Morphological evolution and phylogeny of millipedes (Diplopoda)

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Summary

Millipedes (Diplopoda) count more than 12,000 described species and play, probably since more than 400 million years, a fundamental role as detritivores in terrestrial ecosystems. Despite their great diversity and ecological importance, the biology, morphology, and evolution of the group is only poorly understood. This is especially true for the less diverse taxa, like the Colobognatha and Pentazonia, which only received little attention. Furthermore, several character complexes are poorly studied, and there are large gaps within the fossil record of the Diplopoda. Knowledge of these neglected taxa and structures are essential to understand the evolution of the group through time. The studies presented here aim to contribute to our knowledge of the morphological diversity of extant and extinct Diplopoda, and to understand their structure in a functional, ecological, and evolutionary context. Therefore, micro-computed tomography (µCT), synchrotron-µCT (SR-µCT), scanning electron microscopy (SEM), and histology are used to study extant and extinct Diplopoda, and to document the structure of several character complexes, including the feeding apparatus, the Tömösváry organ and the legs.

For the first time it is shown that the colobognathan Polyzoniida, Siphonocryptida and Siphonophorida possess protractible mandibles and a sucking apparatus, similar to that of suctorial insects. The colobognathan Platydesmida show an intermediate state between those fluid-feeding millipedes and the eugnathan biting-chewing mouthpart configuration (chapters 2 & 3). Further variations from this biting-chewing configuration can be seen within the Pentazonia in the giant pill-millipedes (Sphaerotheriida), in which the tentorial complex is modified. These modifications might be related to its ability to roll into a perfect ball (chapter 4). For the pentazonian Glomeridesmida, which have been assumed to closely represent the ground plan of the Chilognatha, a highly derived character is reported for the first time: the presence of a tarsal thread producing 'spinning' organ (chapter 5). Not only these little-known taxa yielded novel findings, but it is also demonstrated that characters of comparatively well-known and diverse taxa, like the flat-backed millipedes (Polydesmida) need reexamination. For the Polydesmida it is shown, that a Tömösváry organ is absent, although this character has previously been described for several representatives and is frequently used in taxonomic and phylogenetic studies (chapter 6). Finally, 13 of the 16 extant millipede orders are reported from Cretaceous Burmese amber, including the first or oldest fossil record for half the Diplopoda orders. It becomes evident, that the extant taxa with their highly derived morphologies were already present in the Cretaceous almost 100 million years ago (ma) and only changed little since then (chapters 7 & 8).

The presented studies demonstrate how modification of common structures, like the mouthparts, can lead to new functions such as fluid feeding within the primarily biting-chewing

Diplopoda. These distinct morphologies were already present almost 100 ma. However, it remains unknown why the ancient and morphological diverse millipedes, and especially the highly derived Colobognatha, show a low species diversity compared to other arthropod taxa like insects and spiders. The data generated here can be used to correct character statements and to contribute new characters to previous phylogenetic analyses, to better understand the evolution of the Diplopoda. However, it becomes clear that more detailed studies of the whole Diplopoda diversity, as well as the restudy of established character systems are needed to fully understand the diversity and evolution of the group.

Content

List of abbreviations

3D	Three-dimensional
DESY	Deutsches Elektronen-Synchrotron (Hamburg, Germany)
IEZ	Institute of Evolutionary Biology and Ecology (University of Bonn)
LIB	Leibniz Institute for the Analyses of Biodiversity Change (Bonn/Hamburg, Germany)
ma	Million years ago
PSI	Paul Scherrer Institute (Villigen, Switzerland)
SEM	Scanning electron microscopy
SR-µCT	Synchrotron-micro-computed tomography
TEM	Transmission electron microscopy
ZFMK	Zoological Research Museum Alexander Koenig (Bonn, Germany)
ZFMK-MYR	Myriapoda collection of the ZFMK
µCT	Micro-computed tomography

