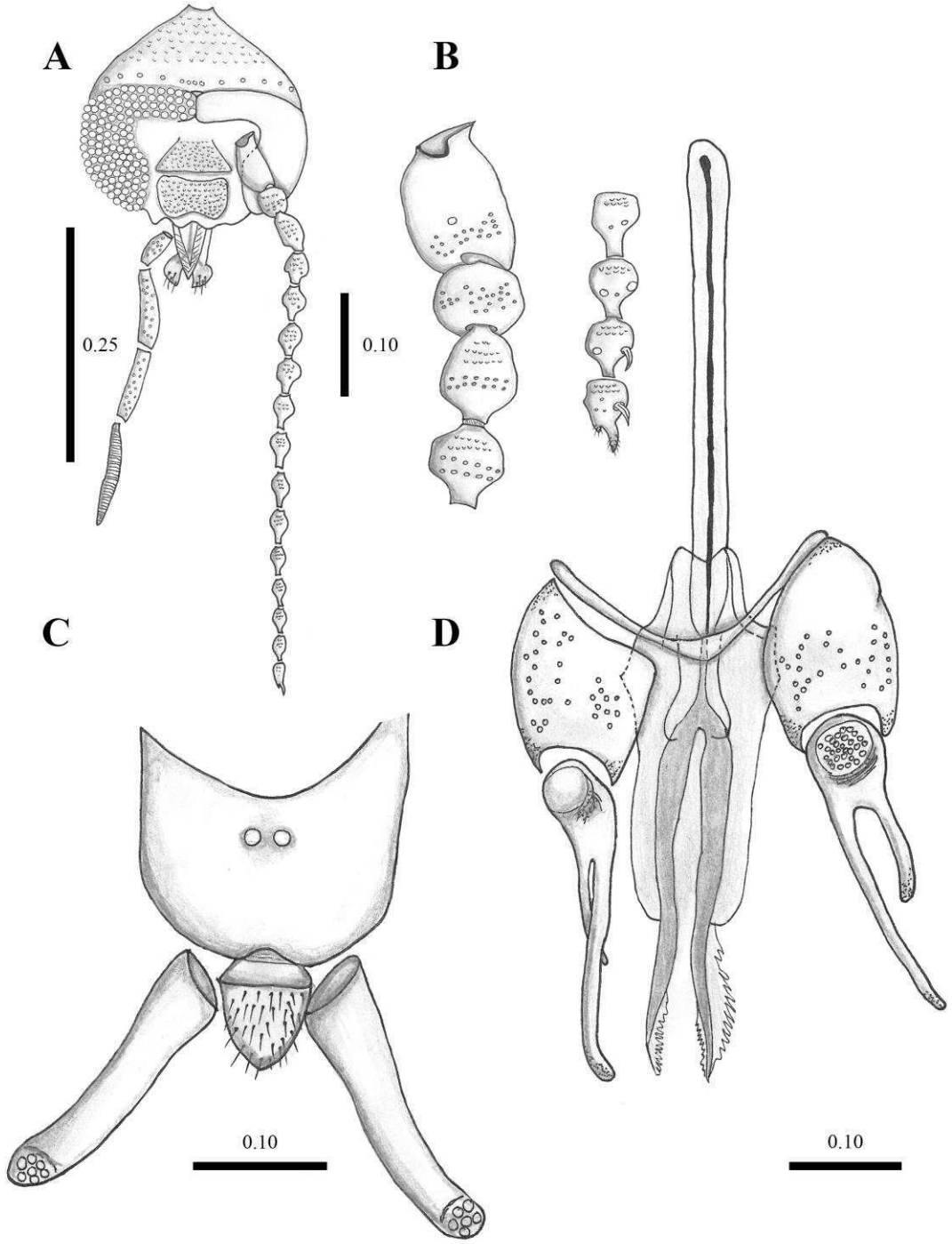

Figure 3. *Gondwanoscurus ostentatus* **sp. nov.** Male holotype. A. Head, B. First and last antennal segments; C. Epandrium and surstyli; D. Aedeagal complex, gonocoxites and gonostyli. Scale lines in millimeters (mm).

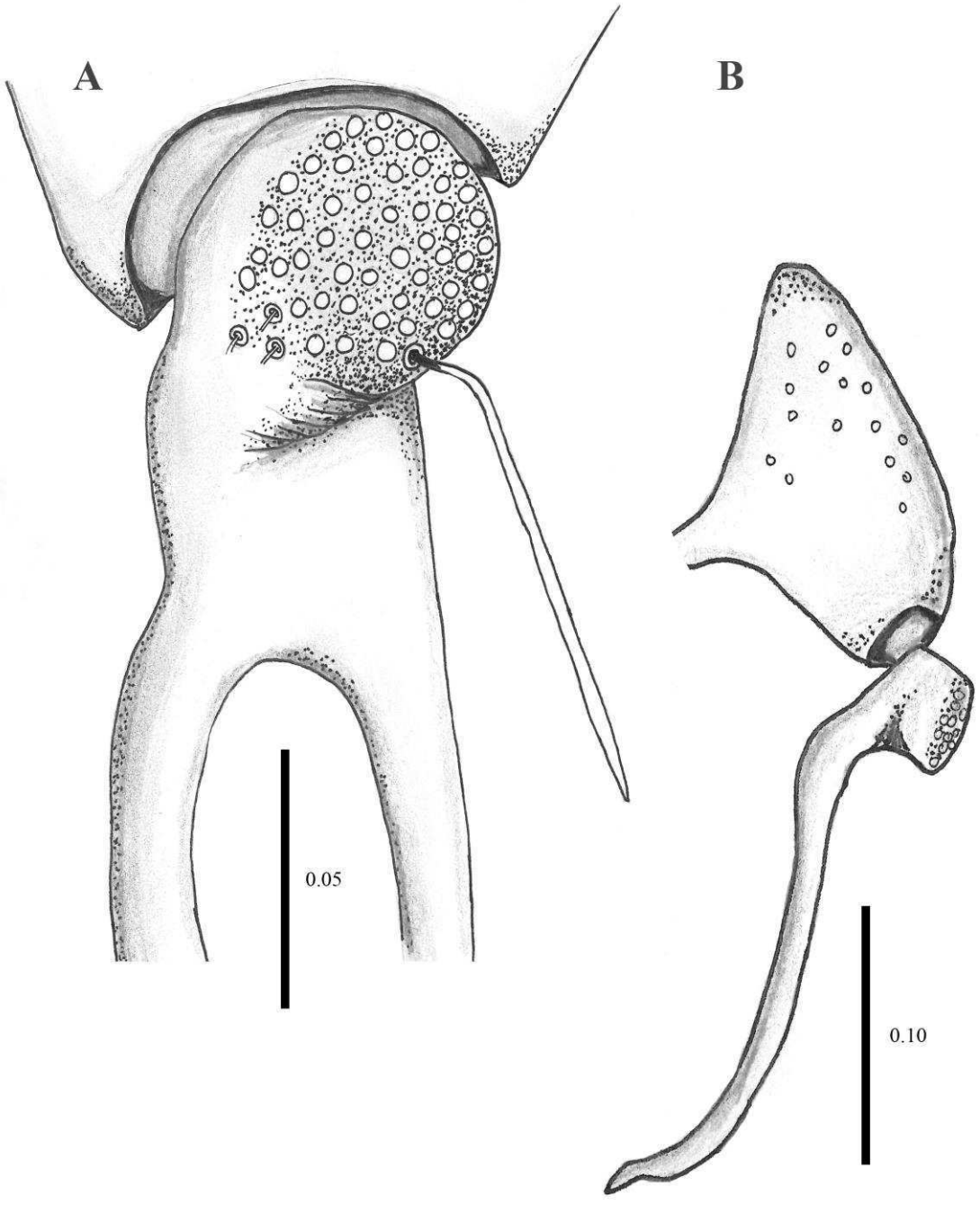

Figure 4. *Gondwanoscurus ostentatus* **sp. nov.** Male paratype. A. Gonostylus; B. Gonocoxite and gonostyli in lateral view. Scale lines in millimeters (mm).

***Gondwanoscurus quadrifurcata* sp. nov.**

Figures 5, 6.

Type locality: LAOS, Xieng Ngeun.

Description. Measurements in mm (n=1) Wing length 2.45, width 1.1; head length 0.46, width 0.44; antennal segments, scape 0.05, pedicel 0.05, flagellomeres 1-4 length 0.09, palpomeres 1: 0.09, 2: 0.14, 3: 0.11, 4: 0.21.

Male. Holotype. Head about the same length as width, subquadrate, margins waisted just above eye level, eye bridge contiguous, with four facet rows, interocular suture absent; two rows of post ocular alveoli present. Antennal scape two times longer than its width, cylindrical; pedicel spherical, about half the length of scape; apical flagellomeres absent in revised material, maximum number of flagellomeres 3; flagellomeres asymmetrical and nodiform; ascoids absent in revised material but multiple insertions present in a single ring in all flagellomeres; frontal alveoli patch trapezoidal; clypeus delimited by posterior suture, labella bulbous, setose. Palpal segments sclerotized, apical segment corrugated, palpal proportions 1.0:1.5:1.2:2.4.

Wing length 2.1 times its width, infuscated with darker infuscation in the costal cell, lighter spots on the area between the apex of veins; Sc vein straight; fork of R_{2+3} distal to fork of M_{1+2}; wing apex rounded, vein R4 ending before, and R5 ending below the apex.

Terminalia. Hypandrium narrow, plate-like, rectangular; gonocoxites 0.64 times the length of gonostyli, gonocoxite longer than wide; gonostyli bifurcated at the base, mesal and lateral ramus about the same length, both rami incurved. Aedeagal symmetrical, aedeagus divided into two sclerotized phallomeres, extending towards the apical third of gonostyli, aedeagal complex with two sclerotized and bifurcated parameres. Epandrium longer than its width,

anterior margin concave, with lateral tips joining the gonocoxites, with two foramina; surstyli about six times longer than the basal width, strongly curved towards in lateral view, slightly tapering towards the apex, with an apical cluster of 14-18 tenacula; tenacula distally with spatulate apex, tenacula length about the same as basal width of surstyli; Epiproct digitate, 3 times longer than its basal width, covered in small setulae.

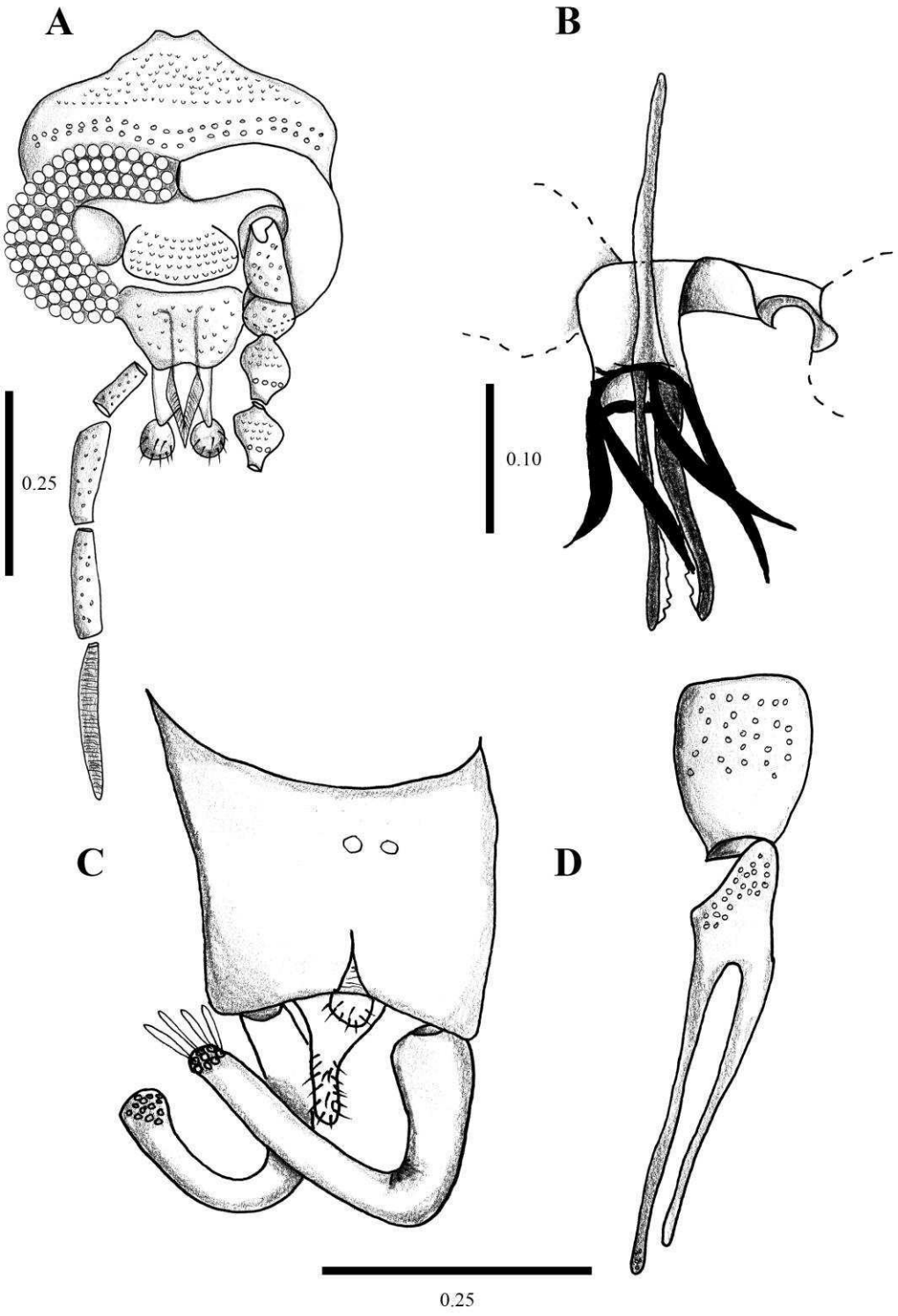

Figure 5. *Gondwanoscurus quadrifurcata* **sp. nov.** Male holotype. A. Head; B aedeagal complex; C. Epandrium and surstyli. D. Gonocoxite and gonostyli. Scale lines in millimeters (mm).

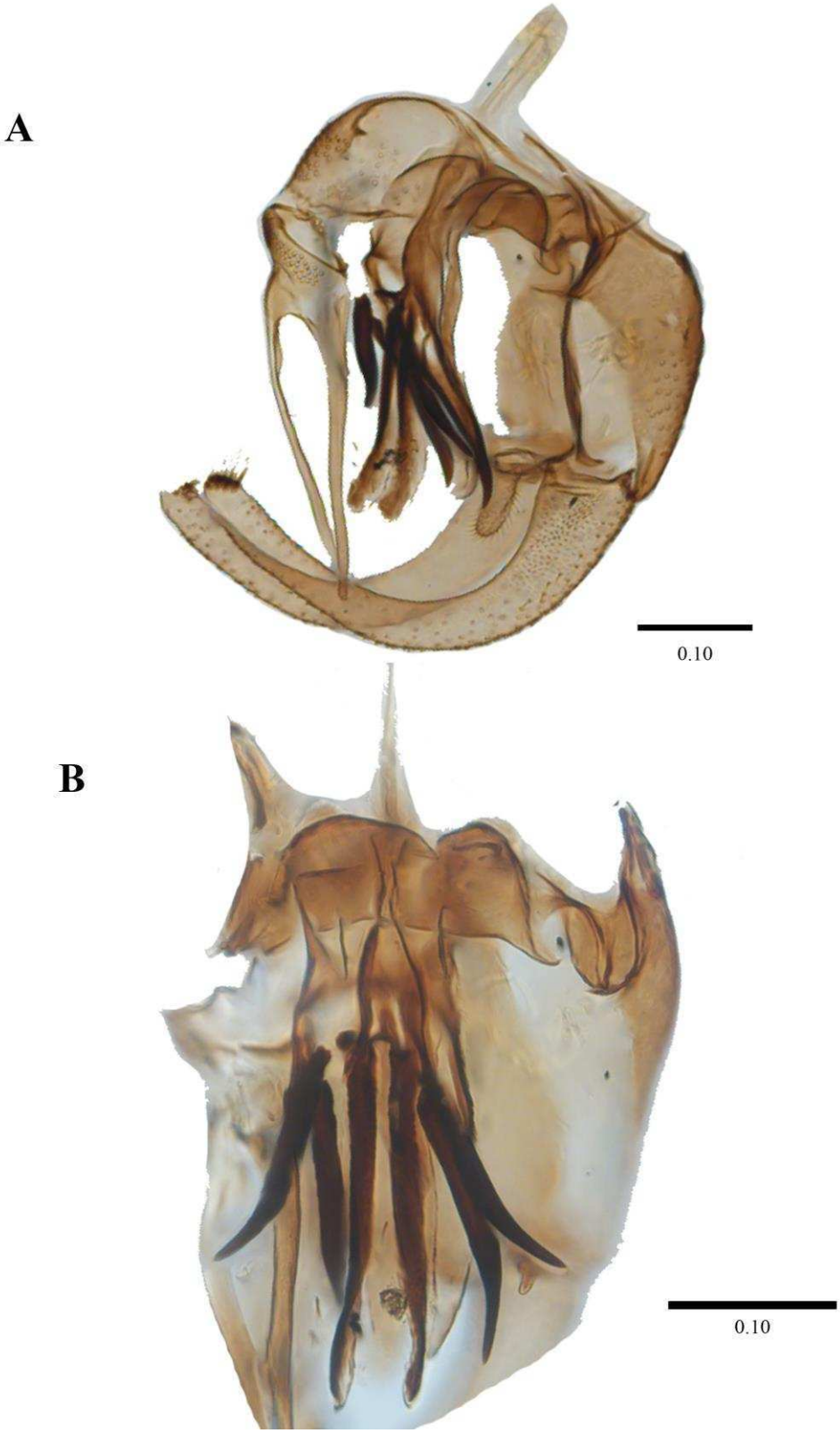

Figure 6. *Gondwanoscurus quadrifurcata* **sp. nov.** Male holotype photographs. **A.** lateral view. **B.** Ventral view. Scale lines in millimeters (mm).

***Gondwanoscurus sagittarius* sp. nov.**

Figure 7.

Type locality: THAILAND, Chiang Mai, Doi Suthep.

Description. Measurements in mm (n=1) Wing length 1.75, width 0.75; head length 0.39, width 0.37; antennal segments, scape 0.10, pedicel 0.05, flagellomeres 1-11: 0.08, palpomeres 1: 0.60, 2: 0.10, 3: 0.11, 4: 0.18.

Male. Holotype. Head longer than wide, vertex about ⅓ of head length, eye bridge contiguous, with four facet rows, interocular suture very faint, appears absent. Antenna scape two times longer than its width, cylindrical; pedicel spherical, about half the length of scape, apical flagellomeres absent in revised material, maximum number of flagellomeres 11, flagellomeres asymmetrical and nodiform, ascoids absent in revised material but multiple insertions present in a single ring in all flagellomeres; frontal alveoli patch oval; clypeus delimited by posterior suture, labella bulbous, setose. Palpal segments sclerotized, apical segment corrugated, palpal proportions 1.0:1.6:1.6:3.0.

Wing length 2.3 times its width, hyaline except in costal cell, Sc vein straight, fork of R_{2+3} distal to fork of M_{1+2}, with dark spots on apices of all veins, wing apex rounded, vein R4 ending before, and R5 ending below the apex.

Terminalia. Only lateral view of Hypandrium is possible in the specimen, seems sclerotized and plate-like; gonocoxites 0.75 the length of gonostyli, about three times longer than wide, broader at the base, tapering towards the junction of gonostyli; gonostyli bifurcated beyond middle, mesal ramus 6 times longer than wide, both rami incurved and tapering towards the apex, lateral ramus two times longer than mesal. Aedeagus symmetrical, extending towards the apex of gonocoxites, aedeagal apodeme not discerned, aedeagus covered by a sheath, with a concave basal margin, apical margin rounded, with a suture in the middle; paramere with

the apex arrow-shaped. Epandrium on lateral view two times longer than its width, rectangular with basal margin extending towards the aedeagus; surstyli more than two times longer than epandrium, curved, almost C-shaped, with depression on upper margin, and a broadening on dorsal margin just before an apical patch of tenacula; tenacula with complete apices, apical tanacula shorter than basal inside the patch. Epiproct in lateral view semi triangular with rounded apex, covered in small setulae.

Examined material. *HOLOTYPE,* #m, slide mounted, deposited at: ZBMN "*Gondwanoscurus kjaerandseni* #m // **THAILAND**: Chiang Mai, // 1 km above Doi Suthep // temple, // 18.805°N, 98.922°E // 11.IV.1991, (hand net) // T. Andersen, Leg. // **ZMBN #:**"

Etymology. From Latin *sagitta* meaning arrow, referencing the arrow-shaped paramere

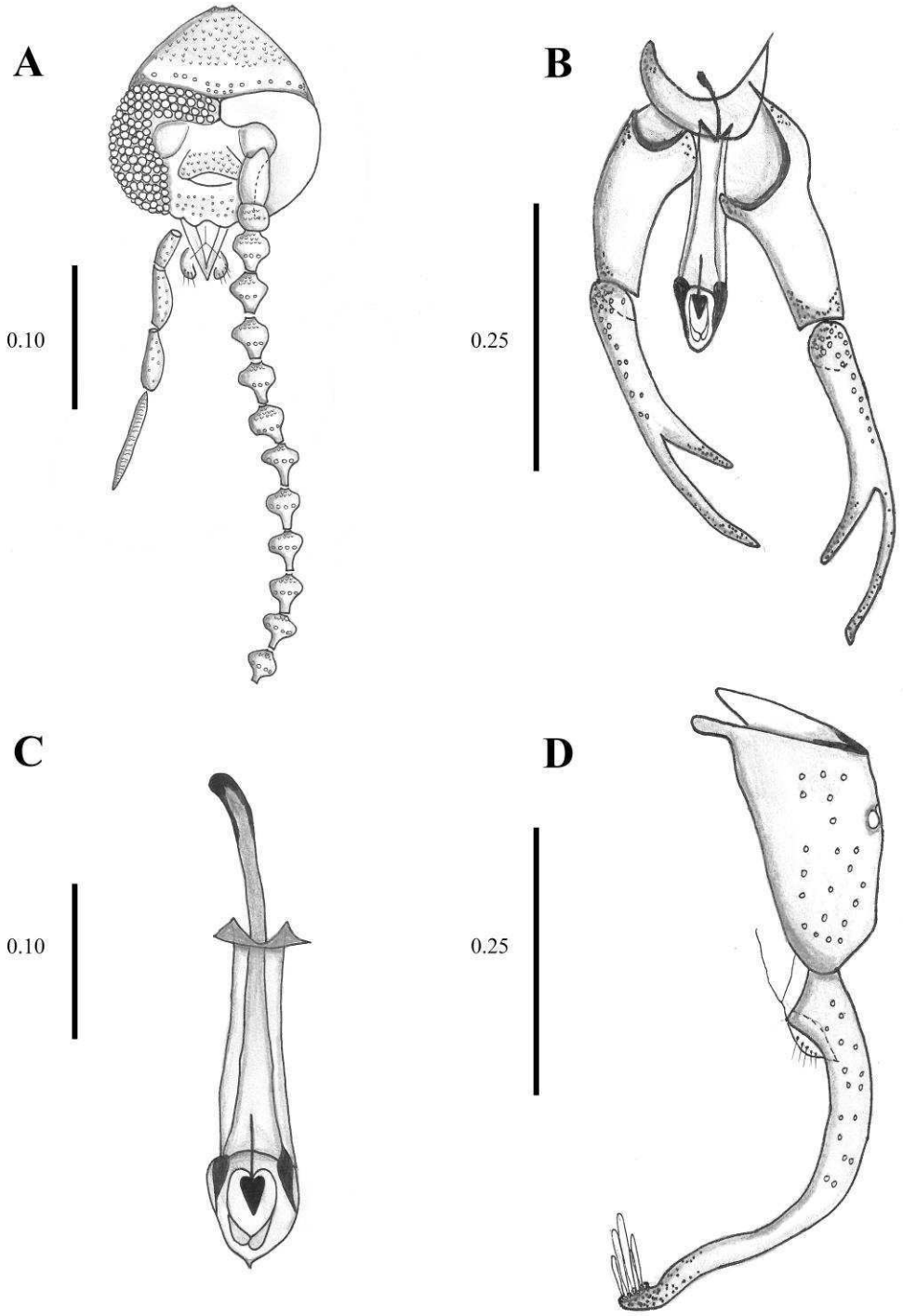

Figure 7. *Gondwanoscurus sagittarius* **sp. nov.** Male holotype. A. Head; B. Aedeagal complex, gonocoxites and gonostyli; C. Aedeagus; D. Epandrium and surstyli. Scale lines in millimeters (mm).