Chapter 1 – Introduction

1.1 Millipede (Diplopoda) diversity and ecology

Millipedes (Diplopoda) are terrestrial Arthropods, which belong to the subphylum Myriapoda (Chilopoda (Fig. 1A), Symphyla (Fig. 1B), Pauropoda (Fig. 1C), Diplopoda (Fig. 1D)), and include >12,000 described species, which are distributed world-wide, except Antarctica (Enghoff et al. 2015). Some estimates suggest as many as 80,000 species (Hoffman 1980; Adis & Harvey 2000), while more conservative and data-based approaches suggest a total of 13,000 – 20,000 extant species worldwide (Brewer et al. 2012a). Currently 16 extant orders with 140 families and more than 3,000 genera are recognized (Shelley 2002; Brewer et al. 2012a; Enghoff et al. 2015), which might indicate inflational use of higher taxa (Brewer et al. 2012a).

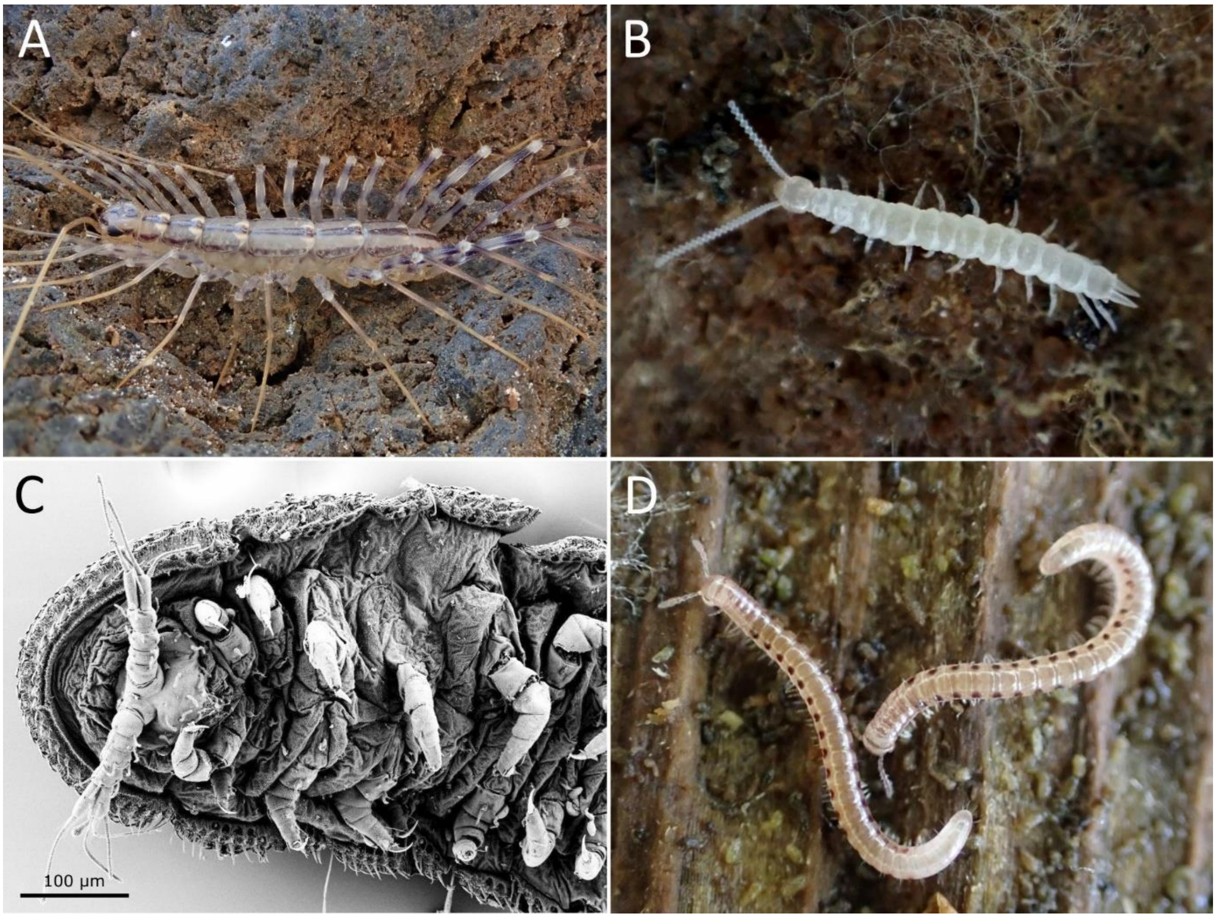

Figure 1. The diversity of the Myriapoda. **A.** *Scutigera coleoptrata* (Linnaeus, 1758) (Chilopoda: Scutigeromorpha) from Tenerife, Spain, photograph **B.** *Scutigerella* sp. (Symphyla) from Saxony, Germany, photograph. **C.** *Trachypauropus cordatus* (Scheller, 1974) (Pauropoda: Tetramerocerata) from North-Rhine-Westphalia, Germany, SEM image of anterior body, ventral view. **D.** *Proteroiulus fuscus* (Am Stein, 1857) (Diplopoda: Julida) from Lower Saxony, Germany, photograph. Not to scale.

Millipedes mainly inhabit the leaf-litter and upper soil layers of temperate, subtropical and tropical forests, but some species have also conquered more extreme habitats like deserts, tundra, caves and high mountains (Kime & Golovatch 2000; Golovatch & Kime 2009). Some "aquatic" millipedes can even be found (temporarily) in freshwater habitats (Adis 1986; Burrows et al. 1994). Often the habitat preference is reflected in the diverse shape of their body (Fig. 2) and distinct ecomorphotypes can be observed, such as cylindrical bodies in burrowers or wedge-shaped bodies in leaf-litter dwellers (Manton 1977; Golovatch & Kime 2009). Several millipede species are habitat specialists (David 2009) and some show specialized feeding habits, like carnivorous Callipodida (Hoffman & Payne 1969), filter feeding Julida (Enghoff 1985), or fungivorous Platydesmida (Macias et al. 2019). For many higher taxa (e.g. Polyzoniida, Siphonocryptida, Siphonophorida) the exact dietary habits are not known for any species. However, most millipedes are assumed to feed on dead organic matter (David 2015) and play a fundamental role in terrestrial ecosystems as detritivores and soil-forming organisms (Crawford 1992). They accelerate the decomposition of dead plant material, thus influencing the nutrient cycle within the soil (Cárcamo et al. 1999; Joly et al. 2020). This way millipedes facilitate the flux of magnesium, calcium, nitrate and carbon into the soil (Cárcamo et al. 1999; Kaneko 1999). Diplopoda might have played this role already since the Paleozoic more than 400 million years ago (ma), when they conquered the land among the first fully terrestrial animals (Wilson & Anderson 2004; Golovatch & Kime 2009). Furthermore, millipedes might also facilitate the dispersal of some algae, which pass undamaged through their gut, within the soil (Pakhomov et al. 2022). Due to their long evolutionary history and limited dispersal ability, millipedes are of high interest for biogeographic studies and show distinct biogeographic patterns.

Despite the great diversity (Fig. 2) and ecological importance of the Diplopoda, the group remains largely understudied in respect to its biology, taxonomy, morphology, phylogeny, and evolution. Therefore, the Diplopoda have been referred to as a "mega-diverse yet understudied Arthropod group" (Brewer et al. 2012a: 1), and it has been suggested that our knowledge regarding their morphology is "on par with that achieved in Crustacea 100 years ago and in Insecta 130 years ago" (Blanke & Wesener 2014: 63). Apparently the "major groups [...] remain *terra incognita*, with respect to their morphology" (Sierwald & Bond 2007: 414). In general, it can be said that these knowledge gaps greatly vary within Diplopoda across taxonomic groups and geographic areas (Brewer et al. 2012a), with a bias towards the more diverse taxa with larger representatives in Europe. Especially species poorer taxa received little attention, as they are often restricted to little studied areas, show a scattered distribution, or are minute in body-size. Furthermore, the taxonomy of some groups has been neglected as they lack well studied diagnostic characters like the highly species specific gonopods (legs in males modified for sperm transfer) of the Eugnatha. This is the case for the minute Polyxenida (< 200 species;