List of species of *Gondanoscurus* of the world.

Distribution map Figure 8.

1. *G. arcuatus* (Vaillant, 1965)

Telmatoscopus arcuatus Vaillant (1965): 222. Distribution: NEPAL.

2. *G. cruciferus* Curler, 2009

Gondwanoscurus cruciferus Curler (2009): 23. Distribution: THAILAND, Sakon, Nahkon Province.

3. *G. curleri* Kvifte & Anderssen, 2019

Gondwanoscurus curleri Kvifte & Anderssen (2019): 60. Distribution: TANZANIA, Tanga Region.

4. *G. ejundicus* (Quate, 1962)

Telmatoscopus ejundicus, Quate (1962a): 8. Distribution: MALAYSIA: Sabah

5. *G. eximius* (Quate, 1962)

Telmatoscopus ejundicus, Quate (1962a): 8. Distribution: MALAYSIA: Sabah

6. *G. Ježeki* Curler & Priyadarsanan, 2015

Gondwanoscurus Ježeki Curler & Priyadarsanan (2015): 478. Distribution: INDIA, Kerala.

7. *G. kjaerandseni* sp. nov.

Gondwanoscurus kjaerandseni **sp. nov.** Distribution: THAILAND: Chiang Mai.

8. *G. malaysiensis* Ježek, 2001

Gondwanoscurus malaysiensis Ježek (2001): 10. Distribution: MALAYSIA, Perak. THAILAND, Phu Phan National Park.

9. *G. mcclurei* (Quate, 1962)

Telmatoscopus mcclurei Quate (1962b): 227. Distribution: MALAYSIA, Batu Caves.

10. *G. ornithostylus* Curler, 2009

Gondwanoscurus ornithostylus Curler (2009): 26. Distribution: THAILAND, Chiang Mai Province.

11. *G. ostentatus* sp. nov.

Gondwanoscurus ostentatus **sp. nov.** Distribution: THAILAND, Chiang Mai.

12. *G. praecipuus* (Quate, 1962)

Telmatoscopus praecipuss Quate (1962a): 11. Distribution: MALAYSIA, Sabah.

13. *G. quadrifurcata* sp. nov.

Gondwanoscurus **sp. nov.** Distribution: LAOS, Luang Parabang, Xieng Ngeun.

14. *G. sagittarius* sp. nov.

Gondwanoscurus sagittarius **sp. nov.** Distribution: THAILAND, Chiang Mai.

15. *G. socotrensis* Ježek & Tkoč 2012

Gondwanoscurus socotrensis Ježek & Tkoč (2001): 548. Distribution: YEMEN, Socotra Island.

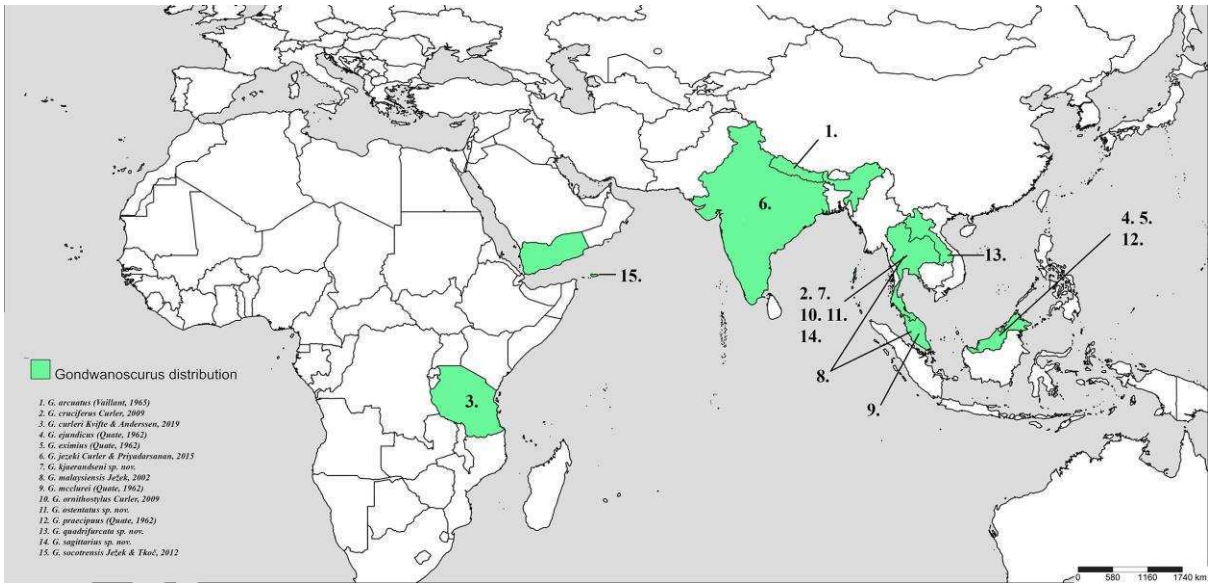

Figure 8. Distribution map of *Gondwanoscurus* species.

Key for the males of *Gondwanoscurus* of the world.

1. Tenacula with apices spatulate or with serrate apex (as in Figure 9 A-B) …**4**

-. Tenacula with clearly two lateral apical projections (as in Figure 9 C-D)…**2**

2. Gonostylus bifurcate (as in Figure 3 D, 4 A, 5 C, 7 B); antennal flagellomeres slightly asymmetrical without nodes exerted laterally …**3**

-. Gonostylus not bifurcate (as in Figure 1 C); antennal flagellomeres strongly asymmetrical, with nodes exerted laterally …***G. cruciferus*** **Curler**

3. Gonostylus bifurcate at mid; surstyli length about six times its width … ***G. arcuatus*** **(Vaillant)**

-. Gonostylus bifurcate at base; surstyli length about 16 times its width … ***G. ejundicus*** **(Quate)**

4. Gonostylus bifurcate … **8**

-. Gonostylus not bifurcate …**5**

5. Gonostylus without a setose lobe …**7**

-. Gonostylus with a setose lobe …**6**

6. Setose lobe at mid; surstylus without a medial protuberance … ***G. ornithostylus*** **Curler**

-. Setose lobe at base (as in Curler 2009, Figure 9); surstylus with a pointed medial protuberance, about twice as long as the width of surstylus apex … ***G. malaysiensis*** **Ježek**

7. First flagellomere with a lateral protuberance; ejaculatory apodeme with anterior margin broad, flat; parameral complex with inner margins not serrated ... ***G. curleri*** **Kvifte**

-. First flagellomere without a lateral protuberance; ejaculatory apodeme with anterior margin narrow, pointed; parameral complex with inner margins serrated …***G. kjaerandseni*** **sp. nov.**

8. Gonostylus bifurcate at basal ⅓, mid or apex … **10**

-. Gonostylus bifurcate at base …**9**

9. Gonostylus with mesal branch less than half of lateral branch; Aedeagus short, ending in basal half of gonostylus …***G. mcclueri*** **(Quate)**

-. Gonostylus with mesal and lateral branches equal in size; aedeagus long, ending in apical half of gonostylus … ***G. quadrifurcata sp nov.***

10. Gonostylus bifurcate at apex ... **13**

-. Gonostylus bifurcate at basal ⅓ or mid … **11.**

11. Flagellomeres 1-3 without spines; Gonostylus bifurcate at basal ⅓ … ***G. ostantatus*** **sp. nov.**

-. Flagelloeres 1-3 with spines; Gonostylus bifurcate at mid … **12.**

12. Flagellomeres 1-3 with three spines; gonostylus shorter than gonocoxites; mesal branch of gonostylus longer than lateral branch … ***G. eximius*** **(Quate).**

-. Flagellomeres 1-2 with three spines, flagellomere 3 with four spines; gonostylus longer than gonocoxites; mesal branch of gonostylus shorter than lateral branch … ***G. preapicuus*** **(Quate).**

13. Gonostylus with basal setose lobe … ***G. Ježeki*** **Curler.**

-. Gonostylus without basal setose lobe … **14.**

14. First flagellomere elongated nodiform, with the node being 3 times longer than neck; apex of aedeagus long, conical-shaped; surstyli bent on lateral view … ***G. socotrensis*** **Ježek & Tkoč**

-. First flagellomere nodiform, with the neck being 2 times longer than node; apex of aedeagus short, apex arrow-tip shaped; surstyli strongly curved in lateral view … ***G. sagittarius*** **sp. nov.**

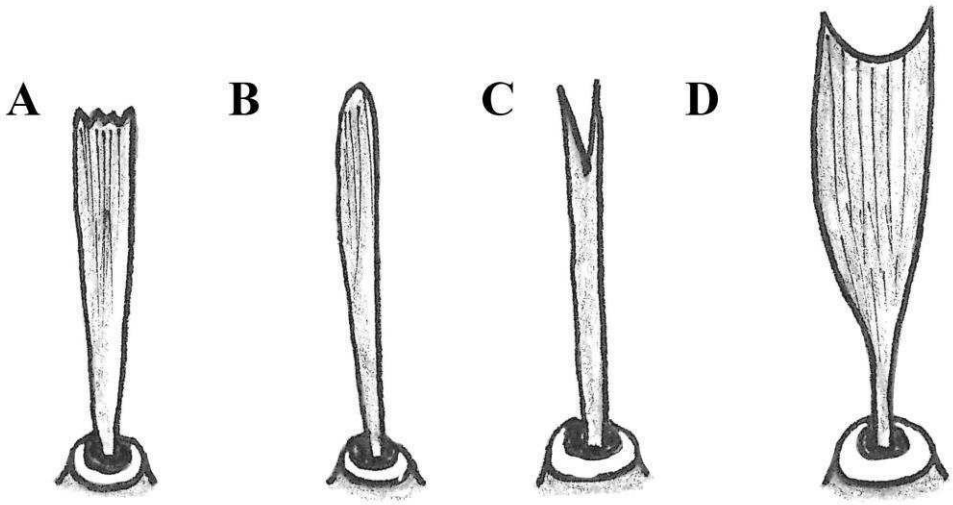

Figure 9. *Gondwanoscurus* spp. Tenacula. A. *G. ostentatus* **sp. nov.;** B. *G. kjaerandseni* **sp. nov.;** C. *G. cruciferus*; D. *G. arcuatus*.

Discussion

Adults of *Gondwanoscurus* have been placed in different tribal classifications inside the Psychodinae, like Paramormiini *sensu* Curler & Courney (2009) and Pericomaini *sensu* Kvifte (2018), however, both tribal classifications have been challenged by both molecular and morphological evidence (Espindola *et al.* 2012; Kvifte 2014) and all the phylogenetic relationships among tribes have not been determined. It has been suggested, based on morphology, that the closest relative of *Gondwanoscurus* is the oriental genus *Neotelmatoscopus* (Ježek 2011; Ježek & Tkoc 2012), however, this is not supported by molecular data (Curler & Moulton 2012). Furthermore, as stated by Curler (2009), inside the genus *Gondwanoscurus* some species groups appear apparent, and further fresh samples of

both imagos and larval stages are desirable to acquire DNA data that would help resolve the phylogeny inside the group.

To date, we elevate the number of described species of *Gondwanoscurus* to 15; those species are mainly present in the Afro Tropical and Oriental Regions. Little is known about their biology, some species were trapped with light traps, while the majority had been collected near water, suggesting that larval stages are aquatic, possibly feeding on decaying organic matter (Curler 2009).

It is likely that several new species are yet to be discovered and described, and the diagnosis and extent of the group will change, especially when it comes to Oriental Psychodinae, as it has been suggested that a large number of species are yet undescribed (Curler 2009; Duckhouse 2004). Further systematic collections and examination of material from both the Afrotropical and Oriental regions is desirable.

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Appendix 16. (Publication chapter 18)

Chapter 18 – Publication

Jaume-Schinkel S, Muller B, Avila-Calero S, Kukowka S, Mengual X (2024) Preserving morphology while extracting DNA: a non-destructive field-to-museum protocol for slide-mounted specimens. *Biodiversity Data Journal* 12: e119448. https://doi.org/10.3897/BDJ.12.e119448

Preserving morphology while extracting DNA: a non-destructive field-to-museum protocol for slide-mounted specimens

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Abstract

Our study aimed to develop an optimized laboratory protocol ensuring the preservation of morphological structures and extraction of high-quality DNA sequences from Psychodidae (Insecta, Diptera) specimens. With 310 analyzed specimens, we investigated the impact of distinct laboratory treatments by employing two shaking categories (constant and interrupted) with five different incubation periods (16, 12, 8, 4, and 2 hours) during the DNA extraction process. Notably, 80.65% of the specimens exhibited morphological changes during DNA extraction. Our results indicated no statistical difference between constant and interrupted shaking for the total of morphological structures lost. However, within each shaking category the loss of structures was influenced significantly by the incubation period. Prolonged incubation correlated with increased structural losses, whereas shorter incubation periods caused minor alterations in structures lost. In addition, our results showed a significant difference between constant and interrupted shaking treatments for DNA concentration. Likewise, the incubation period showed differences within each shaking category. Successful COI sequencing was achieved in 89.6% of specimens, with negligible differences in DNA fragment lengths across treatments. Our findings underscore the importance of an optimized protocol and its potential in systematic research involving nematoceran dipteran specimens by balancing morphological integrity and DNA extraction efficiency.

Keywords

DNA barcoding, Diptera, Psychodidae, moth flies, dark taxa, integrative taxonomy, non-destructive DNA extraction

Introduction

Since the invention of the microscope and Leeuwenhoek's pioneering work with cut specimens in the seventeenth century, microscopy and slide preparation techniques have evolved significantly. A crucial milestone occurred in the 1830s when slide-mounted specimens, similar to those used today, emerged thanks to the discovery of Canada Balsam as a suitable transparent medium (Bracegirdle 1994), known for its remarkable longevity spanning over a century and a half (Neuhaus et al. 2017).

This historical backdrop sets the stage for understanding the enduring nature of insect slide preparation. Despite the advances in microscopy, the methods for mounting insects on permanent slides have remained surprisingly consistent over the past century. The principles and techniques established in the early to mid-1900s (e.g., Carter et al. 2016, Gates 1941, Gibbins 2009, Lutz 1922) remain relevant today, with only minor adjustments to the pre-mounting process (e.g. using chloral hydrate, potassium or sodium hydroxide, lactic acid for specimen diaphanization, see Neuhaus et al. (2017)) or the choice of mounting medium (e.g. Berlese fluid, Canada Balsam, or Euparal see Neuhaus et al. (2017)). These methods have proven effective for decades; thus, major changes have been unnecessary.

On the contrary, from the first known classification systems for insects by Aristotle around 350 BC to the modern post-Linneaus and contemporary classifications, the systematical and taxonomical classifications have constantly changed (Engel and Kristensen 2013). With each shift, taxonomists find themselves expanding the character sets to better comprehend the relationships among different taxa. This often needs the observation of smaller morphological structures, for which microscope slides become invaluable (Neuhaus et al. 2017).

Furthermore, the advent of DNA Barcoding in 2003 (Hebert et al. 2003) and the integrative taxonomy approaches (Dayrat 2005, Yeates et al. 2010) have called for a harmonization of both morphological and molecular needs. This involves preserving taxonomically significant structures while extracting DNA for future molecular studies and preserving specimens as voucher specimens for future morphological reference.

In the case of the family Psychodidae (Insecta, Diptera), the traditional techniques for preparing slides have not changed since the 1900s, starting with the Reverend Eaton's preparations around 1900 (Withers 1989). Microscope slides with all sorts of materials can be found in Natural History Collections around the globe. The permanent slide technique (and its variations) is excellent at preserving morphological structures for a very long time, hence the unnecessity of changing them. However, the story changes when we combine this technique with molecular studies. The widely accepted technique to prepare microscope slides requires a previous maceration of the tissue, removing all traces of DNA in the process (e.g., Ibáñez-Bernal 2005, Curler 2020).

Our study closely matches the objectives of the German Barcode of Life (GBOL) (https://gbol.bolgermany.de/) initiative and its third phase: “GBOL III: Dark Taxa”. GBOL endeavors to establish a comprehensive DNA barcode reference library for all organisms in Germany, aiming to enhance species identification and contribute to biodiversity research. GBOL III: Dark Taxa specifically targets selected groups of Hymenoptera and Diptera, seeking to elucidate the systematics of highly diverse and poorly studied flying insect taxa using genetic information and morphology. By re-evaluating the DNA extraction process and the slide preparation techniques for Psychodidae specimens, our experiment seeks to bridge the gap between traditional slide mounting methods and modern molecular approaches advocated by initiatives such as GBOL III: Dark Taxa.

The aim of the present study is thus to develop a new procedure that balances effective DNA extraction with the preservation of crucial diagnostic morphological characteristics for the accurate identification of slide-mounted specimens of moth flies.

Material and methods

Specimens

A total of 310 specimens of moth flies (Psychodinae subfamily) were used in this study, collected across different years (2013, 2020, and 2021) using Malaise traps as part of the German Barcode of Life (GBOL) project (Geiger et al. 2016, Hausmann et al. 2020; www.bolgermany.de). Upon collection, all specimens were preserved in 96% ethanol and stored at -20°C until the commencement of the experiment. These specimens exclusively comprised adult moth flies and are presently housed at the Leibniz Institute for the Analysis of Biodiversity Change (LIB) - Museum Koenig Bonn (formerly Zoologisches Forschungsmuseum Alexander Koenig, [ZFMK]), Bonn, Germany. Before DNA extraction, the specimens were identified up to genus level and subsequently added to the GBOL barcoding pipeline.

On average, 10 specimens were selected from each collection year (10 from 2013, 10 from 2020, and 10 from 2021) for each of the experimental treatments and the different incubation periods (see laboratory procedures below), summing 102 specimens for 2013, and 104 specimens for 2020 and also for 2021 (310 total). While efforts were made to evenly distribute these specimens across treatments, collection years, and genera, logistical constraints occasionally led to discrepancies in specimen counts. Consequently, in certain instances, 8 or 13 specimens were included from each collection year based on availability (see Suppl. material 1). Likewise, the majority of the 310 specimens selected were adult males (306 males or 98.7 % and 4 females or 1.29%).

All associated specimen data, including collection years, treatment allocations, and genera, is available as supplementary material to enhance the transparency and replicability of our study (see Suppl. materials 1, 2).

Morphological characters

We created a data matrix to annotate the presence or absence of specific morphological structures before and after laboratory procedures (Suppl. material 2). This allowed us to assess the loss of structures during the DNA extraction process. Each specimen was meticulously examined under a LEICA stereomicroscope model M205 C.

The examined morphological characters included: the number of antennal segments, the presence of antennal ascoids on each flagellomere (only for those genera with antennal ascoids), the number of palpal segments, the presence of wings, the number of legs, and the presence of abdominal terminal segments (genitalia)(see Fig. 1).

The 'total number of changes' refers to the overall count of structural alterations. For instance, if a specimen lost flagellomeres on the left antenna, this was counted as a single change, regardless of the number of flagellomeres lost; if the same specimen lost flagellomeres on the right antenna, this was counted as a separate change, totaling two changes (one for the left antenna, and one for the right antenna; see Suppl. material 2). Moreover, if flagellomeres were missing and, as a result, ascoids were lost with them, we counted both separately, as ascoids can be lost without losing flagellomeres. The maximum possible number of changes that could occur in our experiment was 15, including both left and right antennae, ascoids, palpal segments, wings, legs, and terminalia. The minimum number of changes was zero.

To minimize structure loss during specimen handling, we exercised great care. Specimens were transferred from Eppendorf vials to watch-glass before morphological examination, using a plastic pipette to avoid direct contact with the specimens. Before and after DNA extractions, specimens were handled with rigid fine-tipped forceps, ensuring that they were held by one wing to minimize potential damage.

Laboratory procedures (DNA extraction, PCR, and PCR conditions)

A non-destructive DNA extraction was performed using the whole body of the specimens at the molecular laboratory of the LIB – Museum Koenig Bonn. Using Qiagen's magnetic bead-based BioSprint 96 DNA Blood Kit (QIAGEN GmbH - Germany) all specimens were separately lysed in 180 µl ATL buffer and 20 µl Proteinase K in a 12x8 S-Block.

The incubation was carried out in an Eppendorf Thermomixer® comfort, maintaining a constant temperature of 56°C. Within this setup, the lysis duration encompassed five distinct timeframes: 16, 12, 8, 4, and 2 hours. Each of these time treatments was associated with either of two lysis methods: 1) Constant shaking (C; treatment categories including incubation periods abbreviated as C16, C12, C8, C4, and C2); or 2) Interrupted shaking (I; treatment categories including incubation periods abbreviated as I16, I12, I8, I4, and I2). The constant shaking treatments were set at 300 rpm; on the contrary, the interrupted shaking treatment consisted of time periods of 20 minutes divided into one cycle of 30 seconds at 300 rpm followed by a cycle of 19:30 minutes at 0 rpm. Accordingly,

a total of 10 different treatment groups were used for the experiment. All combined treatment variables can be seen in Fig. 2.

After the incubation period elapsed for each treatment, the animals were removed from the DNA-containing ATL buffer and transferred to 96% ethanol for later morphological examination. The lysate washing, DNA extraction, and DNA elution were done with a BioSprint 96 Purification System (Thermo Scientific/ QIAGEN; for more detailed information see Jafari et al. 2023).

Using a QIAGEN Multiplex PCR Kit, the master mix for a 96-PCR-well plate is composed of the following: 2000 µl Multiplex, 400 µl Q-Solution, 880 µl RNase-free water, and 160 µl of each forward and reverse primer (10 pmol/µl). Each sample well was filled with 36 µl PCR Mastermix and 4 µl from the extracted DNA. The standard primers used in GBOL III for the COI barcode region of insect samples are LCO 1490-JJ (forward) and HCO 2198-JJ (reverse) (Astrin and Stüben 2008). The PCR was carried out with a GeneAmp® PCR System 9700 using a touchdown PCR (TD-PCR) as proposed by Korbie and Mattick (2008). The following conditions were used: initial 15 minutes at 95°C, followed by 94°C denaturation for 35 seconds, 55°C annealing for 90 seconds, and 72°C elongation for 90 seconds. Denaturation, annealing, and elongation are repeated 15 times, but the annealing temperature decreases by 1°C in each cycle. Reaching an annealing temperature of 40°C, there is no further reduction in temperature. Denaturation, annealing, and elongation are repeated 25 times as described above. A final elongation at 72°C for 10 minutes terminates the PCR and is then cooled to 12°C permanently.