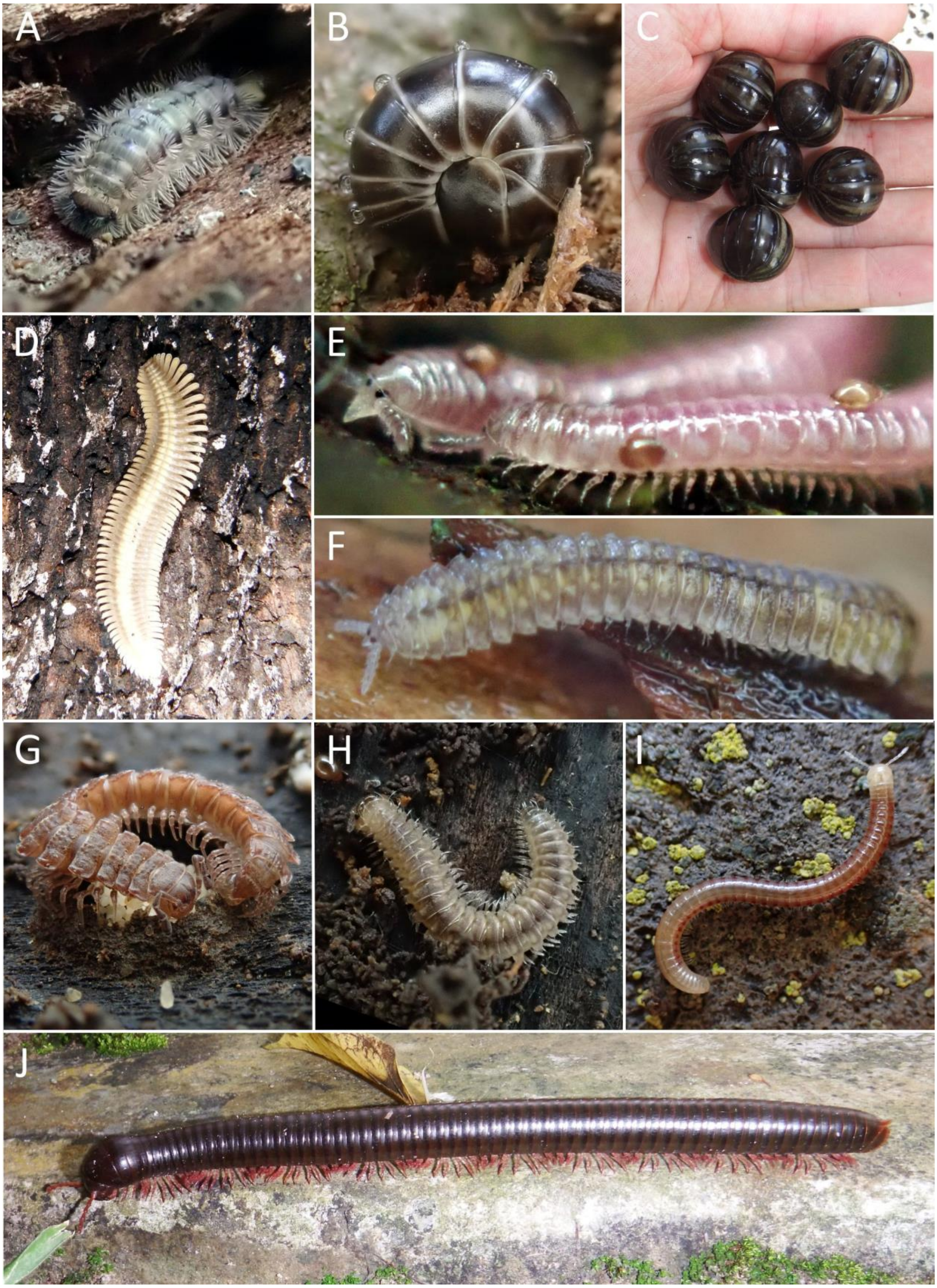

Figure 2. The diversity of the Diplopoda, 10 of the 16 millipede orders. **A.** Polyxenidae indet. (Polyxenida) from Armenia. **B.** *Glomeris marginata* (Glomerida) from Germany, releasing defense fluid from dorsal ozopores. **C.** Zephroniidae (Sphaerotheriida) from Thailand. **D.** Andrognathidae indet. (Platydesmida) from Thailand. **E.** *Rhinotus purpureus* (Polyzoniida) from the Botanical Garden in Bonn,

Germany. **F.** *Hirudicryptus canariensis* from Tenerife, Spain. **G.** *Polydesmus* sp. (Polydesmida) from Germany, female constructing an egg chamber. **H.** Chordeumatida from Germany. **I.** *Syrioiulus armeniacus* (Julida) from Armenia. **J.** Harpagophoridae indet. (Spirostreptida) from Thailand. Not to scale.

Short & Vahtera 2017) and the Pentazonia, including Glomeridesmida (36 species; Wesener et al. 2021) and Sphaerotheriida (ca. 350 species; Wesener 2016), which lack gonopods, and the Colobognatha (ca. 250 species; Enghoff et al. 2015), which often have weakly modified leg-like gonopods (Read & Enghoff 2009; Shorter et al. 2018). However, knowledge of the biology, morphology and fossil record of these little known taxa is essential for our understanding of the evolution of the morphological diversity (Fig. 2) observed in Diplopoda. Therefore, these taxa are studied in chapters 2 – 5, 7 and 8.

To better understand the evolution of the Diplopoda, and how certain characters and habits evolved in these groups it is essential to study the morphology of these taxa across a large taxon sampling covering its morphological diversity, also including extinct taxa. In the following, our current knowledge regarding the morphology, phylogeny, and evolutionary history of the Diplopoda will be summarized as a basis for the next chapters, which will discuss in detail the morphology, evolution and diversity of several neglected millipede groups and character systems, and implications for our understanding of their evolution.

1.2 The morphology of the Diplopoda

In zoology the terms ‘anatomy’ and ‘morphology’ are often used interchangeably for the study of the shape, structure and position of organs and body-parts. However, both words imply a distinct meaning and cannot be used synonymous (Snodgrass 1935, 1951; Gupta 1994). Anatomy, from the greek anátomé meaning cutting up or dissection, is the study of the shape, structure and position of organs within the body of an animal and aims to state structural facts. Morphology, from the greek words morphé (= shape or form) and logos (= word or discourse), aims to interpret structures and their relationship to each other, in a comparative, functional and/or evolutionary context, strongly linked to homology assumptions (Snodgrass 1935, 1951; Gupta 1994; Friedrich et al. 2014). Therefore, anatomy is mainly descriptive, while morphology is a mental construct focusing on why and how certain structures or forms evolved (Snodgrass 1935, 1951; Gupta 1994).

1.2.1 From drawings and dissections to modern imaging techniques

Over the past centuries a wide array of methods has been applied to study the anatomy and morphology of millipedes, reaching from classical dissection and histology to modern techniques, such as electron microscopy, fluorescence microscopy and micro-computed tomography (µCT). Studies of the anatomy of millipedes (Diplopoda), as integral part of taxonomic species descriptions, date as far back as Linnaeus (1758). However, detailed studies of their internal structure with classical dissections started in the 19th century (e.g. Treviranus & Treviranus 1807; Burmeister 1834; Brandt 1837; Newport 1841, 1843; Fabre 1855). These usually focused on certain organ systems (e.g. digestive system, nervous system, reproductive system, respiratory system) of single, often larger species. Later light-microcopy and histology, the sectioning and subsequent staining of embedded specimens or tissues, allowed the study of an organism's microanatomy, with a resolution of down to 0.2 µm (Kässens et al. 2015). Although the roots of histology reach as far back as the 17th century, it only became a well-established method in the study of organisms in the 19th century (Bracegirdle 1977), allowing the detailed investigation of organs and tissues. Thus, detailed histological studies of various millipede taxa and organ systems date back to the end of the 19th and beginning of the 20th century (e.g. Chatin 1882; Sazepin 1884; Bütschli 1885; vom Rath 1886a, b; Heathcote 1889; Rimsky-Korawkow 1895; Rossi 1901; Silvestri 1903; Sinclair 1905; Hennings 1906; Krug 1907; Ziegler 1907; Effenberg 1909; Reinecke 1910; Wernitzsch 1910). Most of these studies focused on the tracheal system or nervous system. In recent years, histological studies in millipedes have been further advanced with fluorescence microscopy, allowing the targeted investigation of the distribution of certain molecules within tissues (Sosinka et al. 2014; Rost-Roszkowska et al. 2018; Chikami & Yahata 2019).