Bidirectional Sanger sequencing of the COI-PCR products was carried out by BGI BIO Solutions Co, Ltd (Hong Kong, China). Assembly analysis and generating consensus sequence of the sequence data was carried out with Geneious v. 7.1.9 (http://www.geneious.com). The total sequence length was set to 658 bp.

DNA concentration was measured with a Quantus™ Fluorometer (Promega GmbH - Germany), and the QuantiFluor® dsDNA Dye System Kit. Each sample underwent three consecutive measurements, from which the average and median values were calculated. For our analysis involving DNA concentration, we used the average.

Based on our DNA concentration measurements, we selected the 99 samples with the highest DNA concentration for DNA fragment length measurement, for this, each sample was measured with a 5200 Fragment Analyzer System (Agilent Technologies Inc.) using the HS Genomic DNA Kit for Genomic DNA. For the fragment analyzer results, we divided the measurements into three groups, fragment lengths from 50 to 500 bp long, 500 to 10,000 bp long, and 10,000 to 40,000 bp long. Using the software ProSize 2.0, we obtained a data matrix stating the percentage of fragments that corresponded to each group for each sample.

One observable but unquantified characteristic was the maceration of soft tissue, as seen in Fig. 1. This outcome was expected during the DNA extraction laboratory procedures, specifically during the lysis process. Tissue maceration is a standard step in the mounting

process for Psychodidae and other nematoceran Diptera families, necessary to make internal structures visible for taxonomical identification. Thus, the tissue maceration as a result of the DNA extraction was helpful for the mounting of specimens.

Preparation of microscope slides

After the lysis and DNA extraction process, specimens were returned to 96% ethanol, and permanent slides were prepared as follows (summarized in Fig. 3):

1. Specimens were rinsed in bi-distilled water, followed by a dehydration process in a series of ethanol solutions with different concentrations: first in 70% ethanol for 10 minutes, then in 96% ethanol for another 10 minutes, and finally in absolute ethanol (100%) for five minutes. Subsequently, the samples were transferred to clove oil and left to soak for at least 10 minutes.
2. Simultaneously, microscope slides were cleaned with 95% ethanol and dried using paper tissue. Four drops (two rows of two drops) of Euparal were distributed in the central part of each microscope slide.
3. Once the dehydration process was complete, specimens were placed in the bottom left drop of Euparal on the microscope slide and dissected directly. The dissected body parts were arranged as follows: wings (or one wing if one was absent) in the top right drop, genitalia in the bottom right drop, head in the top left drop, and thorax in the bottom left drop. All dissected body parts were mounted in a dorsal view, except for the thorax and wings, which were positioned laterally. Slides were then left to dry at room temperature for 24 hours.
4. After the 24-hour period, slides were examined under a stereomicroscope to confirm the proper positioning of dissected parts. If everything was correctly placed, another drop of Euparal was applied on top, and a 9 mm round cover glass was added. If adjustments were needed, another drop of Euparal was placed on top, and after a 5-10 minutes wait for the new drop to soften the dried Euparal, the body part was repositioned. This process was repeated as necessary until a cover glass was added to each drop of Euparal in the slide.
5. Following the addition of the cover glass, slides were allowed to dry at room temperature for a minimum of six months on a flat surface. Alternatively, this process could be expedited by drying the slides in an incubator set at a constant 40°C for at least 30 days before permanent storage.

Data analysis

Following the morphological identification of voucher specimens, their DNA sequences underwent comparison with publicly available sequences in The Barcode of Life Data System (commonly known as BOLD; accessible at https://boldsystems.org/) to ensure the absence of cross-contamination during the DNA extraction process.

Statistical analyses were conducted using R (version 2023.03.1+446). The normality of the data distribution was assessed using the Shapiro-Wilk test (Shapiro and Wilk 1965), with a

significance threshold set at p = 0.05. Initial comparisons of our dataset for morphological changes and DNA concentration were performed using Kruskal-Wallis tests (Kruskal and Wallis (1952). Subsequently, post-hoc pairwise comparisons were conducted using Dunn's test (Dunn 1964) with Bonferroni correction (Abdi 2010) to identify significant differences between treatment groups. The significance level was set at α = 0.05.

To further explore the data, separate analyses were conducted within constant and interrupted treatment for the different incubation periods. Kruskal-Wallis tests were utilized to compare the DNA concentration and the total morphological changes across the different incubation periods within each treatment. Post-hoc pairwise comparisons were conducted using Dunn's test with Bonferroni correction to identify significant differences between treatment groups. The significance level was set at α = 0.05.

To examine the interaction between DNA fragment percentages, the shaking treatment and the incubation periods, a linear mixed-effects model was fitted using the lme4 package in R (Bates et al. 2015). The model incorporated the random effect of shaking conditions to account for variability between different treatment conditions. Model summaries were generated to assess the significance of the interaction effect and evaluate the influence of shaking conditions on the outcome.

Boxplots were generated to visually represent the data using the R package ggplot2 (Wickham 2016), illustrating the distribution of morphological changes and DNA concentration across different treatment conditions and time points. Additionally, line plots were utilized to display mean and median values, facilitating the interpretation of trends and differences within the dataset.

Additionally, we employed a generalized linear model (GLM) approach to assess the relationship between the response variable "Total changes" and the interaction of the predictor variables "shaking treatments" (constant and interrupted), "incubation periods", and collection year. Likewise, we employed a GLM approach to assess the relationship between DNA concentration as the response variable and the interaction of the same predictor variables. Both GLMs were implemented using the 'glm' function in R with a Poisson and Gaussian family, respectively, to account for count data distribution. The significance of the factors and their interaction was evaluated using the 'Anova' function, facilitating the assessment of the overall model fit and the contribution of each predictor to the variation in the response variable.

Results

COI barcoding and species identification

Out of the 310 specimens used for DNA extraction and posterior sequencing, we successfully obtained 278 COI sequences, representing a sequencing success rate of 89.6%. Out of our desired sequence lengh of 658 bp, eight sequences fell short of the desired length (specimen no.: ZFMK-DIP-00097774 [618 bp], ZFMK-DIP-00097780 [624

bp], ZFMK-DIP-00097857 [657 bp], ZFMK-DIP-00097875 [657 bp], ZFMK-DIP-00097881 [631 bp], ZFMK-DIP-00097895 [657 bp], ZFMK-DIP-00097903 [657 bp], ZFMK-DIP-00097906 [608 bp]; see Suppl. material 1 for further information of specimens). Nonetheless, all of the 278 COI sequences obtained are viable to use for species identification. Samples that failed to undergo successful sequencing were slide mounted, and their morphological species determination was subsequently performed.

The 310 specimens used for the study belonged to 13 genera and 24 species, namely: *Clytocerus* Eaton, 1904 [*C. ocellaris* (Meigen, 1804)], *Lepiseodina* Enderlein, 1937 [*L. rothschildi* (Eaton, 1913); *L. tristis* (Meigen, 1830)], *Paramormia* Enderlein, 1935 [*P. polyascoidea* (Krek, 1971); *P. ustulata* (Haliday, 1856)], *Pericoma* Haliday, 1856 (*P. blandula* group], *Peripsychoda* Enderlein, 1936 [*P. auriculata* (Haliday, 1839)], *Philosepedon* Eaton, 1904 [*P. humeralis* (Meigen, 1818)], *Pneumia* Enderlein, 1935 [*P. nubila* (Meigen, 1818)*; P. trivialis* (Eaton, 1893)], *Psychoda* Latreille, 1797 [*P. alternata* Say, 1824; *P. cinerea* Banks, 1894; *P. gemina* (Eaton,1904)*; P. minuta* Banks, 1894; *P. phalaenoides* (Linnaeus, 1758); *Psychoda satchelli* Quate, 1955; *P.* sp.*, P. trinodulosa* Tonnoir, 1922], *Seoda* Enderlein, 1935 [*S. ambigua* (Eaton, 1893)], *Telmatoscopus* Eaton, 1904 [*T. advena* (Eaton, 1893)], *Trichomyia* Haliday, 1839 [*T. parvula* Szabó, 1960; *T. urbica* (Haliday in Curtis, 1839)], *Trichopsychoda* Tonnoir, 1922 [*T. hirtella* (Tonnoir, 1919)), and *Ulomyia* Haliday, 1856 [*U. fuliginosa* (Meigen, 1804)]. Specimen data is given in the Suppl. material 1.

It is worth mentioning that some specimens were not identified to species level (e.g. *Pericoma blandula* group [8 specimens]; *Psychoda* sp. [1 specimen]), corresponding to 2.90 % out of the 310 individuals. This decision was made mainly based on two reasons: 1) the species group requires further examination and a taxonomic revision to properly address the species within, and 2) the morphological characters do not fully match the closest related species and no DNA barcode is available for comparison of molecular data. Therefore, to avoid including a species name that later needs amendment, the determination remains at the genus level.

Data normality

The Shapiro-Wilk normality tests were performed on three variables in our dataset: DNA concentration, total changes in structures lost, and DNA fragment measurements. For DNA concentration, the data was not normaly distributed (W = 0.9062, p = 5.848e-13). Similarly, our change in morphological structures is not normally distributed, as the Shapiro-Wilk yielded a very low p-value (W = 0.9074, p = 7.328e-13). Likewise, the percentage of the DNA fragment measurements are not normally distributed (W = 0.4032, p = 2.2e-16). (NOTE: I am not sure if this section is relevant here. It is my understanding that normality tests are not really results (i.e., answer to your questions), rather they are diagnostic to decide which statistic analysis can be done in the data. I would maybe mention rather this part in methods to justify the statistics you used e.g., non-parametric tests).

Loss of structures

Out of the 310 specimens used in our analyses, 60 (19.35%) did not exhibit any changes in the structures lost during the DNA extraction process, while 250 (80.65%) displayed at least one change (see Fig. 4).

Based on the Kruskal-Wallis test, there were no significant differences detected among shaking treatments (constant and interrupted) in terms of total morphological changes (χ^2 = 3.31976, $p > 0.05$). Therefore, no post-hoc pairwise comparisons were performed. This suggests that the different treatments did not lead to statistically significant variations in total morphological changes among the experimental groups.

To evaluate potential differences in the variable 'Total_changes' (loss of morphological strucutures) among the groups within the treatment 'constant shaking' (hereafter abbreviated as C, followed by the incubation hours, e.g. C2), the Kruskal-Wallis test reported a statistically significant result (χ^2 = 53.429, df = 4, $p < 0.001$). Post-hoc Dunn's tests with Bonferroni correction were performed, revealing significant differences between various group pairs. Specifically, C12 showed no significant difference compared to C16 ($p = 0.280$), but had significantly lower values compared to C2 ($p < 0.001$) and C4 ($p < 0.001$). C16 exhibited significantly higher values than C2 ($p < 0.001$) and C4 ($p = 0.003$). C2 also had significantly lower values compared to C4 ($p < 0.001$). C12 did not significantly differ from C8 ($p = 0.483$), while C16 showed significantly higher values compared to C8 ($p = 0.002$). No significant differences were observed between C2 and C8 ($p = 1$), nor between C4 and C8 ($p = 0.335$) (Fig. 5).

Likewise, when comparing the incubation periods within the interrupted treatment (hereafter abbreviated as I, followed by the incubation hours, e.g. I2), the Kruskal-Wallis test yielded a statistically significant result (χ^2 = 23.301, df = 4, $p < 0.001$). Post-hoc Dunn's tests with Bonferroni correction revealed significant differences between several group pairs. Notably, I16 displayed significantly lower values compared to I2 ($p = 0.002$) and I4 ($p = 0.0004$), while no significant differences were observed between I16 and I12 ($p = 0.151$). Additionally, I2 exhibited significantly higher values than I4 ($p = 0.004$). These findings suggest that the total loss of morphological structures varies significantly across different groups based on the incubation period (Fig. 5).

Our GLM analysis revealed significant effects of the shaking treatment (χ^2 = 5.240, df = 1, $p = 0.02207$) and the incubation period (χ^2 = 96.760, df = 1, $p < 2e\text{-}16$) on the total changes, indicating that both factors independently influence the outcome variable. Specifically, interrupted shaking was associated with a significant increase in the total changes (Estimate = 0.422, Std. Error = 0.1749, z = 2.413, $p = 0.0158$), while an increase in the incubation period was also linked with increased morphological structures lost (Estimate = 0.08573, Std. Error = 0.01126, z = 7.610, $p < 2.74e\text{-}14$). However, the interaction between shaking and incubation period did not reach statistical significance (χ^2

= 2.521, df = 1, p = 0.11232), suggesting that the combined effect of these factors may not be different from their individual effects. Overall, the model provided a good fit to the data, as evidenced by the relatively low residual deviance (447.09 on 306 degrees of freedom) and the corresponding Akaike Information Criterion (AIC) value of 1148.

DNA concentration

The DNA concentration exhibited a range from 0.00 to 1.33 ng/µl, with a mean value of 0.32 ng/µl, a median value of 0.25 ng/µl, and a mode of 0.00 ng/µl. Remarkably, even in cases where the concentration was measured as 0.00 ng/µl, we were able to obtain high-quality COI barcodes of the desired length (658 bp).

The Kruskal-Wallis test was conducted to compare DNA concentrations between constant and interrupted treatments. The test revealed a statistically significant difference in DNA concentration between the two treatment groups ($\chi^2 = 10.92187$, df = 1, $p < 0.05$). Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction further elucidated the findings. The interrupted treatment group exhibited a significantly higher mean DNA concentration (Mean = 0.405) compared to the constant treatment group (Mean = 0.292), with a p-value of 0.0005 (see Fig. 4).

Within the constant shaking treatment, the Kruskal-Wallis test found no significant differences in the average DNA concentration among the different incubation periods ($\chi^2 = 5.5167$, df = 1, p-value > 0.05). Therefore, post-hoc pairwise comparisons using Dunn's test were not performed.

Conversely, the Kruskal-Wallis test revealed a statistically significant difference in average DNA concentration among the different incubation periods within the 'interrupted' treatment ($\chi^2 = 58.657$, df = 4, $p < 0.001$). Post-hoc pairwise comparisons using Dunn's test with Bonferroni correction further elucidated significant differences between certain group pairs. Notably, I2 exhibited significantly lower average DNA concentration compared to I16 ($p = 0.027$) and I4 ($p = 0.0002$). Additionally, I4 displayed significantly lower average DNA concentration compared to I8 ($p < 0.001$). No significant differences were detected between I12 and other incubation periods. (Fig. 6).

DNA fragments

The Shapiro-Wilk test revealed that the DNA fragment measurements were not normally distributed (W = 0.40322, p-value < 2.2e-16) (Suppl. material 3). The linear mixed effects model indicated that the incubation period had a non-significant effect on the response (Estimate = 116.3, Std. Error = 157.5, t-value = 0.738, $p > 0.05$). Random effects analysis revealed substantial variability between different levels of shaking (constant and interrupted), with a significant variance of 538440 (Std. Dev. = 733.8). The model provided a good fit to the data, as indicated by the REML criterion at convergence (2032.8).

Collection year

The results of our GLM show that the collection year variable on both total changes and the DNA concentration is significant. In the case of total changes, the GLM demonstrated a marginally significant effect ($p = 0.084$). The negative coefficient estimate (-0.11728) suggests a potential decreasing trend in the total changes for recent collection years. In other words, specimens that have been collected recently (2020 and 2021) lost fewer structures compared to those collected in 2013. Conversely, when examining DNA concentration, the main effect of the collection year was significant ($p = 0.041$). Although the coefficients for individual years were not statistically significant, the overall effect suggests a potential difference in DNA concentration across different collection years.

Discussion

Loss of structures

Fragile specimens, such as Psychodidae and other nematoceran flies, commonly sustain damage, including the loss of antennae, legs, and wings, during mass-collecting methods such as CDC traps, Malaise traps, or yellow pan traps. Despite the meticulous handling and the precautions taken during transportation and storage, specimen damage remains a persistent issue (Karlsson et al. 2020). Some dipterists prefer hand-netting to mitigate these issues, as up to 50% of specimens in large samples may not be suitable for morphological identification (Jaschhof and Jaschhof 2009).

Moreover, many samples stored for extended periods may not receive proper maintenance or monitoring. Ethanol concentrations may become inadequate for effective specimen preservation, and bulk samples are often stored at room temperature, leading to the deterioration of both specimens and their DNA. Recent projects, such as The Swedish Malaise Trap Project (Karlsson et al. 2020) and the third phase of the German Barcode of Life initiative, GBOL III: Dark Taxa (Hausmann et al. 2020), aim to address these challenges. However, managing large volumes of sampled material remains a persistent issue. Therefore, emphasizing proper management is

crucial to ensure the preservation of both DNA and morphological structures over time.

During our study, we found that the majority of moth fly specimens retrieved from Malaise traps exhibit evident structural changes, such as the loss of ascoids, flagellomeres, and legs preior to our morphological examination. Despite our efforts to minimize handling-induced damage, 80.65% of the specimens displayed further alterations in morphological structures during the experiment. Despite the loss of morphological structures, we successfully identified to species level 95% of the 310 specimens analyzed.

Our results show that there is no significant statistical difference between shaking treatments (constant and interrupted) regarding the loss of morphological structures. However, when evaluating the incubation periods within each treatment, we found statistically significant differences. Within the constant treatment, C2 had the lowest number of structures lost (see Fig. 5), while C12 presented the highest number, although C12 did not differ from C8. Likewise, within the interrupted treatment, there is a trend suggesting that shorter incubation periods result in fewer morphological structures lost, and longer incubation periods result in more structures lost (Fig. 5).

Our findings suggest that varying the incubation period during DNA extraction directly influences the loss of morphological structures. It is important to note the significance of this observation, especially for genera such as *Psychoda*, where terminal flagellomeres are often used for species identification. Likewise, morphological characters found in the antennae (e.g. the shape of antennal segments, the presence, arrangement, and number of ascoids) are often used for genus-level determination. With each structure lost during collection, storage, and specimen handling, we lose valuable diagnostic features, especially when studying hyper-diverse taxa, such as mothflies.

While meticulous handling and storage precautions were undertaken to mitigate damage to the specimens, our statistical analysis reveals a significant effect of the collection year on the total changes ($p = 0.084$). This finding suggests a trend of decreasing morphological changes in recent collection years. Such a trend underscores the importance of understanding the dynamics of specimen damage through time and the need for continued attention to specimen management practices over time.

DNA concentration

Some of the specimens used in our study presented an average DNA concentration of 0.0 ng/µl. Surprisingly, this minimal concentration did not hinder the successful generation of high-quality COI sequences. It's important to clarify that according to the QuantiFluor® user manual, concentrations below the lower limit of 0.01 ng/µl are not displayed during measurement, resulting in registered values of zero. Therefore, a reading of zero does not

imply a lack of DNA extracted but rather a concentration below the machine's detectable threshold.

Our results indicate that there is a significant statistical difference in the average DNA concentration between treatments (constant and interrupted shaking). The interrupted treatment exhibited a higer mean DNA concentration compared to the constant treatment (Fig. 4). Specifically, within the interrupted shaking, I4 yielded the highest average dna concentration (see Fig. 6). On the contrary, C8 and I8 yielded the lowest average DNA concentration (see Figs 5, 6 respectively). Overall, our findings suggest that interrupted shaking during different incubation periods yields higher average DNA concentrations compared to constant shaking. Nonetheless, continuous shaking shows a trend in lower DNA concentrations but fewer losses in morphological structures. Conversely, interrupted shaking leads to higher average DNA concentrations and a higher loss of morphological structures. This discrepancy raises intriguing questions regarding the impact of shaking intervals on DNA concentration across different treatments, warranting further investigation.

Considering the minuscule size of the average adult moth fly (less than 5 millimeters in total body length) and the natural degradation of DNA in preserved specimens, the observed low DNA concentration is not unexpected. Based on a quick exploration of our data, we observed that the genera *Seoda* and *Telmatoscopus* exhibited a notably higher DNA concentration when compared to other genera included in our sampling. This variance may be attributed to the larger body size of species within the *Seoda* and *Telmatoscopus* genera compared to other genera. However, our study's scope did not allow for an in-depth exploration of the specific relationship between body size and DNA concentration and further research is needed to explore the relationship between body size and DNA concentration.

Furthermore, it is crucial to distinguish between DNA concentrations measured directly for our study and the COI sequences obtained from the PCR product sent to BGI. The PCR product amplifies the DNA from the sample, enhancing the potential for obtaining high-quality COI sequences that are not necesarilly dependant on the DNA concentration obtained from our samples.

The Gaussian GLM analysis revealed significant effects of the shaking treatments ($t = 3.966$, $p = 9.10e-05$), especially for the interrupted:incubation period interaction ($t = -2.129$, $p = 0.034$), and for the incubation period ($t = -1.549$, $p = 0.122$) on the DNA concentration. The intercept term was also significant ($t = -7.559$, $p = 4.75e-13$). Specifically, interrupted shaking was associated with a significant increase in DNA concentration (Estimate = 0.64103, Std. Error = 0.16162, $p < 0.001$), while the interaction between interrupted shaking and incubation period demonstrated a significant negative effect (Estimate = -0.04258, Std. Error = 0.02000, $p = 0.034$). Additionally, a decrease in the incubation period was marginally associated with a decrease in DNA concentration (Estimate = -0.02415, Std. Error = 0.01559, $p = 0.122$). The model exhibited a good fit to the data, as evidenced by the low residual deviance (23.807 on 306 degrees of freedom) and the corresponding Akaike Information Criterion (AIC) value of 94.095. Analysis of deviance further confirmed

the significance of the shaking treatment (χ^2 = 17.748, df = 1, p = 2.522e-05), incubation period (χ^2 = 31.684, df = 1, p = 1.814e-08), and their interaction (χ^2 = 3.930, df = 1, p = 0.04743) on DNA concentration.

Our analysis revealed a marginal yet significant effect of the collection year variable on the DNA concentration (p = 0.041). Although individual collection years were not statistically significant, the overall effect suggests a potential difference in DNA concentration across different years. These findings suggest a temporal dynamic that could influence DNA concentration and morphological integrity, underscoring the complexity of factors affecting specimen preservation and DNA extraction efficiency.

General discussion

Our results indicate that groups C2, C4, I2, and I4 were the optimal choices when considering both the number of structures lost and the DNA concentration in our samples (Figs 5, 6). Particularly noteworthy is I4, which demonstrated a relatively low number of lost structures (Fig. 5) coupled with one of the highest DNA concentrations (Fig. 6). Similarly, treatments C4 and C2 also exhibited favorable DNA quantity (Fig. 6), with the level of structural loss remaining on the lower end of the spectrum compared to other categories.

C2 presented the lowest number of morphological structures lost (see Fig. 5), but yielded less DNA than other variables (Fig. 6). However, it is important to highlight that despite the lower DNA median concentration in C2 compared to others, we successfully obtained high-quality COI sequences from 29 out of 30 specimens within this treatment group. This suggests that while the DNA yield might be lower, C2 has an impressive capability to preserve morphological structures, indicating a favorable equilibrium of providing adequate COI sequences whithout compromising the morphological structures of the specimens.

The results of the generalized linear modeling (GLM) analyses shed light on the factors influencing the observed changes in the total changes and DNA concentration. In the case of total changes, our findings indicate significant effects of both shaking and incubation periods, suggesting that interrupted shaking and the incubation period independently impact the total number of changes observed. Interestingly, while the interaction between these factors did not reach statistical significance, their individual effects remain noteworthy. Conversely, for the DNA concentration, the GLM revealed significant effects observed for shaking, incubation period, and their interaction. Interrupted shaking was associated with an increase in DNA concentration, while the interaction effect between the shaking treatment and the incubation period demonstrated a significant negative association with DNA concentration, suggesting a potential moderating effect. Future research could explore additional factors that may influence these outcomes and further elucidate the underlying mechanisms driving these effects.

Overall, our findings suggest that C2 strikes a delicate balance between minimizing structural loss and yielding viable DNA sequences. This observation underscores its potential efficacy in preserving morphological integrity while still ensuring an adequate yield of DNA for downstream analysis. Consequently, a DNA extraction protocol involving

constant shaking with a 2-hour incubation period emerges as a promising choice for future studies involving DNA extraction in mothflies.

The selection of a 2-hour incubation period offers several advantages. Firstly, it promises a relatively short processing time for each set of samples, facilitating efficient throughput in laboratory workflows. With the potential to process multiple sets of samples in a single day, researchers can significantly expedite data collection and analysis compared to protocols requiring longer incubation periods, such as those exceeding 8 hours.

Moreover, the balance achieved by C2 suggests that it may be particularly well-suited for applications where preserving morphological features alongside DNA integrity is crucial. This is especially pertinent in studies involving delicate or rare specimens, where maintaining structural integrity is paramount for accurate taxonomic identification or morphological analysis.

Notes on mounting media

This study used Euparal as mounting media for the microscope slides as it works well, although some researchers prefer Canada Balsam or other mounting media (see Neuhaus et al. 2017). Canada Balsam is also suitable to be used after DNA extractionl. The choice of mounting medium is often a matter of personal preference, costs and availability. At present, both media (Euparal and Canada Balsam) are widely used among taxonomists, and both have proven to be effective for the long-term preservation of specimens (more than 50 and 150 years respectively) (Neuhaus et al. 2017).

During the process of preparing specimens on microscope slides, the application of mounting medium can vary in quantity, typically ranging from one to several drops, with five or six drops often considered the maximum. This variability is contingent upon several factors, including the size of the specimen, the dimensions of the cover glass, the availability of cover glass pieces, and the number of dissected structures per specimen. For our study, we used four small round cover glasses, each with a diameter of 9 mm, for the dissected structures (genitalia, head, thorax, and wings). However, it is common to encounter microscope slides in entomological collections where the entire specimen is covered by a single 12 mm round cover glass or by a square or rectangular cover glass. Researchers frequently employ the practice of cutting square or rectangular cover glasses into smaller pieces, utilizing each fragment to cover dissected structures of the specimens.

Steps 3 and 4 detailed in our proposed method (also see Fig. 3), which pertain to the slide preparation process, can be readily adapted to accommodate the specific requirements and available materials of individual researchers. This adaptability ensures flexibility in the mounting process, allowing researchers to tailor their approach based on the studie taxa, as well as the resources at their disposal.

Conclusions

In summary, the persistent challenges surrounding specimen damage and DNA preservation in entomological studies, particularly among fragile species like Psychodidae and other nematoceran families, highlight the need for effective strategies. Our findings suggest that using different shaking treatments across shorter incubation periods during DNA extraction hold promise in mitigating structural losses while maintaining good DNA yield.

This research underscores the delicate balance between preserving morphological integrity and obtaining viable COI DNA barcodes. Constant shaking with an incubation period of two hours (C2) demonstrates potential efficacy in preserving structures while providing adequate DNA for analysis. Nonetheless, the loss of even minor morphological structures, during sample handling and post-extraction morphological identification, in poorly studied taxa raises concerns about the potential loss of diagnostic information. Therefore, handling specimens with care during collection, DNA extraction, and slide preparation is crucial to avoid missing valuable morphological characters.

The ongoing refinement of methodologies in taxonomical studies remains essential in addressing the enduring challenges of specimen preservation and molecular data analysis. This imperative stems from the continuous evolution of new methods and technologies, especially with next generation sequencing techniques, emphasizing the need for adaptability and innovation to effectively overcome upcoming challenges.

The implications of these findings extend beyond the realm of mothfly research, offering insights into the optimization of DNA extraction protocols across diverse taxa and sample types. By prioritizing both structural preservation and DNA yield, protocols like ours hold promise for enhancing the efficiency and reliability of genetic studies, ultimately advancing our understanding of biological diversity and evolutionary processes. Further investigation and validation across different organisms and experimental conditions will be essential to fully harness the potential of such protocols in molecular research.

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Conflicts of interest

The authors have declared that no competing interests exist.

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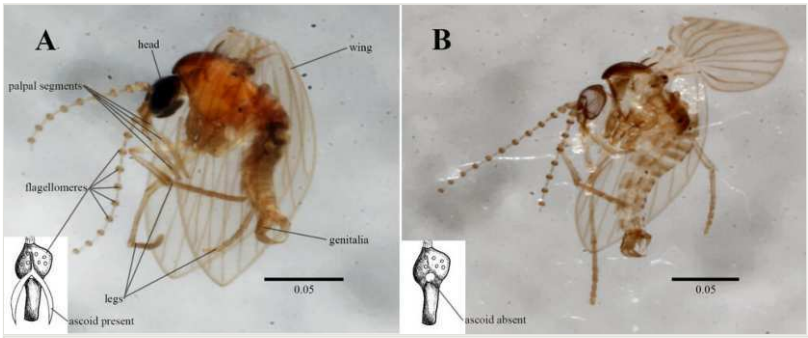

Figure 1.

Specimen of the genus *Psychoda* used during DNA extraction. **A.** habitus before extraction. **B.** habitus after DNA extraction, showing how the extraction helps in the diaphanization of the specimens. Drawing inside a white rectangle shows a flagellomere A) with the ascoid present, and B) with the ascoid absent (i.e., lost after DNA extraction). Scales in millimeters.

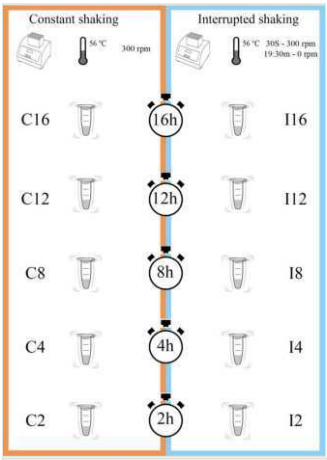

Figure 2.

Graphic representation of treatment variables. Constant shaking (C), Interrupted shaking (I). Abbreviations: m = minutes, rpm = revolutions per minute, h = hours, s = seconds.

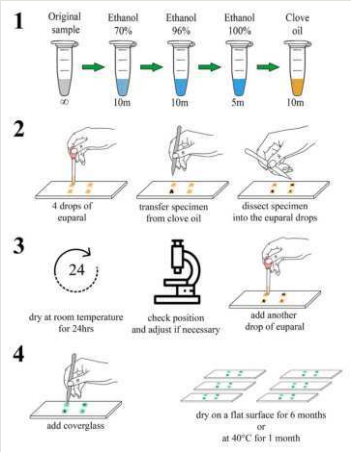

Figure 3.

Graphic representation of the slide mounting technique. **1.** dehydration process using different ethanol concentrations and clove oil (m = minutes). **2.** preparation of the microscope slide and dissection of the specimens. **3.** 24-hour lapse for drying the euparal and checking the correct positions of dissected body parts. **4.** Placement cover glasses and drying process.

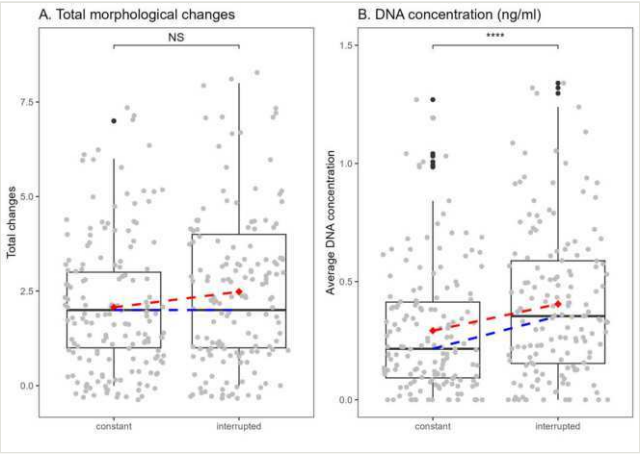

Figure 4.

A. Total morphological changes occurred during DNA extraction. B. DNA concentration (ng/ml) obtained during DNA extraction. Significance values obtained from Kruskal-Wallis test with posthoc Dunn test, Bonferroni corrected. Values: NS = not significant, **** = $p < 0.0001$. The Red dashed line indicates the mean, and the blue dashed line indicates the median.

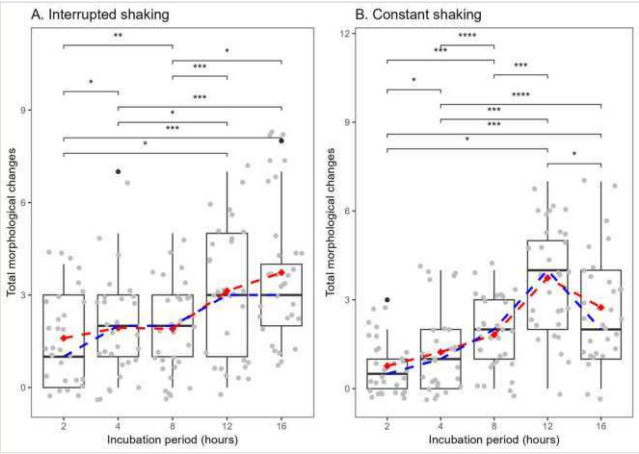

Figure 5.

Total morphological structures that were lost within each shaking category. A. Interrupted shaking. B. Constant shaking. Significance values obtained from Kruskal-Wallis test with posthoc Dunn test, Bonferroni corrected. Values: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The Red dashed line indicates the mean, and the blue dashed line indicates the median.

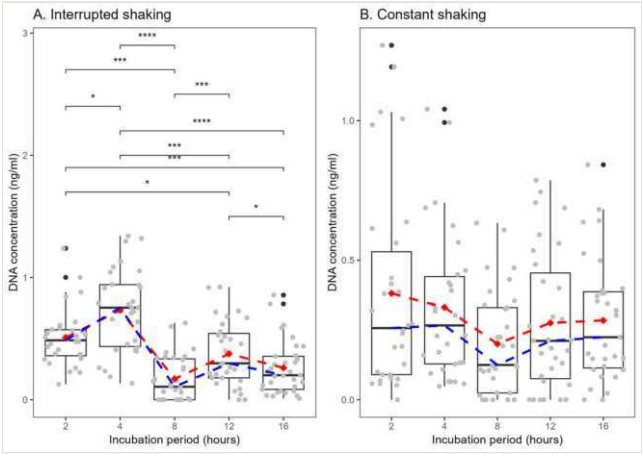

Figure 6.

DNA concentration within each shaking category. A. Interrupted shaking. B. Constant shaking. Significance values obtained from Kruskal-Wallis test with posthoc Dunn test, Bonferroni corrected. Values: NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The Red dashed line indicates the mean, and the blue dashed line indicates the median.

Supplementary materials

Suppl. material 1: Specimen Collection Data

Authors: Jaume-Schinkel, S. et al
Data type: collection data
Brief description: Collection data for all specimens used in the study
Download file (41.95 kb)

Suppl. material 2: Experiment data

Authors: Jaume-Schinkel, S. et al
Data type: numerical
Brief description: Data used for statistical analysis
Download file (32.63 kb)

Suppl. material 3: Fragment Analyzer Data

Authors: Jaume-Schinkel, S. et al
Data type: Numerical
Brief description: Data obtained from the fragment analyzer in percentage of fragment lengths
Download file (6.37 kb)

Appendix 17. (Publication chapter 19)

Chapter 19 – Publication

Jaume-Schinkel S, Avila-Calero S, Kvifte GM, Kukowka S, Martin S, Mengual X (in prep) Phylogeny of Psychodidae using exon-capture sequencing.

Phylogeny of Psychodidae using exon-capture sequencing

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Summary

In the present study, high-throughput sequencing was used to capture and enrich phylogenetically and evolutionary informative exonic regions within Psychodidae. With the help of the BAITFISHER software, we developed a new bait kit to capture1445 coding regions belonging to 1161 orthologous genes. This new bait kit was successfully used to target regions from three infraoders, namely, Tipulomorpha, Psychodomorpha, and Culicomorpha. Specifically, in Psychodidae, we were able to effectively capture targeted loci for 82 species from 46 genera, representing over 40% of the total Psychodidae genera. Based on our phylogenetic inferences, we present a subdivision of Psychodinae into seven tribes: Brunettiini Vaillant, 1971, Maruinini Enderlein, 1937 **stat. rev.**, Mormiini Enderlein, 1937 **stat. rev.**, Paramormiini Enderlein, 1937 **stat. rev.**, Pericomaini Enderlein, 1935 **stat. rev.**, Psychodini Newman, 1834, and Setomimini Vaillant, 1982 **stat. rev.**

Introduction

The family Psychodidae (Diptera: Nematocera: Psychodomorpha), commonly known as moth flies, sand flies, or owl flies, encompasses over 100 genera with nearly 3,500 described species distributed worldwide (Cordeiro & Wagner, 2018; Curler et al., 2019; Galati, 2018; Galati & Rodrigues, 2023; Wagner & Ibáñez-Bernal, 2009). Remarkably, the total global species richness of Psychodidae is estimated to exceed 25,000 species (Wagner & Ibáñez-Bernal, 2009). These small flies, usually ranging from 1-5 mm, are easily identifiable by their setose appearance, distinctive wing shape, and wing venation. They often hold their wings horizontally over the abdomen when at rest, resembling small moths, which led to their common name (Curler & Courtney, 2009; Wagner & Ibáñez-Bernal, 2009).

Throughout history various intrafamilial classifications have been proposed and the family's subdivision is still a topic of debate (Curler & Moulton, 2012; Galati & Rodrigues, 2023). Furthermore, the exact evolutionary relationships between these subfamilies remain unresolved, leading to a lack of universal acceptance of the classification system (Bertone et al., 2008; Curler & Moulton, 2012; Hennig, 1972; Kvifte & Wagner, 2017; Quate & Vockeroth 1981; Wiegmann et al., 2011). Nevertheless, a broadly used consensus classification recognizes six extant subfamilies within Psychodidae: Bruchomyiinae Alexander, 1921 (74 species), Horaiellinae Enderlein, 1937 (6 species), Phlebotominae Rondani, 1840 (1060 species), Psychodinae Newman, 1834 (2050 species), Sycoracinae Jung, 1954 (46 species), and

Trichomyiinae Tonnoir, 1922 (215 species), along with one extinct subfamily, Datziinae Stebner, Solórzano-Kraemer, Ibañez-Bernal & Wagner, 2015 (5 species) (Galati & Rodrigues, 2023).

Initially, the subfamily Bruchomyiinae was described and considered a subfamily of Tanyderidae (Alexander, 1920). Likewise, the subfamily Trichomyiinae has been regarded as a separate family (Rohdendorf, 1962). Species of *Sycorax* (Sycoracinae) were originally described as part of the family Dixidae (Müller, 1927), and others suggested *Sycorax* should be part of Trichomyinae (Edwards,1928) or a subfamily within Psychodidae (Alexander, 1929; Jung, 1954; Fairchild, 1955). Some authors have suggested treating Phlebotominae as a tribe within Psychodidae, or treat it as a separate family (e.g., Azar et al., 1999; Fairchild, 1955; Rohdendorf, 1974; Lewis, 1973; Williams, 1993). However, the latter classification would require elevating all subfamilies to the family level.

The subfamily Psychodinae has undergone several changes in its subclassification, particularly in the tribal classification. Enderlein (1935, 1937) proposed the initial tribal classification mainly based on wing venation. However, he overlooked important character systems in the male genitalia, leading subsequent researchers to disregard his work (Kvifte, 2018). Vaillant (1971, 1982, 1986, 1990) later developed a tribal classification using a broader range of morphological characters, although his early work faced criticism for violating the International Code of Zoological Nomenclature (ICZN) (Duckhouse, 1978). Despite this, some authors continued to use Vaillant's classification (e.g. Andersen & Håland, 1995.; Bernotienè, 2002; Krek, 1999; Salamanna & Raggio, 1985; Svensson, 2009; Wagner, 1990, 1997; Wagner, 2004). Vaillant (1990) recognized 7 tribes within Psychodinae, namely, Brunettiini, Maruinini, Mormiini, Neomaruinini, Pericomaini [as Pericomini], Psychodini, and Setomimini.

Ježek (Ježek, 1983, 1984, 1985, 1990; Ježek & Goutner, 1993; Ježek & van Harten, 2005) proposed an alternative tribal classification, based on morphological characters. Ježek recognized 5 tribes, namely, Mormiini, Paramormiini [with two subtribes, Paramormiina and Trichopsychodina], Pericomaini [as Pericomini], and Psychodini. Similarly, Duckhouse (1985, 1987) proposed a different tribal classification with 5 tribes (Maruinini, Mormiini, Paramormiini, Pericomaini [as Pericomini], and Psychodini). Duckhouse classification was later followed by Quate (Quate, 1996, 1999) and Kvifte (2012). Subsequently, Quate & Brown (2004) recognized 6 tribes, i.e., Maruinini, Mormiini, Paramormiini, Pericomaini [as Pericomini], Psychodini, and Setomimini. Besides these studies, other morphological

discussions relevant to the tribal classification have been presented by Quate (1959), Curler & Courtney (2009) and Cordeiro (2013), although with limited taxon sampling.

While earlier classifications were primarily based on morphological characters, recent studies have explored phylogenetic inferences using molecular data (Curler & Moulton (2012) [nuclear DNA sequence data]; Espíndola et al. (2012) [mitochondrial DNA sequence data]; Kvifte (2018) [nuclear and mitochondrial DNA sequence data]). Among these studies, only Kvifte (2018) proposed significant changes in the tribal classification based on a combination of molecular and morphological evidence and recognized 4 tribes, namely Brunettiini, Maruinini, Pericomaini, and Psychodini.

While adult morphology has traditionally served as the cornerstone for understanding the family's diversity, as we delve into the relationships within moth flies, it becomes apparent that the advancement in genomic research offers a transformative perspective to explore the relationships within Psychodidae. High-throughput sequencing (HTS) enables cost-effective and genome-scale data collection, facilitating the processing of large amounts of taxa (Andermann et al., 2020; Lemmon & Lemmon, 2013). Sequence-capture methods (also referred to as target enrichment or targeted sequencing) are used to enrich sequence libraries for specific regions of the genome (Faircloth et al., 2012; Gnirke et al., 2009; Lemmon & Lemmon, 2013; Lemmon et al., 2012).

The BAITFISHER software (Mayer et al., 2016) was created to design hybrid enrichment baits for various scenarios, including capturing exons from less similar sequences, to reduce the number of required baits for the taxonomic group of interest. The approach of enriching orthologous single-copy protein-coding genes has been effectively applied to multiple plant families (Li et al., 2017) and various metazoan taxa, such as stony corals (Hexacorallia: Scleractinia, Anthozoa: Scleractinia; Quek et al., 2020), sea spiders [Pycnogonida: Pantopoda; Dietz et al. (2019)], isopods [Malacostraca: Isopoda; Stringer et al. (2021)], wasps [Insecta: Hymenoptera; Bank et al. (2017); Klopfstein et al. (2019); Maletti et al. (2021); Mayer et al. (2016)], butterflies and moths [Insecta: Lepidoptera; Call et al. (2021); Mayer et al. (2021); Mayer et al. (2016)], cockroaches [Insecta: Blattodea; Evangelista et al. (2021)], and hover flies [Insecta: Diptera; Mengual et al. (2023)].

Despite efforts to construct a robust phylogenetic hypothesis for moth flies, their subfamilies, tribes, and genera, there are still taxa with ambiguous phylogenetic relationships or whose phylogenetic placement has not yet been studied. Interpreting the evolutionary history

of the group relies on a better understanding of their phylogenetic relationships to have a more stable classification. In the present study, we aimed to infer a robust phylogeny of Psychodidae to address the problems of the current tribal classification within Psychodinae.

Materials and methods

Taxon sampling

Taxa were selected to cover as much generic and geographical diversity as possible within the family Psychodidae with special emphasis on the subfamily Psychodinae. Specimens of Blephariceridae, Culicidae, Limoniidae, Ptychopteridae, and Tanyderidae were selected to include representatives of the infraorders Culicomorpha, Tipulimorpha, and Psychodomorpha. The selection of these taxa as outgroups was done following the results by Weigmann et al. (2011) as these taxa represent groups closely related to Psychodidae.

The following Psychodidae subfamilies are represented by a single genus in our analysis: Sycoracinae (*Aposycorax* Duckhouse, 1972 [1 species]), Phlebotominae (*Lutzomyia* França, 1924 [2 species]), and Trichomyiinae (*Trichomyia* Haliday, 1839 [5 species]). For the subfamily Psychodinae, we included 74 species belonging to 43 genera and four species of undescribed genera [based on morphology three of these species belong to Psychodini and one to Pericomaini] (see Table 3 as Suppl. Material). Among the 43 sampled genera in Psychodinae, 34 contained a single species and nine genera included two or more taxa. These 43 genera included in our analysis correspond to 40.6 % of the total of described genera within Psychodinae (106).

Specimen documentation

For the Psychodidae samples, the head, the wings, and the genitalia were dissected and mounted on permanent slides. The abdomen, thorax, and legs were used for DNA extraction. Dissected parts were mounted in microscope slides with the following procedure: wings were mounted right after dissection, using Euparal as a mounting medium. The head and genitalia were diaphanized in NaOH 10 % overnight (approximately 12 hours at room temperature). After diaphanization, the head and genitalia were rinsed in bidestilled water twice, then dehydrated in a series of ethanol at different concentrations, 70 % for ten minutes, 95 % for ten minutes, and 100 % for five minutes. After ethanol, dissected parts were transferred to clove oil for ten minutes. Finally, dissected parts were transferred to Euparal droplets previously

placed in a microscope slide on which the corresponding specimen wings were previously mounted. Slides were left to dry at room temperature for 24 hours, then, another drop of Euparal was added followed by a cover slide. Prepared slides were left to dry at room temperature for six months on a flat surface before permanent storage. The abdomen, thorax, and legs used for DNA extraction were crushed during the process and posteriorly discarded, thus, these structures are no longer available for morphological study.

All microscope slides holding the dissected body parts are deposited in the Museum Koenig Bonn (ZFMK), Leibniz Institute for the Analysis of Biodiversity Change (previously known as the Zoological Research Museum Alexander Koenig).

Molecular laboratory

Genomic DNA extraction was performed on dissected samples using Qiagen Blood & Tissue Kits (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. Samples were lysed at 56°C overnight with agitation at 350 rpm using an Eppendorf Thermomixer for approximately 23 hours. Subsequently, RNAse digestion and washing steps were carried out before elution in 100 µL of water. Finally, the DNA concentration of each sample was determined using a Quantus Fluorometer (Promega), while fragment lengths were assessed using a Fragment Analyzer (Agilent Technologies Inc.).