The establishment of commercial electron microscopes and advances in sample preparation in the 1960s greatly advanced the field (Colliex 1998; Haguenau et al. 2003; Kässens et al. 2015) and allowed ultrastructural investigations of tissues and surfaces with a resolution below 1 nm (Wipfler et al. 2016), also in Diplopoda. Electron microscopy uses accelerated electrons, which are focused on an object. Transmission electron microscopy (TEM) relies on the scattering and diffraction of electrons, when passing through a thin object like histological sections. Scanning electron microscopy (SEM) relies on the interaction of the electrons with a surface, which mainly leads to the emission of secondary electrons, but also backscattered electrons and x-rays from the sample. These carry information about the object and can be measured with various detectors (Colliex 1998). In millipedes TEM has been mainly used for the study of glandular tissue and the nervous system (Barth 1967; Weatherstone & Percy 1969; El-Hifnawi & Seifert 1971, 1972, 1973; Seifert 1971; Müller et al. 2007; Sosinka et al. 2014; Rost-Roszkowska et al. 2018), while SEM has been used for the visualization of surfaces of cuticular structures like the mouthparts (e.g. Enghoff 1979, 1985; Köhler & Alberti 1990; Ishii & Tamura 1995; Semenyuk et al. 2011; Oeyen & Wesener 2018), and is today mainly used to document external characters in taxonomic studies (Cheung et al. 2013; Akkari et al. 2013).

Micro-computed tomography (µCT), which allows the three-dimensional in situ investigation of internal organs with a resolution down to 0.5 µm (Lin & Alessio 2009; Wipfler et al. 2016), was only employed since the last decade for the study of millipedes (Wojcieszek et al. 2012). In contrast, in entomology µCT has been used to study organ systems like the musculature since the early 2000s (Hörnschemeyer et al. 2002; Friedrich et al. 2014). In general, µCT relies on the acquisition of two-dimensional radiographic projections of an object around a rotation axis. For µCT a specimen is placed between an x-ray source and a detector, and x-ray images are taken at different angles while the object rotates around an axis. When passing through the specimen (object) x-ray photons are absorbed or scattered (attenuation), depending on the properties and especially density of the object's material. The detector measures the x-rays transmitted through the object, the so-called absorption contrast. In the resulting two-dimensional images (projections), the higher absorbing (denser) areas are represented by darker pixels, while the less absorbing (less dense) areas are shown as lighter pixels. This absorption can be influenced by adequate treatment of the specimen, such as the application of staining agents or drying. From these projections virtual cross-sections of the specimen are reconstructed using a so called filtered back-projection algorithm, resulting in a three-dimensional (3D) image, in which the absorption at each point within the specimen is represented by a voxel, which has a x-, y- and z-value (Stauber & Müller 2008; Boyd 2009; Keklikoglou et al. 2019). The resulting 3D-data can then be used to analyze and visualize the inner and outer structure of a specimen with rendering and segmentation software. Synchrotron-micro-computed tomography (SR-µCT) follows the same principle but uses synchrotron radiation and offers some advantages over conventional µCT. SR-µCT offers higher resolutions, down to 0.25 µm, less noise and scanning artefacts, faster data acquisition, and the option to use phase-contrast for the study of material properties (Betz et al. 2007). µCT and SR-µCT allow the detailed, non-destructive study of the internal morphology of a large number of specimens in a relatively short timeframe compared to histological methods (Stauber & Müller 2008; Friedrich et al. 2014). Therefore, also valuable and rare specimens from natural history collections can be studied in detail (Keklikoglou et al. 2019) or the same specimens can be investigated with other techniques (e.g. Histology, SEM) afterwards. Furthermore, it allows the detailed three-dimensional study of otherwise obscured specimens, such as fossil inclusions in amber (Dierick et al. 2007). However, compared to vertebrates and insects, µCT has only been employed in few studies on millipedes, including several studies of this dissertation (Wojcieszek et al. 2012; Blanke & Wesener 2014; Riquelme et al. 2014; Akkari et al. 2015; Liu et al. 2017; Moritz & Wesener 2017, 2019; Sagorny & Wesener 2017;