The quality assessment revealed a predominance of low-concentration samples, although most exhibited satisfactory quality. To proceed, we concentrated the samples to the required volume using a SpeedVac R SPD 111V (ThermoFisher Scientific, Waltham, MA, USA). Subsequently, for higher-quality samples with longer fragment lengths (>500bp), a fragmentation step was necessary. This was accomplished using a Bioruptor PICO sonicator (Diagenode S.A.) to achieve DNA fragments approximately 350 bp in length. Following fragmentation, samples were reassessed using a Fragment Analyzer. Conversely, lower-quality samples, already fragmented (<500 bp), required no further fragmentation. These samples underwent an FFPE DNA repair step using NEBNext FFPE DNA Repair Mix (NEB, USA), according to the manufacturer's protocol. Post-repair, sample concentrations were reassessed using a Quantus Fluorometer (Promega).

After standardizing the samples, we proceeded with library preparation using the Agilent SureSelect XT HS2 Library Preparation Kit for ILM, adhering to the manufacturer's protocol. To ensure accurate library PCR, a Quantus measurement step was incorporated to

determine the optimal number of cycles. For samples with low concentrations, 16 cycles were utilized, while 9 cycles were used for higher-concentration samples. Following the PCR reaction, purification was carried out at a ratio of 1:0.8 using Agencourt AMPure XP beads. Subsequently, library quantification was performed using a Quantus Fluorometer (Promega), and quality assessment was conducted using a Fragment Analyzer (Agilent Technologies Inc.).

We pooled 8 samples, each at an equimolar absolute amount of 93 ng, for the hybridization stage, reducing the volume to 3.5 µL using a SpeedVac R SPD 111V (ThermoFisher Scientific, Waltham, MA, USA). During hybridization capture, we encountered performance issues with the newer HS XT2 kit, specifically with the hybridization buffer when used with our insect samples. To address this, we opted to utilize the hybridization buffer from the older version of the XT2 kit, which had yielded successful results in previous projects. Subsequently, we proceeded with the enrichment following the Agilent SureSelect XT2 protocol outlined in (Mayer et al., 2021), with minor adjustments. We utilized SureSelect Custom baits Tier2 0.5-2.9 Mb and adjusted the volumes to accommodate captured library sizes <3.0 Mb by reducing them by 50%, adhering to the manufacturer's protocol. For two pools (comprising 16 samples), we had to switch to the new SureSelect HS XT2 kit due to the discontinuation of the older version. To optimize the process, we made a few modifications to the manufacturer's protocol: we increased the input DNA amount to 250 ng per sample in these pools and reduced the specified volumes by 50%.

The capture and washing steps after hybridization were done for all pools according to the HS XT2 manufacturers' protocol. Captured Library PCR was done with 16 cycles and purified with Agencourt AMPure XP beads in a ratio of 1:0.75. Quantity and quality checks were done with a Quantus Fluorometer (Promega) and Fragment Analyzer (Agilent Technologies Inc.), respectively.

Paired-end sequencing was done at Macrogen Europe (Amsterdam, Netherlands) on a NovaSeq 6000 System with a read length of 150 bp and an estimated output of 15 Gbps per library pool.

Gene selection

For an overview, we used OrthoDB v10.1 with the Phyloprofile "Present in all species" and "Single-copy in >90% species". The output was: "1161 ortholog groups (OGs) that span in all species and single-copy in >90% species".

From all the species listed for “Nematocera”, 11 were selected as “reference species” based on the quality of their data: *Aedes aegypti* (Linnaeus, 1762), *Anopheles culicifacies* Giles, 1901, *Anopheles gambiae* Giles, 1902, *Anopheles minimus* Theobald, 1901, *Culex quinquefasciatus* Say, 1823 [family Culicidae]; *Belgica antarctica* Jacobs, 1900, *Clunio marinus* Haliday, 1855, *Polypedilum nubifer* Skuse, 1889, *Polypedilum vanderplanki* Hinton, 1951 [family Chironomidae]; and *Lutzomyia longipalpis* (Lutz & Neiva, 1912), and *Phlebotomus papatasi* (Scopoli, 1786) [family Psychodidae].

Bait design for target enrichment

We downloaded the genomes of the 11 nematoceran species listed above. This included FASTA files of all annotated genes as amino acid sequences and the corresponding coding nucleotide sequences. We then designated baits for the selected coding regions with the BaitFisher software v. 1.2.7 (Mayer et al., 2016). For each locus, the baits should reflect the variability of the gene of interest in the whole taxonomic group of interest.

De novo assembly and orthology prediction and sequence alignment

The sequence analysis methodology involved a comprehensive workflow. Initially, we used fastp (Chen et al., 2018) for precise sequence trimming, followed by SPAdes (Prjibelski et al., 2020) for sequence assembly. Post-assembly, we used the tool Ortograph for pinpointing the coding sequences (CDS) of interest. Our analytical pipeline, designed in a Snakemake workflow following (Mayer et al., 2021), encompassed multiple steps (eg. sequence trimming, assembly, identification of orthologous loci, alignment, and stringent data filtering). To unravel evolutionary relationships, we conducted phylogenetic analyses using IQ-TREE 1.6.3 (Nguyen et al., 2015), see also the approach outlined in (Mayer et al., 2021).

Results

Phylogenetic analyses

Our phylogenetic inference (Fig. 1) recovers *Protoplasa* cf. *fitchii* (Tanyderidae) as the sister taxon of *Aposycorax chilensis* (Tonnoir, 1929) (Sycoracinae) with high support (Ultrafast Bootstrap or UFB = 95), questioning the monophyly of Psychodidae. This group (Tanyderidae

+ Sycoracinae) was resolved as sister to all other moth flies (UFB = 100). Our analyses resulted in well-supported relationships among the remaining subfamilies (namely Phlebotominae, Trichomyiinae, and Psychodinae; UFB = 100). Phlebotominae was recovered as sister to Trichomyiinae (UFB = 100), and this group (Phlebotominae + Trichoyiimae) was resolved as sister to Psychodinae (UFB = 100).

Within Psychodinae, our analyses recovered multiple caldes with high support. Setomimini was placed as sister to Psychodini with high support (UFB = 97). Likewise, this group (Setomimini + Psychodini) was recovered as the sister group of the remaining Psychodinae with high support (UFB = 100). Within the remaining Psychodinae, our inference recovered Brunettiini as sister to Maruinini (UFB = 100), and this group (Brunettiini + Maruinini) was resolved as the sister group to the remaining taxa (UFB = 100). Within the remaining nodes, *Tonnoiriella* Vaillant, 1982 was recovered as a distinct clade with high support (UFB = 100), positioned as the sister group to the remaining taxa. Likewise, Pericomaini was recovered as the sister group to the remaining nodes with moderate support (UFB = 81). Mormiini was recovered as sister to a group including the genera *Peripsychoda* Enderlein, 1935 and *Mystropsychoda* Duckhouse, 1975, although with very low support (UFB = 52). The cluster including Mormiini + *Peripsychoda* + *Mystropsychoda*, was recovered as sister to a cluster containing the genera *Lepidiella* Enderlein, 1937, *Clytocerus* Eaton, 1904 and *Clogmia* Enderlein, 1937, with virtually no support (UFB = 47). Finally, this cluster (*Lepidiella* + *Clytocerus* + *Clogmia*) was recovered as sister to Paramormiini with moderate support (UFB = 85).

Our phylogenetic analysis revealed that several of genera with more than one species formed monophyletic groups, such as *Psychoda* Latreille, 1797, *Atrichobrunettia* Satchell, 1953, *Tonnoira* Enderlein, 1937, and *Mystropsychoda*. However, *Alepia* Enderlein, 1937, *Thornburghiella* Vaillant, 1982, *Pneumia* Enderlein, 1935, *Pericoma* Haliday, 1856, and *Telmatoscopus* Eaton, 1904 were resolved as non-monophyletic.

A visual representation comparing our revised tribal classification, with the included taxa in our analysis, and the previous tribal classifications can be seen in Figure 2.

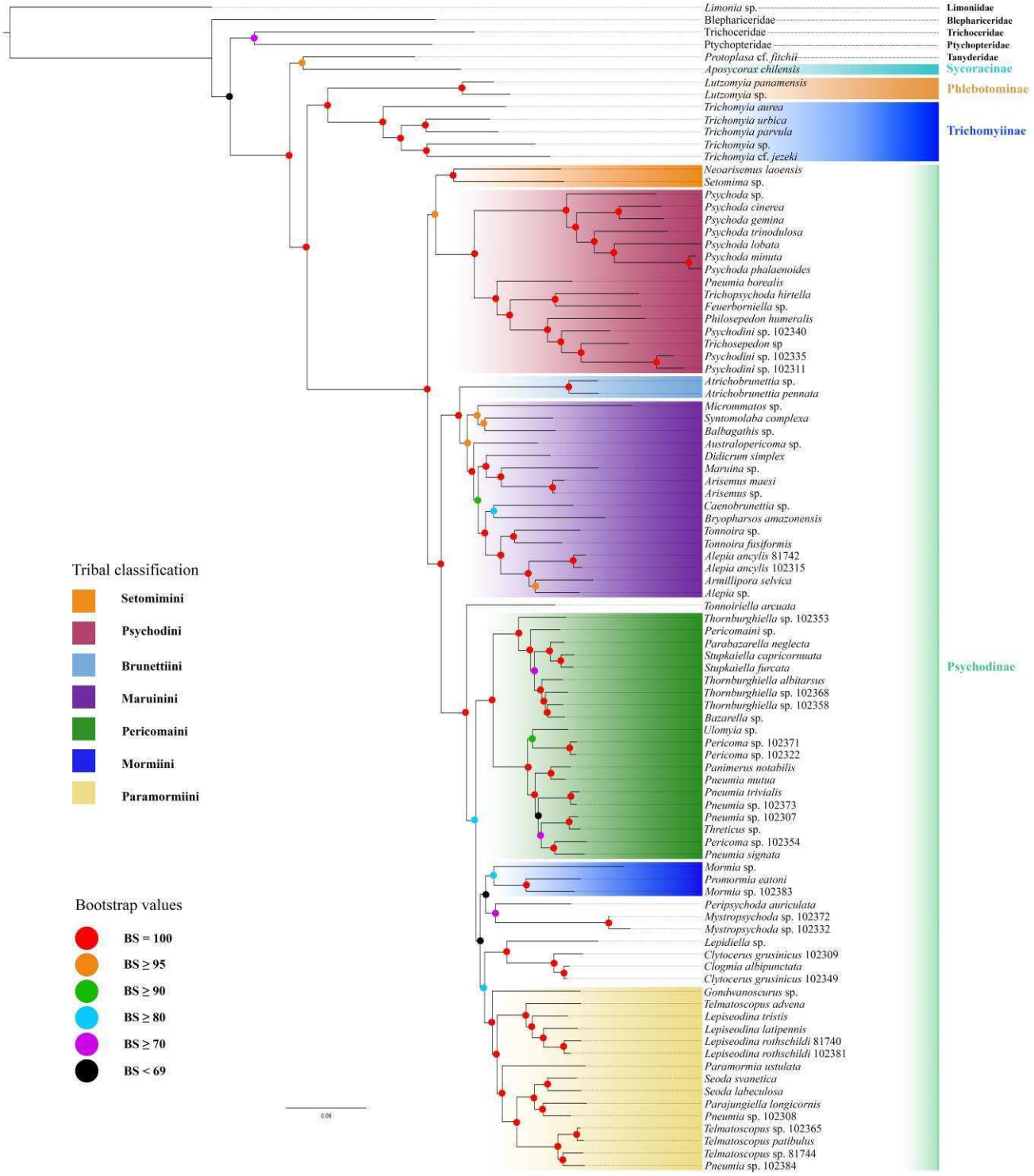

Fig. 1. Phylogeny of Psychodidae inferred in IQ-TREE. Ultrafast bootstrap values are depicted on branches. Node labels with species with the same name, or unidentified species of the same genus ending in sp. are followed by a unique identifier number.

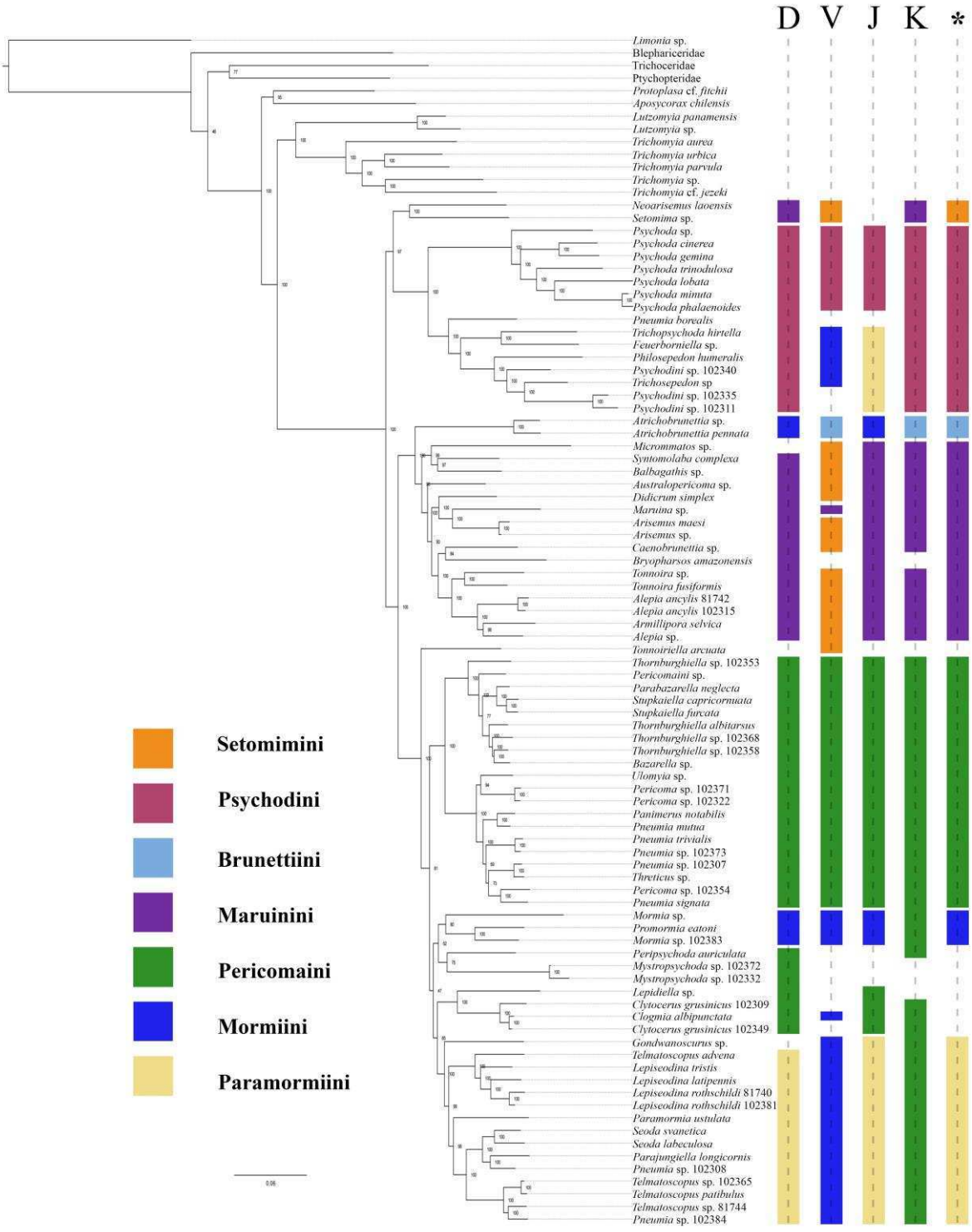

Fig. 2. Comparison of the tribal classification of Psychodidae by different authors and this study. Phylogeny inferred in IQ-TREE. Ultrafast bootstrap values are depicted on branches. Authors following Table 2. Abbreviations: D = Duckhouse; J = Ježek; K = Kvifte; V = Vaillant; * = this study.

Discussion

Subfamilies and outgroups

The placement of Sycoracinae as sister to Tanyderidae renders Psychodidae non-monophyletic. The close relationship between Tanyderidae and Psychodidae lines up with results from several prior studies (e.g., Crampton, 1926; Hennig, 1972; Krzeminski & Krzeminska, 2003; Bertone et al., 2008; Wiegmann et al., 2011, Curler & Moulton, 2012). Specifically, our placement of *Protoplasa* cf. *fitchii* (Tanyderidae) as the sister group of *Aposycorax chilensis* (Psychodidae: Sycoracinae) mirrors the outcomes reported by Curler & Moulton (2012), although with higher support in our analysis (UFB = 100). Both nodes (Tanyderidae + Sycoracinae) are placed as sister to all the remaining Psychodidae (UFB = 100). The non-monophyly of moth flies is a vivid debate (see Bertone et al. (2008) and Curler & Moulton (2012)). However, caution is warranted when interpreting this result, as the relative scarcity of representatives from Tanyderidae and Sycoracinae, coupled with the absence of representatives from the subfamilies Bruchomyiinae and Horaiellinae, might significantly influence the outcomes of further analysis where these groups are included.

Our phylogenetic inference recovers well-supported relationships among the other three studied subfamilies, confirming the monophyletic nature of groups containing multiple representatives, specifically, Trichomyiinae, Psychodinae, and Phlebotominae. In our study, the clade ((Phlebotominae + Trichomyiinae) + Psychodinae) is well-supported (UFB = 100). Notably, our findings differ from the hypothesis proposed by Azar et al. (1999) (also see Curler & Moulton, 2012), who suggested a closer association between Phlebotominae and Psychodinae based on fossil data.

The subfamily Psychodinae was represented in this study by significantly more species than any other subfamily. Psychodinae is the most taxonomically diverse subfamily within the moth flies and was the main goal in our survey, as our main objective was to assess the validity of its putative tribes and their relationships within this subfamily. The phylogenetic placement of Psychodinae (sister to Phlebotominae + Trichomyiinae) and its monophyly are unambiguously supported in our analyses (BS = 100), matching the results of previous molecular studies (Curler & Moulton, 2012; Espíndola et al., 2012; Kvifte, 2018). In addition, our results recovered seven well-supported tribes within psychodines: Setomimini, Psychodini, Brunettiini, Maruinini, Paramormiini, Pericomaini, and Mormiini (Fig. 1, 2).

Tribe Setomimini Vaillant, 1982 stat. rev.

Vaillant (1982) created Setomimini to include three genera (i.e., *Setomima*, *Neoarisemus* and *Alepia*) that did not fit into his concept of Brunettiini. Simultaneously, Vaillant (1982) proposed the tribe Arisemini for the genus *Arisemus*. Later, Vaillant (1986) synonymized Arisemini under Setomimini, a concept followed later by himself (Vaillant, 1990). Building upon this, Duckhouse (1987) included *Maruina* and Vaillant's Setomimini in the tribe Maruinini (see Table 1, 2). The proposal of Duckhouse (1987) to treat Maruinini as a broader assemblage including *Maruina* and Setomimini was later followed by Quate (1996, 1999). Subsequently, Quate & Brown (2004) regarded Maruinini and Setomimini as distinct tribes, mirroring the classification by Vaillant (1990). In their classification, Quate & Brown (2004) left *Maruina* in Maruinini alone and included in their Setomimini 15 Neotropical genera (Quate & Brown, 2004; Tkoč et al., 2017). Their differentiation between these tribes was primarily based on the presence in Maruinini of an aedeagal sheath, as described by Hogue (1973) (absent in Setomimini). Quate & Brown (2004) studied solely representatives from the Neotropical region and acknowledged the lack of strong evidence supporting Setomimini as a monophyletic tribe. Contrary to previous studies and mainly based on wing and antennal characters, Ježek et al. (2011) suggested that Maruinini, including Setomimini, was a polyphyletic assemblage of species belonging to their concept of Pericomaini and Mormiini (see Table 1, 2). Afterwards, other authors continued to treat the combined assemblage of *Maruina* and Setomimini as a single tribe, under the name Maruinini (e.g. Wagner & Andersen, 2007; Kvifte, 2012; Afzan & Belquat, 2016; Kvifte, 2018; Curler, 2020). There is a lack of consensus about the monophyly of the broader assemblage of Maruinini (including Setomimini), and Kvifte (2018) argued that the complexity of the morphological characters on their own may not be sufficient to resolve the phylogenetic relationships of the assemblage.

In our analyses, two Setomimini *sensu* Vaillant (1982, 1990) taxa (*Neoarisemus* and *Setomima*) are resolved together as the sister group of the tribe Psychodini with high support (UFB = 97). The close relationship between *Setomima* and *Neoarisemus* has been already suggested using molecular characters (Curler & Moulton, 2012; Kvifte, 2018). In both studies the cluster (*Setomima* + *Arisemus*) was recovered as sister to *Maruina* (Maruinini). Curler & Moulton (2012) and Kvifte (2018) treated these three genera (*Arisemus*, *Maruina*, and *Setomima*) as part of the broader concept of Maruinini (including Setomimini). Our dataset contains 13 genera classified in Maruinini *sensu* Kvifte (2018) (*Micrommatos*, *Syntomolaba*, *Balbagathis*, *Australopericoma*, *Didicrum*, *Maruina*, *Arisemus*, *Caenobrunettia*, *Bryopharsos*, *Tonnoira*, *Alepia*, and *Armillipora*) and two genera of Setomimini *sensu* Vaillant (1982, 1990)

(*Neoarisemus* and *Setomima*). Our phylogenetic inference, based on a much broader taxon sampling and a larger dataset, recovers two clades: Setomimini as sister to Psychodini (UFB = 97), and Maurinini as sister to Pericomaini (UFB = 100).

Based on our results, our concept of Setomimini **stat. rev.** includes the type genus *Setomima* Enderlein, 1937 and *Neoarisemus* Botosaneanu & Vaillant, 1970. At this point, we cannot make any assumptions on the other genera previously included in Setomimini. The morphological characters available to properly diagnose this tribe are not sufficient and further studies are required to properly define the limits of the tribe Setomimini in its current state.

Tribe Psychodini Newman, 1834

Members of Pscychodini *sensu* Kvifte (2018) have been placed in other tribes, such as Telmatoscopini or Neomaruinini (Vaillant, 1972, 1990; see Table 1, 2), or Paramormiini (subtribes Trichopsychodina and Threticina) (Ježek, 1985; see Table 1, 2). Morphological evidence supports the concept of a monophyletic Psychodini as defined by Quate (1959) and Duckhouse (1985) (see also Ibañez-Bernal (2004) and Kvifte (2015, 2018)). In recent molecular studies based on mitochondrial DNA sequence data (Curler & Moulton, 2012) and nuclear DNA sequence data (Espíndola et al., 2012), the tribe Psychodini was resolved as non-monophyletic. However, Kvifte (2018), using both nuclear and mitochondrial data, resolved Psychodini as a monophyletic group with strong support.

Our phylogenetic inference recovers a monophyletic Psychodini with high support (UFB = 100), except for the placement of *Pneumia* cf. *borealis* (member of the tribe Pericomaini) within the Psychodini clade. After a meticulous morphological re-examination of the specimen, we think that the recovery of *Pneumia* cf. *borealis* within Psychodini has likely a bioinformatics explanation. Further analysis of this problem is ongoing.