Moritz et al. 2018, 2021a, 2022; Oeyen & Wesener 2018; Wesener 2019; Stoev et al. 2019; Wesener et al. 2019; Moritz & Koch 2020; Naumann et al. 2020; Zahnle et al. 2020, 2022; Rosenmejer et al. 2021; Evsyukov et al. 2022; Bhansali & Wesener 2022).

The most comprehensive works on the morphology of the Diplopoda, treating a great variety of taxa and most organ systems comparatively, including a detailed review of the available literature at that time, have been provided by Silvestri (1903), Attems (1926), and Verhoeff (1928, 1932). These studies are still relevant today and are a "treasure trove for morphological characters" (Blanke & Wesener 2014: 73). However, most early works and many articles until the 1970s on millipede anatomy and morphology have been published in Italian (e.g. Rossi 1901; Silvestri 1903), French (e.g. Chatin 1882), German (e.g. Attems 1926; Verhoeff 1928; vom Rath 1886; El-Hifnawi & Seifert 1971) or Russian (e.g. Rimsky-Korsakow 1895). Therefore, these contributions are hard to access or ignored by the mainly English-speaking community, and Blanke & Wesener (2014: 64) even conclude that "Millipedes are one of the classes of organisms for which it appears that the scientific community has unfortunately lost knowledge compared to work done at the beginning of the last century (1903–1932)". However, a comprehensive overview of our current knowledge on the morphology of millipedes has been given by Hopkin and Read (1992), Dunger (1993) and especially Koch (2015).

1.2.2 General morphology of the Diplopoda

The body of the Diplopoda can be divided into a head and a trunk. The trunk consists of a highly variable number of units, reaching from ten in some Polyxenida (Short & Vahtera 2017) to 330 in the polyzonidan *Eumillipes persephone* Marek, 2021, which is with 1,306 legs (653 leg-pairs) the leggiest animal known (Marek et al. 2021). These units are mainly considered to represent diplosegments, carrying two pairs of legs, which gives the group the name Diplopoda (from greek dipló = double; pódi = foot). Exceptions are the first four post-cephalic units including the leg-less collum, followed by three halposegments with a single leg-pair each (Fig. 3A, B). This anterior region with collum and haplosegments is sometimes referred to as thoracic region (Latzel 1884; Verhoeff 1928; Koch 2015), while the remaining body, consisting of diplosegments and a posterior telson, has been referred to as abdomen by some authors (Latzel 1884; Verhoeff 1928). The units consist of a dorsal tergite, lateral pleurites, ventral leg-bearing sternites, and stigmatic plates, which are fused or reduced to varying degrees (Silvestri 1903; Wesener et al. 2014) (Fig. 3C – G). For example, the tergites, pleurites and sternites are completely free in Polyxenida, while these are fused to complete rings in Polydesmida (Fig. 3F), Juliformia (Fig. 3G) and Siphoniulida (Blower 1985; Sierwald et al. 2003). Thus, these units are often referred to as body-rings.