The monophyly of the tribe is consistent with the phylogenetic hypothesis by Kvifte (2018). Within our Psychodini clade, all the species of the genus *Psychoda* [*P.* sp 1, *P. cinerea* Banks, 1894, *P. gemina* (Eaton, 1904), *P. trinodulosa* Tonnoir, 1922, *P. lobata* Tonnoir, 1940, *P. minuta* Banks, 1894, and, *P. phalaenoides* (Linnaeus, 1758)] form a well-supported clade (UFB = 100). Our results suggest that the genus *Psychoda* constitutes a monophyletic lineage, contrasting with previous molecular studies. Nevertheless, this worldwide distributed genus has a remarkably morphological variability and diversity. The study of more and distinct *Psychoda* species are crucial to understand the relationships within the genus.

Likewise, the remaining representatives of Psychodini (*Philosepedon, Trichopsychoda*, *Trichosepedon,* and two taxa belonging to undescribed genera) are resolved in another well-supported clade (UFB = 100). The genera *Philosepedon, Trichopsychoda* and *Trichosepedon* have been considered part of other tribes, for example members of the group with Y-shaped ascoids of Mormiini (Vaillant, 1990), or members of the subtribe Trichopsychodina within Paramormiini (Ježek et al., 2011) (see Table 1, 2). However, our phylogenetic inference supports their placement within Psychodini, in agreement with the tribal classification by Duckhouse (1987), Quate (1996, 1999), and Kvifte (2018). The morphology of the studied specimens of the undescribed genera supports their inclusion in Psychodini, but they require further study to understand their relationships with other genera within the tribe.

Based on our analysis and further study, the tribe Psychodini is defined the following morphological diagnostic characters: 1) palpal segment 4 sclerotized; 2) antenna with basal flagellomeres nodiform; 3) antenna with distal flagellomeres fusiform and diminutive; 4) flagellomeres with ascoids digitiform or foliform, with one to three anterior branches and one posterior branch; 5) ejaculatory apodeme laterally compressed, narrow in dorsal view; 6) epandrium with one aperture; 7) cercus reduced, vestigial or present as lateral lobes of proctiger; 8) epandrial appendages (surstyli) well developed with apical tenacula (Quate, 1959; Duckhouse, 1987; Kvifte, 2015, 2018). Psychodini includes the type genus *Psychoda* Latreille, 1797*,* and the genera *Cookiellocapsa* Ježek & Le Pont, 2016; *Epacretron* Quate, 1965; *Eurygarka* Quate, 1959; *Feuerborniella* Vaillant, 1971; *Mucomyia* Kvifte & Curler, 2018; *Neomaruina* Vaillant, 1963; *Nielseniella* Vaillant, 1971; *Perithreticus* Vaillant, 1973; *Philosepedon* Eaton, 1904; *Quatiella* Botosaneanu & Vaillant, 1970; *Rhipidopsychoda* Vaillant, 1991; *Soeliella* Kvifte, 2015; *Threticus* Eaton, 1904; and *Trichopsychoda* Tonnoir, 1922.

Tribe Brunettiini Vaillant, 1971

Vaillant (1971) created Brunettiini to group the genera *Brunettia* Annandale, 1910 and *Neoarisemus* Botosaneanu & Vaillant, 1970. Later, Vaillant (1982) redefined this tribe using morphological characters and included *Brunettia*, *Atrichobrunettia*, *Gerobrunettia* Quate & Quate, 1967 and *Parasetomima* Duckhouse, 1968 (also followed by Vaillant, 1986); although, *Parasetomima* was excluded from Brunettini later (Vaillant, 1990). Members of Brunettiini *sensu* Vaillant (1990) and *sensu* Kvifte (2018) have been assigned to Mormiini in the past (Quate, 1999; Ježek, 1984; Duckhouse, 1985, 1987).

Curler & Moulton (2012) recovered *Brunettia* (represented in their analysis by a single specimen identified as *Brunettia* sp. as sister to *Maruina* in their Maximum Likelihood analysis, or as sister to *Mystropsychoda* and *Neotelmatoscopus* Tonnoir, 1933 in their Bayesian inference (both placements with relatively low support; ML = 49, Bayseian = 0.75). Espíndola *et al*. (2012) also included a single individual of the Bruneittiini (identified as *Atrichobrunettia graeca* Ježek & Goutner, 1993) in their phylogenetic studies, which was resolved embedded within the Psychodini. Later, Kvifte (2018) recovered an unidentified Brunettiini sister to *Mystropsychoda* and *Neotelmatoscopus* virtually without support (Bayesian Posterior Probability = 0.55).

Our analysis places the two studied specimens of *Atrichobrunettia* as sister to Maurinini with high support (UFB = 100); a similar position that the one presented by Curler & Moulton (2012). Nevertheless, our dataset includes a larger number of Maruinini members *sensu* Kvifte (2018), i.e., 13 genera namely *Micrommatos*, *Syntomoloba*, *Balbagathis*, *Australopericoma*, *Didicrum*, *Maruina*, *Arisemus*, *Caenobrunettia*, *Bryopharsos*, *Tonnoira*, *Alepia*, and *Armillipora*. Based on our phylogenetic inference we consider Brunettiini a well-supported tribe (UFB = 100), in agreement with the tribal concept of Vaillant (1990), followed by Kvifte (2018). The inclusion of members of *Brunettia* Annandale and *Gerobrunettia* Quate & Quate is desirable to understand the relationships within Brunettiini.

Based on our results and further study, the tribe Brunettiini is defined by the following morphological diagnostic characters: 1) palpal segment 4 sclerotized or corrugated; 2) antenna with distal 1–3 flagellomeres diminutive and fusiform; 3) antenna with flagellomeres nodiform; 4) flagellomeres with ascoids variable but never Y-shaped; 5) ejaculatory apodeme laterally compressed, narrow in dorsal view; 6) parameres paired and triangular; 7) gonostyli distally with spiniform setae; 8) epandrial appendages (surstyli) with tenacula distally expanded, polyseriate, and occasionally in two separated patches (Vaillant, 1990; Kvifte, 2018). Brunettini includes the type genus *Brunettia* Annandale, 1910 and the genera *Atrichobrunettia* Satchell, 1953, and *Gerobrunettia* Quate & Quate, 1967.

Tribe Maruinini Enderlein, 1937 stat. rev.

Maruinini was originally placed within the subfamily Phlebotominae based on wing venation (Enderlein, 1937). Species within Maruinini *sensu* Kvifte (2018) have been placed in separate tribes, such as Arisemini (Vaillant, 1971; later synonymyzed under Setomimini by

Vaillant (1986)), Pericomaini and Mormiini (Ježek et al., 2011). Some authors consider the genus *Maruina* as the only representative of Maruinini (Quate, 1996, 1999; Quate & Brown, 2004; Duckhouse 1985, 1987; Vaillant, 1990; and Ježek, 1985). Others treat it in a broader concept of Maruinini (including Setomimini) (Duckhouse, 1978; Quate & Brown, 2004; Kvifte, 2018;). However, the monophyly of this broader concept of Maurinini has been questioned (see discussion under tribe Setomimini).

Our phylogenetic inference recovers a monophyletic Maruinini with high support (UFB = 98) as sister to Brunetiini (UFB = 100). Curler & Moulton (2012) included single specimen identified as *Maruina lanceolata* (Kincaid, 1899) in their analysis and recovered it as sister to *Brunettia* in their Maximum Likelihood analysis, or as sister to *Mystropsychoda* and *Neotelmatoscopus* in their Bayesian inference (both placements with relatively low support, ML = 49; Bayesian = 0.75). Later, Kvifte (2018) recovered an unidentified *Maruina* as sister to *Neoarisemus* and *Setomima* with high support (Bayesian Posterior probability = 0.91).

Within our Maruinini taxa, the genus *Armillipora* renders *Alepia* paraphyletic. Quate & Brown (2004) stated that *Alepia* is morphologically highly variable and likely paraphyletic. On the other hand, Ježek et al. (2020) and Jaume-Schinkel & Mengual (2024) have discussed that species of *Armillipora* and *Alepia* are morphologically very similar.

The genus *Bryopharsos* was treated by Quate (1996, 1999) as a member of his Mormiini, but Kvifte (2018) considered it as unplaced within his tribal classification. Our inference recovers this taxon embedded within Maruinini. This is the first time that a tribal classification is inferred for this genus based on molecular characters. It is desirable to further study the morphological characters to understand how this genus relates to others included within Maruinini.

Our results align in part with the Maruinini concept by Kvifte (2018), although without the inclusion of Setomimini. Further examination of the morphological characters traditionally used to define both tribes, Setomimini and Maruinini, is necessary, particularly to clarify the morphological boundaries of the included genera. Nevertheless, our findings provide support for classifying the tribe Maruinini as a distinct lineage within Psychodinae.

Based on our results and further study, we diagnose the tribe Maruinini with the following morphological characters: 1) antenna with flagellomeres primitively fusiform, if flask-shape, flagellomere 1 retains its fusiform shape; 2) antenna with apical flagellomeres reduced, without ascoids; 3) flagellomeres with ascoids with two to many branches; 4) wing

vein R_{2+3} arising from R_4 beyond the apex of basal radial cell (R_4 pectinate); 5) wing vein R fork generally before M fork, and both basal of wing centre; 6) aedeagal complex asymmetrical; 7) ejaculatory apodeme broad on ventral view (Duckhouse, 1990; Kvifte, 2018). Maruinini **stat. rev.** includes the type genus *Maruina* Muller, 1895, and the genera *Alepia Enderlein, 1937*; *Alloeodidicrum* Duckhouse, 1990; *Ancyroaspis* Duckhouse, 1990; *Armillipora* Quate, 1996; *Arisemus* Satchell, 1955; *Australopericoma* Vaillant, 1975; *Balbagathis* Quate, 1996; *Caenobrunettia* Wagner, 1981; *Desmioza* Enderlein, 1937; *Dictyocampsa* Enderlein, 1937; *Didicrum* Enderlein, 1937; *Didimioza* Quate & Brown, 2004; *Eremolobulosa* Dcukhouse, 1990; *Lobulosa* Szabó, 1960; *Micrommatos* Quate & Brown, 2004; *Myiomystax* Curler, 2020; *Nemoneura* Tonnoir, 1929; *Neurosystasis* Satchell, 1955; *Platyplastinx* Enderlein, 1937; *Polletomyia* Curler, 2020; *Rotundopteryx* Duckhouse, 1990; *Satchellomyia* Duckhouse, 1990; *Synmormia* Enderlein, 1937; *Syntomolaba* Enderlein, 1937; *Thrysocanthus* Enderlein, 1937; *Tonnoira* Enderlein, 1937; and *Valerianna* Quate & Brown, 2004.

Placement of *Tonnoiriella* Vaillant, 1971

The genus *Tonnoiriella* currently contains 23 extant species, 18 from the Palaearctic and 5 from the Afrotropical Region (Wagner & Withers, 2020). This genus has been placed in separate tribes such as Pericomaini (Vaillant, 1971), Setomimini (Vaillant, 1982, 1990) or Maruinini (Duckhouse, 1985, 1987). Wagner & Withers (2020) revised the genus and discussed its tribal position. They suggested that additional morphological and genetic data are necessary to elucidate the phylogenetic relationships. Moreover, Kvifte (2018) treated *Tonnoiriella* as unplaced in his tribal classification. Nonetheless, it has been suggested that the genus *Tonnoiriella* may warrant a placement within or basal to Pericomaini *sensu* Kvifte (2018) (Jaume-Schinkel & Kvifte, in press; refer to chapter 15 and appendix 13).

Our analysis recovered *Tonnoiriella* as a monotypic lineage with high support (UFB = 100), as sister to Pericomaini + Paramormiini. Consequently, our findings are consistent with the proposed tribal placement discussed in Jaume-Schinkel & Kvifte (in press). Jaume-Schinkel & Kvifte (in press) discussed based on morphology (in particular the narrow eyebridge, the fusiform flagellomeres, the male genitalia with a single paramere, and the Y-shaped linkage of the aedeagal-parameral complex with the subepandrial sclerite) that *Tonnoiriella* is likely to be situated within or basal to Pericomaini. It is likely that *Tonnoiriella* warrants recognition as a distinct tribe.

Tribe Pericomaini Enderlein, 1935 stat. rev.

Species of Pericomaini have been placed in different tribes, such as Ulomyiini (Enderlein, 1935, 1937), Pericomaini (Kvifte, 2018; Ježek 1985; Duckhouse, 1985, 1987; Vaillant, 1990), and Telmatoscopini (Vaillant, 1971) (Table 1, 2). Morphological evidence supports the monophyletic concept of Pericomaini (Vaillant, 1990; Duckhouse, 1985, 1987; Ježek, 1985), although with minor differences with the genera included in this tribe and the diagnostic morphological characters. Recent molecular studies based on mitochondrial DNA sequence data (Curler & Moulton, 2012) recovered Pericomaini as a clade with good support (Bayesian = 1; ML = 78). On the contrary, studies based on nuclear DNA sequence data, recovered Pericomaini as non-monophyletic (Espíndola et al., 2012). Kvifte (2018) analysed nuclear and mitochondrial data and resolved Pericomaini (*sensu* Kvifte (2018)) as a clade with high support (Bayesian Posterior probability = 0.99). Nonetheless, Pericomaini *sensu* Kvifte (2018) remains a very heterogeneous group without clear diagnostic characters.

Our phylogenetic inference recovers a monophyletic Pericomaini with high support (UFB = 100), except for the placement within the Pericomaini clade of *Threticus* sp. (member of the Psychodini) and *Panimerus notabilis* (Eaton, 1893) (member of the Paramormiini). *Threticus* has been placed in distinct tribes such as Psychodini (Quate, 1959; Duckhouse, 1985; Cordeiro et al., 2014; Kvifte, 2018), Mormiini (Vaillant, 1990), and Paramormiini (Ježek, 1985). Likewise, *Panimerus* has been treated as part of Mormiini (Vaillant, 1990), Paramormiini (Ježek, 1984; Duckhouse 1985, 1987) or Pericomaini (Kvifte, 2018). After a meticulous morphological re-examination of both specimens, we think that the recovery of *Threticus* sp. and *Panimerus notabilis* within Pericomaini has likely a bioinformatics explanation. Further analysis of this problem is ongoing.

Within our Pericomaini taxa the genera *Bazarella*, *Parabazarella*, *Stupkaiella*, and a specimen from an undetermined genus (Pericomaini sp-03) render the genus *Thornburghiella* as non-monophyletic. Several authors have treated *Stupkaiella* as a distinct genus (Vaillant, 1973, 1982; Wagner, 1984; Ježek, 1992, 2001; Curler & Moulton, 2010; Oboňa et al., 2017), while Duckhouse (1987) treated it as a subgenus of *Thornburghiella*. Ježek (2001) discussed the close affinity of the genera *Bazarella*, *Thornburghiella*, *Stupkaiella*, *Tokunagaiella* Vaillant, 1983, and *Joostiella* Vaillant, 1983 (although the latter two are not included in our analysis). Morphological evidence (adult and larval characters) have suggested that the genus *Stupkaiella* is distinct from closely related genera (such as *Bazarella* and *Thornburghiella*) (Curler & Moulton, 2010; Oboňa et al., 2017; Ježek, 2001). However, Curler & Moulton (2010)

discussed that reliable identification of larvae in these Pericomaini genera requires the rearing the adults, comparing DNA, and exhaustive comparison of morphological characters due to remarkable larval uniformity. Likewise, the genus *Parabazarella* Vaillant, 1983 was originally described as a subgenus of *Bazarella* (Vaillant, 1961, 1983). Ježek (2001) gave *Parabazarella* a genus status, suggesting that both *Bazarella* and *Parabazarella* are sister groups (based on morphological characters) and that both are closely related to *Thornburghiella*, *Stupkaiella*, *Joostiella*, and *Tokunagaiella*. Further evidence is required to properly define these genera.

Our phylogenetic inference recovers the genus *Pneumia* as non-monophyletic. This result is similar to the one found by Espíndola et al (2012) where the genera *Pneumia*, *Pericoma*, and *Ulomyia* were resolved as non-monophyletic.

Our recovery is consistent with the tribal classification of Duckhouse (1985, 1987), Vaillant (1990), Ježek (1985) and Quate (1996, 1999). Based on our results, our concept of Pericomaini **stat. rev.** has the following morphological diagnostic characters: 1) antenna with flagellomeres fusiform; 2) flagellomeres with ascoids short, usually digitiform; 3) interocullar suture present; 4) gonocoxites close to each other at base; 5) wing vein R and M forks almost at the same level, or R fork beyond M fork; 6) aedeagal complex usually symmetrical; 7) epandrial appendages (surstyli) with one to multiple apical tenacula. Pericomaini includes the type genus *Pericoma* Haliday, 1856 and the genera *Bazarella* Vaillant, 1961; *Berdeniella* Vaillant, 1975; *Clytocerus* Eaton, 1904; *Eugenys* Quate, 1996; *Joostiella* Vaillant, 1983; *Pneumia* Enderlein, 1937; *Saraiella* Vaillant, 1981; *Stupkaiella* Vaillant, 1973; *Szaboiella* Vaillant, 1979; *Thornburghiella* Vaillant, 1982; *Tokunagaiella* Vaillant, 1983; and *Ulomyia* Walker, 1856.

Tribe Mormiini Enderlein, 1937 stat. rev.

Members of Mormiini *sensu* Ježek (1984) have been placed in different tribes, such as Telmatoscopini or Mormiini (Vaillant, 1971, 1990), Mormiini (Duckhouse, 1978, 1985; Vaillant, 1974; Ježek & Goutner, 1993), Paramormiini (Ježek, 1985), and Pericomaini (Kvifte, 2018) (see Table 1, 2). Different morphological characters have been used to define the tribe and different genera have been grouped within Mormiini. No general consensus on what constitutes Mormiini has been achieved in the past.

Curler & Moulton (2012) recovered two species of Mormiini (identified as *Mormia* sp. nov. 1, and *Mormia* sp. nov. 2) as a sister of *Clytocerus americanus* (Kincaid, 1901) in their

Maximum Likelihood analysis (with relatively low support, 66) and Bayesian Inference (with high support, 0.98). Espíndola et al. (2012) included a single specimen of Mormiini (identified as *Oomormia andrenipes* (Strobl, 1910)) in their phylogenetic studies, which was resolved as sister to the Paramormiini genera *Telmatoscopus*, *Parajungiella*, *Jungiella* Vaillant, 1972 and *Panimerus*, virtually without support (ML = 52). Later, Kvifte (2018) recovered an unidentified Mormiini as sister to *Clytocerus* with high support (Bayesian Posterior probability = 0.99).

Our analyses recovered Mormiini (*Mormia* sp., *Mormia (Promormia) eatoni* and one undetermined genus) with moderate support (UFB = 80). Our Mormiini was recovered as sister to *Peripsychoda* and *Mystropsychoda*, although with low support (UFB =52). In comparison to the previous molecular inferences, our phylogenetic inference did not recover Mormiini as sister to *Clytocerus* nor to Paramormiini.

Based on our phylogenetic inference and further study, our tribe Mormiini **stat. rev.** is defined with the following morphological diagnostic characters: 1) antenna with flagellomeres nodiform; 2) flagellomeres with multiple-branched ascoids; 3) anterior additional sclerite of the pteropleurite developed; 4) pteropleurite with a break; 5) wing vein R_4 connected with R_{2+3} which is conspicuously prolonged basally; 6) wing vein Sc long, thin and straight. Mormiini includes the type genus *Mormia* Enderlein, 1935, and the genera *Hemimormia* Krek, 1971; *Lepimormia* Enderlein, 1937; *Limomormia* Vaillant, 1983; *Oomormia* Ježek, 1984; *Promormia* Ježek, 1983; *Psychomormia* Ježek, 1983; *Jovamormia* Ježek, 1983; *Katamormia* Ježek, 1984; *Rhadinomormia* Vaillant, 1975; *Taramormia* Ježek, 1984; *Telomormia* Ježek, 1984; *Yomormia* Ježek, 1984; and an undescribed genus.

Placement of *Peripsychoda* and *Mystropsychoda*

The genus *Peripsychoda* has been placed in Mormiini (Vaillant, 1990), Paramormiini (Ježek, 1984; Duckhouse, 1985, 1987), or Pericomaini (Kvifte, 2018) based on morphological characters. Likewise, *Mystropsychoda* has been placed in Psychodini (Duckhouse, 1985, 1987) or left unplaced (Kvifte, 2018). In previous molecular studies, the genus *Mystropsychoda* was resolved as sister to *Neotelmatoscopus* (Curler & Moulton, 2012; Kvifte, 2018), or both genera (*Mystropsychoda* + *Neotelmatoscopus*) were recovered as sister to Brunettiini (Curler & Moulton, 2012; Kvifte, 2018).

Our phylogenetic inference recovers *Mystropsychoda* and *Peripsychoda* together as sister to Mormiini, albeit with low support (UFB = 52). This finding is consistent with Vaillant's

(1990) classification (see Table 1, 2). While both genera may potentially be included within Mormiini in the future, additional evidence is needed to confirm their placement.

Placement of *Lepidiella*, *Clogmia*, and *Clytocerus*

Our analysis recovers *Lepidiella*, *Clogmia*, and *Clytocerus* together as sister to Paramormiini with moderate support (UFB = 85). Quate (1996, 1999) treated *Lepidiella* as part of Pericomaini. Kvifte (2018) treated the genus as unplaced in his tribal classification. Jaume-Schinkel & Kvifte (2022) suggested that *Lepidiella* represents either a plesiomorphic sister group to *Clytocerus* or even the paraphyletic ancestral taxon to it. Our recovery of *Lepidiella* as sister to *Clytocerus* aligns with the hypothesis by Jaume-Schinkel & Kvifte (2022) where *Lepidiella* is closely related to *Clytocerus*.

The genus *Clogmia* has been placed in different tribes, such as Mormiini (Vaillant, 1990), Paramormiini (Duckhouse, 1985, 1987), and Pericomaini (Kvifte, 2018). In recent phylogenetic analysis, *Clogmia* has been placed as sister to *Lepiseodina* (Curler & Moulton, 2012), as sister to *Paramormia* (Espíndola et al., 2012) or as sister to *Panimerus* and *Paramormia* (Kvifte, 2018). Our placement of *Clogmia* embedded within two specimens of *Clytocerus grusinicus* has likely a bioinformatics explanation. Further analysis of this problem is ongoing.

Tribe Paramormiini Enderlein, 1937 stat. rev.

Members of Paramormiini have been placed in different tribes, such as Mormiini (Vaillant, 1990), Paramormiini (Duckhouse, 1985, 1987; Ježek, 1985), or Pericomaini (Kvifte, 2018). In recent molecular phylogenies based on mitochondrial DNA sequence data (Curler & Moulton, 2012) studied genera of Paramormiini were recovered as a clade with good support in their Bayesian inference (Bayesian = 1), but with relatively low support in their maximum likelihood analysis (ML = 54); . Furthermore, Curler & Moulton (2012) argued that the tribes Mormiini *sensu* Vaillant (1990) and Paramormiini *sensu* Ježek (1985) are probably non-monophyletic groups.

Our analysis recovered Paramormiini as a well-supported clade (UFB = 100). Our results are consistent with the tribal classification of Duckhouse (1985, 1987) and Quate (1996, 1999). Our inference recovers the genus *Gondwanoscurus* as sister to the remaining

Paramormiini, matching the outcome by Curler & Moulton (2012). Moreover, our analysis recovers *Telmatoscopus* in three different placements, thus rendering *Telmatoscopus* as a non-monophyletic genus as currently defined. Curler & Moulton (2012) recovered (*Clytocerus* + *Mormia*) as sister to the Paramormiini taxa included in their analysis. This result aligns with our recovery of ((*Clytocerus* + *Clogmia*) + *Lepidiella*) as sister to the Paramormiini (also see discussion above)

Based on our analysis and further study, Paramormiini **stat. rev.** is defined with the following morphological diagnostic characters: 1) antenna with flagellomeres flask-shaped; 2) antenna with the first flagellomere not fusiform; 3) antenna with the last three flagellomeres sometimes smaller and showing reduction of necks, but never subspherical and diminutive; 4) wings held horizontally; 5) wing membrane naked; 6) wing vein origin of R_{2+3} at or before the apex of basal radial cell; 7) wing vein Sc sometimes long, but never especially short; 8) aedeagus not forming the ball-and-socket (primitively linked to it by a V-shaped furca; 9) epandrial appendages (surstyli) with more than one tenaculum present (Duckhouse, 1987). Our concept of Paramormiini includes the type genus *Paramormia* Enderlein, 1937 and the genera *Crenopanimerus* Vaillant, 1983; *Elsahowia* Duckhouse, 1978; *Eutelmatoscopus* Satchell, 1953; *Gondwanoscurus* Ježek, 2002; *Iranotelmatoscopus* Ježek, 1987; *Jezekiella* Wagner & Kvifte, 2015; *Jungiella* Vaillant, 1972; *Lepiseodina* Enderlein, 1937; *Moruseodina* Bravo & Cordeiro, 2014; *Nototelmatoscopus* Satchell, 1953; *Panimerus* Eaton, 1913; *Parajungiella* Vaillant, 1972; *Perakomyia* Ježek, 2010; *Phyllotelmatoscopus* Vaillant, 1983; *Psychomasina* Ježek, 2004; *Rhadinoscopus* Quate & Quate, 1067; *Satoba* Ježek, 1984; *Seoda* Enderlein, 1935; *Telmatoscopus* Eaton, 1904; and *Vaillantodes* Wagner, 2001.

Unplaced genera

The following genera are not included within our revised tribal classification *Abcharis* Tkoč & Ježek, 2013; *Breviscapus* Quate, 1955; *Chirolepia* Enderlein, 1937; *Hyrcanoressliella* Ježek, 1997; *Mormopericomiella* Ježek & van Harten, 2002; *Neoquatiella* Vaillant, 1973; *Neothreticus* Vaillant, 1973; *Parasetomima* Duckhouse, 1968; *Potophila* Kvifte, 2014; *Saximormia* Ježek, 1984; and *Vagmania* Krek, 1972.

Conclusions

In this study we have designed 18651 hybrid-capture baits to target 1445 coding regions belonging to 1161 ortholog groups. We were able to effectively capture targeted loci for 82 moth fly species from 46 genera, representing over 40% of the total Psychodidae genera.

Psychodidae is recovered as non-monophyletic and our inferred phylograms agree with previous morphological classifications suggested for Psychodinae. Our results allowed us to revise the status of the tribes included in this subfamily and present a subdivision of Psychodinae into seven tribes: Brunettiini Vaillant, 1971, Maruinini Enderlein, 1937 **stat. rev.**, Mormiini Enderlein, 1937 **stat. rev.**, Paramormiini Enderlein, 1937 **stat. rev.**, Pericomaini Enderlein, 1935 **stat. rev.**, Psychodini Newman, 1834, and Setomimini Vaillant, 1982 **stat. rev.** Nevertheless, further analyses are required to properly assess the relationships and tribal classification of the following genera: *Clogmia*, *Clytocerus*, *Lepidiella*, *Mystropsychoda*, *Peripsychoda*, and *Tonnoiriella*. Moreover, further morphological evidence is desirable to diagnose the tribe Setomimini **stat. rev.**

The application of the exon-capture method, assisted by BAITFISHER, proved highly successful in capturing all targeted loci and accurately inferring well-supported and highly congruent phylogenies within Psychodidae. Besides Psychodidae, using our bait kit led to the successful capture and sequencing of several CDS regions within the infraorders Tipulomorpha [including Limoniidae and Trichoceridae], Psychodomorpha [Ptychopteridae, Blephariceridae, and Tanyderidae], and Culicomorpha [Culicidae and Chironomidae (although Chironomidae was not included in our analysis)]. This versatility underscores the balance our kit strikes between precision and broader applicability, as it effectively targets CDS regions across diverse Diptera families.

Moreover, our kit can be used to infer strong phylogenies within medically significant groups like biting midges (Ceratopogonidae, Corethrellidae), black flies (Simuliidae), and mosquitoes (Culicidae). By facilitating robust phylogenetic analyses within these groups, our bait kit not only enhances our understanding of their evolutionary histories but also holds immense promise for applications in disease vector control and management strategies. Moreover, its ability to enable phylogenetic analyses across various "Nematocera" taxa highlights its potential to contribute significantly to broader ecological and evolutionary studies. Our bait kit represents a pivotal tool for advancing research in phylogenomics within Diptera and holds particular significance for “Nematocera” and medically important groups within. Leveraging this sequence-capture method promises to catalyze future in-depth

phylogenetic analyses across various Diptera lineages, which are pivotal for unravelling the evolutionary dynamics within this group.

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Tables.

Table 1 Summary of the tribal classification of Psychodinae. Compiled from Duckhouse (1987), Enderlein (1935; 1937), Ježek (1984; 1985; 1990; Ježek & Goutner, 1993; Ježek & van Harten, 2005; Ježek et al., 2011), Kvifte (2018), Quate (1996; 1999), Quate & Brown (2004), and Vaillant (1990).

	Author						
Tribe name	Duckhouse 1987	Enderlein 1935-1937	Ježek 1984-2011	Kvifte 2018	Quate 1996-1999	Quate & Brown 2004	Vaillant 1990
Brunetiini Vaillant, 1971	Not recognized, considered synonym of Mormiini	Not recognized	Recognized as subtribe of Mormiini	Recognized	Not treated	Not treated	Recognized
Maruinini Enderlein, 1937	Recognized	Considered Maruinini as part of Phlebotominae. Treated only in the identification Key.	Not recognized	Recognized	Recognized	Recognized, restricted to *Maruina* Müller, 1895	Recognized, restricted to *Maruina* Müller, 1895
Mormiini Enderlein, 1937	Recognized	Recognized with two subtribes: Mormiina and Paramormiina	Recognized, comprising *Mormia* and Vaillant (1990) Brunettiini	Not recognized, considered part of Pericomaini	Recognized	Not treated	Recognized
Neomaruinini Vaillant, 1990	Not treated	Not recognized	Not recognized	Not recognized, considered part of Psychodini	Not treated	Not treated	Recognized
Paramormiini Enderlein, 1937	Recognized	Recognized as a subtribe	Two subtribes Paramormiina and Trichopsychodina. Equivalent to Vaillant (1990) Mormiini, with the exclusion of *Mormia*	Not recognized, considered part of Pericomaini	Recognized	Not treated	Not recognized

Pericomaini Enderlein, 1935	Recognized	Recognized in 1935, not treated	Recognized	Recognized	Recognized	Not treated	Recognized
Psychodini Newman, 1834	Recognized	Recognized with two subtribes: Psychodina and Clytocerina	Recognized. Restricted to *Psychoda* Latreille, 1797	Recognized	Recognized	Not treated	Recognized
Setomimini Vaillant, 1982	Not recognized, considered synonym of Maruinini	Not recognized	Not recognized, considered part of Pericomaini and Mormiini	Not recognized, considered part of Maruinini	Considered part of Maruinini	Recognized	Recognized
Telmatoscopini Vaillant, 1971	Considered synonym of Paramormiini	Not recognized	Not recognized	Considered part of Pericomaini	Not treated	Not treated	Considered a synonym of Mormiini
Ulomyiini Enderlein, 1935	Not treated	Recognized in 1935, not treated in 1937	Not treated	Considered part of Pericomaini	Not treated	Not treated	Not treated

Table 2 Historic tribal classification of Psychodinae. Authors are presented alphabetically and their works arranged chronologically. Compiled from Duckhouse (1985; 1987; 1990), Enderlein (1935; 1937), Ježek (1984; 1985; 1990; Ježek & Goutner, 1993; Ježek & van Harten, 2005; Ježek et al., 2011), Kvifte (2018), Quate (1969; 1996; 1999; Quate & Brown, 2004), and Vaillant (1971; 1982; 1986; 1990).

Author	Year	Tribe	Characters	Included Genera	Comments
Duckhouse	1985	Psychodini	1) Antenna with flagellar segments flask-shaped, except for terminal tree which are reduced. 2) Ascoids Y-shaped, or derived by loss of one branch, of development of a third. 3) Wing pointed and R5 ending at apex. 4) Sc short and thick. 5) wings held folded in tectiform position. 6) Thorax with stigma anteriorly on mesopleuron. 7) Erect hair present on R1. 8) ninth tergite of male with single pseudospiracular opening. 9) Labellum flattened with terminal fringe of sensory rods (teeth). 10) Larval antenna with two mushroon-shaped elements flanking sensory rod, or secondarily reduced from this condition.	*Epacretron* Quate, 1955; *Feuerborniella* Vaillant, 1971; *Neomaruina* Vaillant, 1963; *Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797; Threticus, Eaton, 1904; *Trichopsychoda* Tonnoir, 1922.	He follows Psychodini as stablished by Quate (1959). Of the apomorphies he enlisted, 4-8 are newly recognised. 3 and 8 occur sporadically in Telmatoscopini, the rest are absent from Telmatoscopini. *Neomaruina* is added to Psychodini because it presents characters 1-9.
	1987	Pericomaini	1) flagellomeres barrel-shaped. 2) ascoids small, paired and digitate. 3) males do not form a "ball-and-socket" articulation (as in Maruinini). 4) R fork usually at almost same level as M fork, or beyond. 5) stem of R fork usually arising at, or befor apex of basal radial cell.	*Notiocharis* Eaton, 1913 [now *Abcharis* Tkoc & Ježek, 2013]; *Pericoma* Haliday, 1856; *Pneumia* Enderlein, 1935; *Ulomyia* Haliday, 1856.	

		Maruinini	1) flagellomeres primitively barrel-shaped and narrower than scape and pedicel, or if flask-shaped, flagellomere 1 usually retains the barrel shape. 2) Ascoids frequently larger than in Pericomini, or branched. 3) males have the ball-and-socket articularion. 4) R fork generally before M fork, and both placed basally of wing center. 5)stem of R fork is several times as long as R2+3.	*Neoarisemus* Botosaneanu & Vaillant, 1970; *Setomima* Enderlein, 1937; *Tonnoiriella* Vaillant, 1982. And other genera not mentioned	The ball-and-socket refers to the gonocoxal apodemes, which are large and have a median dorsal suture (that looks like a ball in dorsal view). The gonocoxal apodemes fit into a concavity on the underside of the aedeagal apodeme, giving it the "ball-and-socket". He states that *Setomima*, *Alepia*, *Neoarisemus*, and *Paratelmatoscopus* form a "taxonomic, but not necessarily genealogical soubgroup of Maruinini. 1) several to many tenacula. 2) hair on ventral side of epandrium. 3) distinc articulation between epandrium and hypandrium. 4) a direct connection between anterior end of ventral epandrial plate and the gonocoxal apodemes. 5) vestiture on the wing membrae. 6) pedicel with internal sclerotised collar. Furthermore, he referes to Duckhouse (1978) saying that *Seotmima* is linked with *Gerobrunettia* by several apomorphisms.
		Mormiini		*Atrichobrunettia* Satchell, 1953; *Brunettia* Annandale, 1910; *Gerobrunettia* Quate & Quate, 1967; *Mormia* Enderlein, 1937.	Closely related to Maruinini, but have lost the "ball-and-socket" linkage, and there is not a furca. He synonymyzes Vaillant (1975) Brunettini under Mormiini Enderlein, 1937.

		Paramormiini	1) flagellomeres flask-shaped. 2) first flagellomere not fusiform. 3) last three flagellomeres sometimes smaller and showing reduction of necks, but never subspherical and diminutive. 4) Winges held horizontally. 5) wing membrane naked. 6) origin of R2+3 at or before the apex of basal radial cell. 7) Sc sometimes long, but never especially short. 8) aedeagus not forming the ball-and-socket (primitively linked to it by a V-shaped furca. 9) More than one tenaculum present.	*Clogmia* Enderlein, 1937; *Crenopanimerus* Vaillant, 1983; *Elsahowia* Duckhouse, 1978, *Eutelmatoscopus* Satchell, 1953 [now subgenus of *Telmatoscopus*], *Jungiella* Vaillant, 1972; *Panimerus* Eaton, 1912; *Telmatoscopus* Eaton, 1904; *Xenapates* Eaton, 1904 [now *Vaillantodes* Wagner, 2001].	
		Psychodini	Characters as discussed by Duckhouse (1985)	Adding *Clytocerus* Eaton, 1904 and *Mystropsychoda* Duckhouse, 1975 to his Psychodini	He states that the tribal placement of these two genera is “obscure”.
	1990	Maruinini	1) Flagellomeres primitively fusiform, if flask-shape, flagellomere 1 retains its fusiform shape. 2) ascoids frequently larger than in Pericomaini, with two to many branches. 3) apical flagellomeres reduced, without ascoids. 4) R2+3 arising from R4 beyond the apex of basal radial cell (R4 pectinate). 5) R fork generally beofore M fork, and both basal of wing center. 6) aedeagal complex asymmetrical. 7) Ejaculatory apodeme broad on ventral view.	*Alloeodidicrum* Duckhouse, 1990; *Ancyroaspis* Duckhouse, 1990; *Didicrum* Enderlein, 1937; *Eremolobulosa* Duckhouse, 1990; *Notiocharis* Eaton, 1913 [now *Abcharis* Tkoc & Ježek, 2013]; *Rotundopteryx* Duckhouse, 1990; *Satchellomyia* Duckhouse, 1990.	Restricted to Australasian region.
Enderlein	1935	Ulomyiini	1) R4 & R5 merging as a single stem. 2)Stem of R2+3 and M1+2 extends to the first third of the wing. 3) wing broad	*Ulomyia* Haliday, 1856	
		Pericomini		*Clytocerus* Eaton, 1904; *Lepiseoda* Enderlein, 1935 [now *Panimerus* Eaton,	

				1913]; *Pericoma* Haliday, 1856; *Pneumia* Enderlein, 1935; *Sciria* Enderlein, 1935 [now *Telmatoscopus*]; *Seoda* Enderlein, 1935; *Telmatoscopus* Eaton, 1904; *Xenapates* Eaton, 1904 [now *Vaillantodes* Wagner, 2001; misspelled as *Xenapanthes*].	
		Psychodini		*Logima* Eaton, 1904; *Mormia* Enderlein, 1937; *Paramormia* Enderlein, 1935, *Peripsychoda* Enderlein, 1935, *Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797;Threticus, Eaton, 1904.	
	1937	Psychodini, with two subtribes, Clytocerina and Psychodina	Vein R5 originates on R4 near the origin of R4	Clytocerina: *Alepia* Enderlein, 1937; *Clytocerus* Eaton, 1904; *Lepipneumia* Enderlein, 1937; *Lepiseodina* Enderlein, 1937, *Mogisetia* Enderlein, 1937 [now *Panimerus*]; *Panimerus* Eaton, 1913; *Pneumia* Enderlein, 1935; *Seoda* Enderlein, 1935; *Synseoda* Enderlein, 1937 [now *Clytocerus*]. Psychodina: *Psychoda* Latreille, 1797; *Pericoma* Haliday, 1856; *Pericomina* Enderlien, 1937 [now *Ulomyia* Haliday, 1856]; *Colpopteryx* Enderlein, 1937	

				[now *Ulomyia*]; *Tinearia* Schellenberg, 1803 [now *Psychoda*]; *Marsypia* Enderlein, 1937 [now *Ulomyia*]; *Trichopsychoda* Tonnoir, 1922; *Sciria* Enderlein, 1937 [now *Telmatoscopus*];Clogmia Enderlein, 1937 Enderlein, 1937; *Telmatoscopus* Eaton, 1904; *Xenapates* Eaton, 1904 [now *Vaillantodes* Wagner, 2001; misspelled as *Xenapanthes*]; *Syntomoza* Enderlein, 1937 [now *Lepidiella*]; *Lepidiella* [Enderlein, 1937].	
		Mormiini, with two subtribes, Mormiina and Paramormiina	Vein R5 originates from a common stem of the fork R4 + R2+3, or originates freely at the base of the wing	Mormiina: *Desmioza* Enderlein, 1937; *Dictyocampsa* Enderlein, 1937; *Eophlebotomus* Cockerell, 1920 [part of Phlebotominae]; *Lepimormia* Enderlein, 1937; *Mormia* Enderlein, 1937; *Notiocharis* Eaton, 1913 [now *Abcharis* Tkoc & Ježek, 2013]; *Peripsychoda* Enderlein, 1935; *Setomima* Enderlein, 1937; *Synmormia* Enderlein, 1937; *Syntomolaba* Enderlein, 1937. Paramormiina: *Brunettia* Annandale, 1910; *Chirolepia*	

				Enderlein, 1937; *Didicrum* Enderlein, 1937; *Mecysmia* Enderlein, 1937 [now *Didicrum*]; *Nemoneura* Tonnoir, 1929; *Paramormia* Enderlein, 1935; *Parabrunettia* Brunetti, 1911 [now *Brunettia*]; *Platyplastinx* Enderlein, 1937; *Podolepria* Enderlein, 1937 [now *Didicrum*]; *Tonnoira* Enderlein, 1937.	
		Maruinini	separated from Phlebotomus in the Key. R2 short, R2+3 long. Wing narrow. CuA2 not shortened, ending close to the middle of the wing.	*Maruina* Müller, 1895	Maruinini was included within the subfamily Phlebotominae. Phlebotominae and Psychodinae are separated in the key by the presence/absence of proboscis.
Ježek	1983 1984	Mormiini	1) Anterior additional sclerite of the pteropleurite developed. 2) pteropleurite with a break. 3) R4 connected with R2+3 which is conspicuosly prolonged basally. 4) Sc long, thin and straight.	*Jovamormia* Ježek, 1983; *Promormia* Ježek, 1983; *Psychomormia* Ježek, 1983.	
		Paramormiini	1) Anterior additional sclerite of the pteropleurite developed. 2) pteropleurite with a break. 3) R2+3 connected with R4 which is at least inconspicuously prolonged basally.	*Feuerborniella* Vaillant, 1971; *Jungiella* Vaillant, 1972; *Panimerus* Eaton, 1912; *Paramormia* Enderlein, 1935; *Parajungiella* Vaillant, 1972; *Philosepedon* Eaton, 1904; *Peripsychoda* Enderlein, 1935; *Telmatoscopus* Eaton, 1904; *Threticus*, Eaton, 1905, Eaton, 1904; *Trichopsychoda* Tonnoir, 1922.	

		Psychodini	1) Anterior additional sclerite of the pteropleurite not developed. 2) pteropleurite without a break.	*Chodopsycha* Ježek, 1984; *Copropsychoda* Ježek, 1984; Logima Eaton, 1904; *Psycha* Ježek, 1984; *Psychomora* Ježek, 1984; *Psychoda* Latreille, 1797; *Psychodocha* Ježek, 1984; *Psychodula* Ježek, 1984; *Tinearia* Schellenberg, 1803 [now *Psychoda*]; *Ypsydocha* Ježek, 1984.	
		Mormiini	1) Origin of R4 behind the origin of R2+3. 2) Sc long, thin and straight.	*Hemimormia* Krek, 1971; *Jovamormia* Ježek, 1983; *Keatamormia* Ježek, 1984; *Lepimormia* Enderlein, 1937; *Limomormia* Vaillant, 1982; *Mormia* Enderlein, 1935; *Oomormia* Ježek, 1984; *Promormia* Ježek, 1984; *Psychomormia*, Ježek, 1984; *Rhadiomormia* Vaillant, 1974; *Saximormia* Ježek, 1984;, *Taramormia* Ježek, 1984; *Telomormia* Ježek, 1984; *Yomormia* Ježek, 1984.	
		Paramormiini	1) origin of R2+3 behind the origin of R4. 2) Sc short and thick.	*Clogmia* Enderlein, 1937; *Paramormia* Enderlein, 1935; *Telmatoscopus* Eaton, 1904; *Panimerus* Eaton, 1913; *Psycmera* Ježek, 1984; *Parajungiella* Vaillant, 1972; *Satoba* Ježek, 1984; *Jungiella* Vaillant, 1972; *Psychogella* Ježek, 1984 [now *Jungiella*];	He synonymized Vaillants 1971 Telmatoscopini with his Paramormiini

				Psychocha Ježek, 1983 [now *Jungiella*]	
	1985	Paramormiini with two subtribes: Paramormiina, Trichopsychodina	Trichopsychodina. 1) Ascoids with two or three arms. 2) apical antennal segments reduced. 3) last palpal segment not annulated. 4) Sc short. 5) hypandrium not developed. Paramormiina. 1)Ascoids simple, finger like. 2) apical antennal segments with developed hals. 3)last palpal segment annulated. 4) Sc long. 5) hypandrium developed	Trichopsychodina: *Feuerborniella* Vaillant, 1971; *Philosepedon* Eaton, 1904; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	The genera included in his Paramormiina are not specified, only states 7 genera present.
	1990	Paramormiini with two subtribes: Paramormiina, Trichopsychodina		*Clogmia* Enderlein, 1937; *Iranotelmatoscopus* Ježek, 1987; *Jungiella* Vaillant, 1972; Karakovounimerus Ježek, 1990 [now *Panimerus*]; *Lepiseodina* Enderlein, 1937; *Panimerus* Eaton, 1913; ; *Parajungiella* Vaillant, 1972; *Paramormia* Enderlein, 1935; Peripsychoda Enderlein, 1935; *Telmatoscopus* Eaton, 1904.	He follows his 1985 classification of Paramormiini with two subtribes. In this paper he only deals with Paramormiina, and the intergeneric relationships.
	2005	Pericomaini	1) basal flagellomeres spindle-shaped	not mentioned	
		Mormiini	1) Basal flagellomeres with bulbous base and neck. 2) Wing basally with prolonged R2+3, with connection to R4. 3) Pteropleurite not trapezoidal, upper suture partially developed or missing	not mentioned	
		Paramormiini	1) basal flagelloermes with bulbous base and neck. 2) Wing basally with prolonged R4, with connection to R2+3. 3) Pteropleurite not trapezoidal, upper suture partially developed or missing	not mentioned	

		Psychodini	1) Basal flagellomeres with bulbous base and neck. 2) Pteropleurite trapezoidal, always conspicuously marked upper suture	*Chodopsycha* Ježek, 1984; *Copropsychoda* Ježek, 1984; *Falsologima* Ježek & Harten, 1996; *Logima* Eaton, 1904; *Psycha* Ježek, 1984; *Psychana* Ježek & Harten, 2005 [now *Psychoda*]; *Psychodocha* Ježek, 1984; *Psychodula* Ježek, 1984; *Psychmora* Ježek, 1984; *Psychoda* Latreille, 1797; *Tinearia* Schellenberg, 1803 [now *Psychoda*]; *Ypsydocha* Ježek, 1984.	They state that the genera *Neopsychoda* Duckhouse, 1966 [now *Epacretron*]; *Mystropsychoda* Duckhouse, 1975, and *Rhipidopsychoda* Vaillant, 1991, probably do not belong to Psychodini and were left out of the key.
	2011	Paramormiini with two subtribes: Paramormiina, Trichopsychodina		Trichopsychodina: *Eurygarka* Quate, 1959; *Feuerborniella* Vaillant, 1971; *Neothreticus* Vaillant, 1973; *Neoquatiella* Vaillant, 1973; *Nielseniella* Vaillant, 1972; *Perithreticus* Vaillant, 1973; *Philosepedon* Eaton, 1904; *Quatiella* Botosaneanu & Vaillant, 1970; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	He synonymizes Vaillants 1991 Threticina under Ježeks trichopsychodina
		Psychodini			Makes comments about his classification of *Psychoda*, and treats *Tinearia* [as a valid genus]
	2020	Mormiini		*Lepimormia* Enderlein, 1937; *Mormia* Enderlein, 1935; *Promormia* Ježek, 1983; *Yomormia* Ježek, 1984.	Following the classification of Ježek (1990a, 1994a, 1999a, 2007), Ježek & van Harten (2005), Omelkova & Ježek (2012a), Oboňa & Ježek (2014), and Kroča & Ježek (2015, 2019).

		Paramormiini with two subtribes: Paramormiina		Paramormiina: Jungiella Vaillant, 1972; Lepiseodina Enderlein, 1937; Panimerus Eaton, 1913; *Paramormia* Enderlein, 1935; Parajungiella Vaillant, 1972; Peripsychoda Enderlein, 1935; Seoda Enderlein, 1935. Trichopsychodina: *Feuerborniella* Vaillant, 1971; *Philosepedon* Eaton, 1904; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	
		Psychodini		*Chodopsycha* Ježek, 1984; *Logima* Eaton, 1904; *Psycha* Ježek, 1984; *Psychoda* Latreille, 1797; *Psychodocha* Ježek, 1984; *Psychodula* Ježek, 1984; *Psychomora* Ježek, 1984; *Tinearia* Schellenberg, 1803 [now *Psychoda*].	
		Pericomaini		*Berdeniella* Vaillant, 1976; *Clytocerus* Eaton, 1904; *Parabazarella* Vaillant, 1983; *Pericoma* Haliday, 1856; *Pneumia* Enderlein, 1935; *Saraiella* Vaillant, 1981; *Tonnoiriella* Vaillant, 1982; *Ulomyia* Haliday, 1856	

Kvifte	2012	Psychodini	1) Flagellomeres nodiform. 2) apical flagellomeres flobular and reduced. 3) ascoids with two anterior and one posterior branches (sometimes one or three anterior). 4) fourth palpomere fully sclerotized. 5) anepisternum with a large anterior spiracle. 6) anepimeron rectangular or triangular. 7) Sc thivkened relative to other wing veins. 8) R5 ending at wing apex. 9) aedeagus symmetrical or asymmetrical. 10) surstyli with one to several distal tenacula (Trichopsychoda with complex subapical tenacula present)	*Neomaruina* Vaillant, 1963; *Perithreticus* Vaillant, 1973; *Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797; *Rhipidopsychoda* Vaillant, 1991; *Soeliella* Kvifte, 2015; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	He redifined the Psychodini tribe, but only treated the afrotropical genera.
	2018	Psychodini	Palpus with segment 4 sclerotized; antenna with basal flagellomeres nodiform, distal 1–3 fusiform and diminutive, ascoids digitiform or foliform, with one to three anterior branches and one posterior branch. Ejaculatory apodeme laterally compressed, narrow in dorsal view. Epandrium with one aperture. Cercus reduced, vestigialor present as lateral lobes of proctiger. Surstylus well developed with apical tenacula	*Cookiellocapsa* Ježek & Le Pont, 2016; *Epacretron* Quate, 1965; *Eurygarka* Quate, 1959; *Feuerborniella* Vaillant, 1971; *Mucomyia* Kvifte & Curler, 2018; *Neomaruina* Vaillant, 1963; *Nielseniella* Vaillant, 1972; *Perithreticus* Vaillant, 1973; *Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797; *Quatiella* Botosaneanu & Vaillant, 1970; *Rhipidopsychoda* Vaillant, 1991; *Soeliella* Kvifte, 2015; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	Kviftes Psychodini corresponds to the previous Psychodini and includes Vaillants 1990 Neomaruinini, and Jezek Trychopsychodina.

		Brunettiini	Palpus with segment 4 sclerotized or corrugated. Flagellomeres nodiform, with ascoids variable but never Y-shaped; distal 1–3 flagellomeres diminutive, fusiform. Ejaculatory apodeme laterally compressed, narrow in dorsal view. Parameres paired, triangular. Gonostyli distally with spiniform setae. Surstylus with tenacula distally expanded, polyseriate, occasionally in two separated patches.	Atrichobrunettia Satchell, 1953; Brunettia Annandale, 1910; Gerobrunettia Quate & Quate, 1967.	Kviftes Brunettiini = Vaillants Brunettiini, which = Duckhouses Mormiini in part.
		Maruinini	Palpus with segment 4 sclerotized or corrugated; flagellomeres nodiform or fusiform. Gonocoxal condyles usually forming large plates with median keel linking to ejaculatory apodeme. Ejaculatory apodeme ovoid, hollow internally, broad in dorsal view. Surstylus sometimes reduced. Tenacula uniseriate, polyseriate or aseriate	*Alepia* Enderlein, 1937; *Alloeodidicrum* Duckhouse, 1990; *Ancyroaspis* Duckhouse, 1990; *Arisemus* Satchell, 1955; *Armillipora* Quate, 1996; *Australopericoma* Vaillant, 1975; *Balbagathis* Quate, 1996; *Caenobrunettia* Wagner, 1981; *Desmioza* Enderlein, 1937; *Dictyocampsa* Enderlein, 1937; *Didicrum* Enderlein, 1937; *Didimioza* Quate & Brown, 2004 *Eremolobulosa* Duckhouse, 1990; *Lobulosa* Szabó, 1960; *Maruina* Müller, 1895; *Micrommatos* Quate & Brown, 2004; *Nemoneura* Tonnoir, 1929; *Neoarisemus* Botosaneanu & Vaillant, 1970; *Neurosystasis* Satchell, 1955; *Platyplastinx* Enderlein, 1937;	Kviftes Maruinini = Vaillants Setomimini in part.

				Rotundopteryx Duckhouse, 1990; *Satchellomyia* Duckhouse, 1990; *Setomima* Enderlein, 1937; *Synmormia* Enderlein, 1937; *Syntomolaba* Enderlein, 1937; *Thrysocanthus* Enderlein, 1937; *Tonnoira* Enderlein, 1937; *Valerianna* Quate & Brown, 2004.	
		Pericomaini	Palpus with segment 4 corrugated. Flagellomeres nodiform or fusiform, first flagellomere in nodiform species sometimes fusiform, ascoids with one to several anterior branches. Gonocoxal condyles present as arched sclerites or reduced. Dorsal paramere (furca) often present, linking tuje ejaculatory apodeme to the gonocoxal condyles and/or the subepandrial plate. Ejaculatory apodeme laterally or dorsoventrally compressed, broad or narrow in dorsal view. Surstyli with tenacula polyseriate or aseriate	*Bazarella* Vaillant, 1961; *Berdeniella* Vaillant, 1976; *Clogmi*a Enderlein, 1937; *Clytocerus* Eaton, 1904; *Crenopanimerus* Vaillant, 1983; *Elsahowia* Duckhouse, 1978; *Eutelmatoscopus* Satchell, 1953 [now *Telmatoscopus*]; *Gondwanoscurus* Ježek, 2001; *Iranotelmatoscopus* Ježek, 1987; *Jezekiella* Wagner & Kvifte, 2015; *Joostiella* Vaillant, 1983; *Jungiella* Vaillant, 1972; *Lepiseodina* Enderlein, 1937; *Mormia* Enderlein, 1935; *Moruseodina* Bravo & Cordeiro, 2014; *Nototelmatoscopus* Satchell, 1953; *Panimerus* Eaton, 1913; *Parabazarella* Vaillant, 1983; *Parajungiella* Vaillant, 1972; *Paramormia* Enderlein, 1935; *Perakomyia*	Kviftes Pericomaini = Vaillants 1971 Telmatoscopini, Vaillants 1990 Mormiini (excluding the y-shaped group) and Vaillant 1990 Pericomini. Kvifte (2018) states that the Pericomaini (sensu Kvifte) is a very heterogenous group without clear diagnostic characters, and the morphological delimitations of the names palced in synonymy rely on characters prone to homoplasy with all other tribes.

				Ježek, 2010; *Pericoma* Haliday, 1846; *Peripsychoda* Enderlein, 1935; *Phyllotelmatoscopus* Vaillant, 1983; *Pneumia* Enderlein, 1935; *Promormia* Ježek, 1983; *Psychomasina* Ježek, 2004; *Rhadinomormia* Vaillant, 1975 [now *Mormia*]; *Rhadinoscopus* Quate & Quate, 1967; *Saraiella* Vaillant, 1981; ; *Satoba* Ježek, 1984; *Seoda* Enderlein, 1935; *Stupkaiella* Vaillant, 1973; *Szaboiella* Vaillant, 1979; *Telmatoscopus* Eaton, 1904; *Thornburghiella* Vaillant, 1982; *Tokunagaiella* Vaillant, 1983 *Ulomyia* Haliday, 1856; *Vaillantodes* Wagner, 2001.	
		Unplaced Genera		*Abcharis* Tkoc & Ježek, 2013; *Breviscapus* Quate, 1955; *Bryopharsos* Quate, 1996; *Chirolepia* Enderlein, 1937; *Eugenys* Quate, 1996; *Hyrcanoressliella* Ježek, 1997; *Lepidiella* Enderlein, 1935; *Mormopericomiella* Ježek & van Harten, 2002; *Mystropsychoda* Duckhouse, 1975; *Neoquatiella* Vaillant, 1973; *Neotelmatoscopus* Tonnoir, 1933; *Neothreticus*	

				Vaillant, 1973; *Potophila* Kvifte, 2014; *Saximormia* Ježek, 1984 *Synmormia* Enderlein, 1937; *Tonnoiriella* Vaillant, 1982; *Vagmania* Krek, 1972.	
Quate	1959	Psychodini	1) Antenna with 14-16 segments. 2) Ascoids generally Y-shaped and longer than segment. 3) Flagellomeres nodiform. 4) terminal segments beyond 13 always reducedin size. 5) Wing with acute apex. 6) Radial fork distad of medial. 7) R5 ending at wing apex.	*Eurygarka* Quate, 1959; *Lepidopsychoda* Edwards, 1928 [now *Philosepedon*]; *Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	
	1996	Pericomaini	1) flagellomeres fusiform. 2) R and M forks near the center of the wing. 3) Radial sector not pectinate. 4) Male genitalia lack the keel between the gonocoxal apodemes	*Pericoma* Haliday, 1856; *Syntomoza* Enderlein, 1937 [now *Lepidiella*].	Following Duckhouse (1987).
		Maruinini	1) Basal flagellomere fusiform. 2) radial and medial forks usually basal to wing center. 3) possession of keel in the male genitalia conecting the gonocoxal apodemes. 4) Surstylus with a single tenaculum	*Alepia* Enderlein, 1937; *Armillipora* Quate, 1996; *Arisemus* Satchell, 1955; *Balbagathis* Quate, 1996; *Caenobrunettia* Wagner, 1981; *Maruina* Müller, 1895; *Setomima* Enderlein, 1937; *Tonnoira* Enderlein, 1937.	Following Duckhouse (1987).
		Telmatoscopini		*Duckhousiella* Vaillant, 1972 [now *Paramormia*]; *Telmatoscopus* Eaton, 1904.	Following Vaillant (1971, 1990). Quate states that Duckhouse (1987) synonymizes part of Telmatoscopini *sensu* Vaillant with Paramormiini. But Quate will stick to Vaillants tribal designation.

		Mormiini	1) Flagellomeres nodiform. 2) flagellomeres with multiple ascoids varying from digitate to bifurcate or palmate. 3) Vein radial sector pectinate. 4) R and M forks basal to wing center. 5) R5 ending in wing apex. 6) Male genitalia lack the keel between the gonocoxal apodemes.	*Brunettia* Annandale, 1910; *Bryopharsos* Quate, 1996; *Eugenys* Quate, 1996.	
		Psychodini		*Philosepedon* Eaton, 1904; *Psychoda* Latreille, 1797.	Follows Quate (1959) and Duckhouse (1985).
	1999	Pericomaini		Syntomoza Enderlein, 1937 [now *Lepidiella*]	Following Duckhouse (1987) and Quate (1996).
		Maruinini		*Alepia* Enderlein, 1937; *Armillipora* Quate, 1996; *Arisemus* Satchell, 1955; *Balbagathis* Quate, 1996; *Caenobrunettia* Wagner, 1981; *Desmioza* Enderlein, 1937; *Eugenys* Quate, 1996; *Maruina* Müller, 1895; *Platyplastinx* Enderlein, 1937; *Setomima* Enderlein, 1937; *Tonnoira* Enderlein, 1937.	Following Duckhouse (1987) and Quate (1996).
		Mormiini		*Brunettia* Annandale, 1910; *Bryopharsos* Quate, 1996.	In 1996, the genus *Eugenys* was treated as part of Mormiini, later in 1999 as part of Maruinini.
		Paramormiini		*Duckhousiella* Vaillant, 1972 [now *Paramormia*]; *Telmatoscopus* Eaton, 1904.	Rectifies the usage of Telmatoscopini Vaillant to this tribe, after misinterpreting the synonymy by Duckhouse (1987).
		Psychodini		*Philosepedon* Eaton, 1904, *Psychoda* Latreille, 1797.	Following Quate (1959) and Duckhouse (1985).
Quate & Brown	2004	Maruinini	Maruina possess an aedeagal sheath (as described by Hogue, 1973)	*Maruina* Müller, 1895.	

		Setomimini	Aedeagal sheath absent in Setomimini	*Alepia* Enderlein, 1937; *Arisemus*, Satchell, 1955; *Australopericoma* Vaillant, 1975; *Balbagathis* Quate, 1996; *Caenobrunettia* Wagner, 1981; *Desmioza* Enderlein, 1937; *Didicrum* Enderlein, 1937; *Didimioza* Quate & Brown, 2004; *Micrommatos* Quate & Brown, 2004; *Nemoneura* Tonnoir, 1929; *Neurosystasis* Satchell, 1955; *Patyplastinx* Enderlein, 1937; *Thrysocanthus* Enderlein, 1937; *Tonnoira* Enderlein, 1937; *Valerianna* Quate & Brown, 2004.	They state that at the present level of knowledge, there is no strong evidence that suggest Stomimini is a monophyletic tribe.
Vaillant	1971-1975	Telmatoscopini	1) flagellomeres bottle-shaped. 2) Labella always spherical and swollen. 3) Wing R and M forks distant from wing base.	*Duckhousiella* Vaillant, 1972 [now *Paramormia*]; *Feuerborniella* Vaillant, 1971; *Jungiella* Vaillant, 1972; *Mormia* Enderlein, 1935; *Panimerus* Eaton, 1913; *Peripsychoda* Enderlein, 1935; *Philosepedon* Eaton, 1904; *Telmatoscopus* Eaton, 1904; *Threticus*, Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	Character 1 is shared with Psychodini and Brunettiini, but not with Pericomaini. Character 2 is shared with Pericomaini and Brunetiini. character 3 is shared with Pericomaini and Psychodini.
		Psychodini	1) apical flagellomeres reduced. 2) base of gonocoxites separated. 3) hypandrium dividing gonocoxites	*Copropsychoda* Ježek, 1984; *Psychoda* Latreille, 1797;	Characters and genera only treated in the key in 1971.

		Pericomaini	1) Flagellomeres fusiform. 2) single pair of simple and short ascoids. 3) labella as in Telmatoscopini and Brunettiini.	*Bazarella* Vaillant, 1961; *Berdeniella* Vaillant, 1976; *Clytocerus* Eaton, 1904; *Pericoma* Haliday, 1856; *Satchelliella* Vaillant, 1979; *Saraiella* Vaillant, 1981; *Szaboiella* Vaillant, 1979; *Tokunagaiella* Vaillant, 1983; *Tonnoiriella* Vaillant, 1982; *Ulomyia* Haliday, 1856; *Stupkaiella* Vaillant, 1973; *Thornburghiella* Vaillant, 1982.	Characters treated in the key in 1971, further characters and genera included in 1975.
		Brunettiini	1) R and M forks are near the base of the wing	1971: *Brunettia* Annandale, 1910, *Neoarisemus* Botosaneanu & Vaillant, 1970. 1975: *Alepia* Enderlein, 1937; *Arisemus* Satchell, 1956, *Atrichobrunettia* Satchell, 1953, *Brunettia* Annandale, 1910; *Gerobrunettia* Quate & Quate, 1967, *Neoarisemus* Botosaneanu & Vaillant, 1970, *Parasetomima* Duckhouse, 1968; *Paratelmatoscopus* Satchell, 1953; *Platyplastinx* Enderlein, 1937; *Setomima* Enderlein, 1937, *Trichobrunettia* Tonnoir, 1939 [now *Brunettia*]	Characters treated in the key in 1971. Further genera included in 1975.

	1982	Brunettiini	1) Eye bdrige with 3 or 4 facet rows. 2) antennal flagellomeres bulbiform, but with short neck. 3) scoids digitiform. 4) last antennal segments narrower than preceding ones. 5) Ejaculatory apodeme laterally compressed. 6) distal part of aedeagus comprisses a chitinous stem	*Atrichobrunettia* Satchell, 1953; *Brunettia* Annandale, 1910; *Gerobrunettia* Quate & Quate, 1967; *Parasetomima* Duckhouse, 1968.	
		Setomimini	1) eye bridge with 4 facet rows. 2) Flagellomeres bulbiform, with usually a sharp stem. 3) Ascoids diverse shapes (simple, branched or leaf-shaped). 4)Terminal flagellmeres same wiedth as preceding. 5) Aedeagus is asymmetrical. 6) ejaculatory apodeme broad (laterally expanded).	*Alepia* Enderlein, 1937; *Neoarisemus* Botosaneanu & Vaillant, 1970; *Setomima* Enderlein, 1937.	Vaillant created Setomimini to fit the genera that did not fit his concept of Brunettiini.
		Arisemini	1) Flgallomeres bulbiform with a very distinct stem. 2) Ascoids digitiform. 3) ejaculatory apodeme broad	*Arisemus* Satchell, 1955	
	1986	Setomimini		*Alepia* Enderlein, 1937; *Arisemus* Satchell, 1955; *Bazara* Vaillant, 1987 [now *Arisemus*]; *Neoarisemus* Botosaneanu & Vaillant, 1970; *Neurosystasis* Satchell, 1955; *Paratelmatoscopus* Satchel, 1953; *Setomima* Enderlein, 1937; *Tonnoiriella* Vaillant, 1982.	Vaillant (1986) synonymzes Arisemini under Setomimini. Also states that Neomaruina Vaillant, 1963 and Neotelmatoscopus might be synonyms of the other genera included in Setomimini.
		Brunetiini	1) M and R forks near the base on the wings. 2) gonocoxites widely separated. 3) ejaculatory apodeme laterally compressed. 4) Surstyli short. 5) Flagellomeres onion-shaped (with short neck).	*Atrichobrunettia* Satchell, 1953; *Brunettia* Annandale, 1910; *Gerobrunettia* Quate & Quate, 1967; *Parasetomima* Duckhouse, 1968.	Parasetomima Duckhouse, 1968 is in the border between Setomimini and Brunettiini.

	1990	Psychodini	Interocular suture absent, or very weak. Labella flatenned with apical teeth. Gonocoxites separated, without a dorsal extension. Surstyli apically pointed, with 1 apical tenaculum. Aedeagus asymmetrical	*Copropsychoda* Vaillant, 1971; *Psychoda* Latreille, 1797.	Vaillants 1990 Psychodini = Duckhouse, 1987 Psychodini (in part). Vaillant in 1990 mentioned that in all Psychodini sensu Vaillant, without exception, the gonocoxites are widely separated and completely lack a dorsal extension. These characters, are not found in any species of Mormiini (group with Y-shaped ascoids). However, they are present in almost all Brunettiini sensu Vaillant.
		Mormiini	Interocular suture always present. Labella bulbouse and fleshy, with apical bristles. Gonocoxites close to each other, with a dorsal extension. Surstyli apically rounded, with miltuple tenacula. Aedeagus symmetrical	*Clogmia* Enderlein, 1937; *Crenopanimerus* Vaillant, 1983; *Feuerborniella* Vaillant, 1971; *Jungiella* Vaillant, 1972; *Mormia* Enderlein, 1935; *Nielseniella* Vaillant, 1972; *Panimerus* Eaton, 1913; *Paramormia* Enderlein, 1935; *Peripsychoda* Enderlein, 1935; *Philosepedon* Eaton, 1904; *Phyllotelmatoscopus* Vaillant, 1983; *Quatiella* Botosaneanu & Vaillant, 1970; *Telmatoscopus* Eaton, 1904; *Threticus* Eaton, 1905; *Trichopsychoda* Tonnoir, 1922.	Vaillants 1990 Mormiini = Vaillants 1971 Telmatoscopini = Duckhouse, 1987 Paramormiini + part of Psychodini. the Y-shaped group of Vaillants 1990 Mormiini, is what Vaillant in 1971 referes too as Telmatoscopini with Y-shaped ascoids, which also corresponds to the part of Psychodini from Duckhouse 1985+1987.
		Pericomini	Interocular suture always present. Labella bulbouse and fleshy, with apical bristels. Gonocoxites close to each other, with a dorsal extension. Aedeagus symmetrical	*Bazarella* Vaillant, 1961; *Berdeniella* Vaillant, 1976; *Clytocerus* Eaton, 1904; *Joostiella* Vaillant, 1983; *Pericoma* Haliday, 1856; *Satchelliella* Vaillant, 1979;	Vaillant, 1990 states that the Mormiini (excluding the Y-shaped ascoids group, = to Vaillans, 1971 Telmatoscopini, are very well separated from the Psychodini, and not so clearly separated from the

				Saraiella Vaillant, 1981; *Stupkaiella* Vaillant, 1973; *Szaboiella* Vaillant, 1979; *Thornburghiella* Vaillant, 1982; *Tokunagaiella* Vaillant, 1983; *Ulomyia* Haliday, 1856.	Pericomaini, as they share multiple characters.
		Brunettiini	Gonocoxites separated, without a dorsal extension	*Atrichobrunettia* Satchell, 1953; *Brunettia* Annandale, 1910; *Gerobrunettia* Quate & Quate, 1967	
		Setomimini	Characters not explicitly mentioned	*Alepia* Enderlein, 1937; *Arisemus* Satchell, 1955; *Bazara* Vaillant, 1987 [now *Arisemus*]; *Neoarisemus* Botosaneanu & Vaillant, 1970; *Neurosystasis* Satchell, 1955; *Parasetomima* Duckhouse, 1968; *Paratelmatoscopus* Satchell, 1953; *Setomima* Enderlein, 1937; *Tonnoiriella* Vaillant, 1982.	Vaillant 1990 argues that the characters provided by Quate that separate Maruinini from Setomimini are good to distinguish them. He contradicts the posture of Duckhouse (1987) where he unites both tribes.
		Maruinini	Characters not explicitly mentioned	*Maruina* Müller, 1895	
		Neomaruinini	Characters not explicitly mentioned	*Neomaruina* Vaillant, 1963	Vaillant mantains *Neomaruina* Vaillant, 1963 as a separate tribe from Psychodini mainly using larval characters, and the habitat in which the larvae develop. Thus, contradictinv the placement of *Neomaruina* Vaillant, 1963 within Psychodini by Duckhouse, 1987.