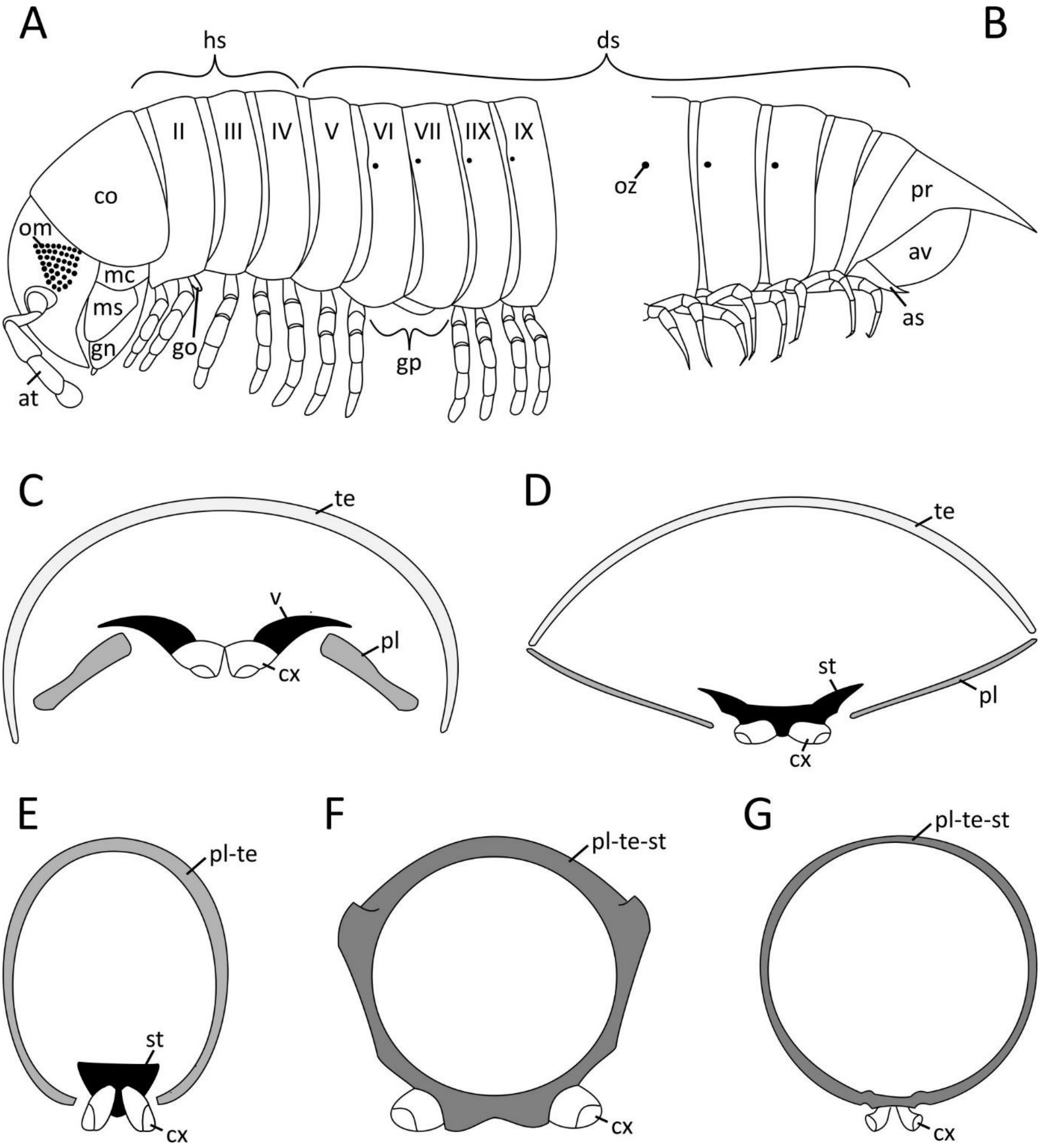

Figure 3. Habitus and body-ring architecture of the Diplopoda, based on Blower (1985: fig. 3), modified after Wesener et al. (2014). **A.** Anterior body of a Julidan millipede, lateral view. **B.** Posterior body of a Julidan millipede, lateral view. **C – G.** Cross-sections through the body-rings of different millipede taxa. **C.** Body-ring with free stigmatic plates, pleurites and tergites, as in Pentazonia. **D.** Body-ring with free sternites, pleurites and tergites, as in most Colobognatha. **E.** Body-ring with free sternites and fused pleurotergite as in Nematophora and some Colobognatha. **F.** Completely fused body-ring as in Polydesmida. **G.** Completely fused body-ring as in Juliformia. Not to scale. **Abbreviations:** as = anal scale, at = antennae, av = anal valve, co = collum, cx = coxa, dp = diplosegments, gn = gnathochilarium, go = gonopore, gp = gonopods (here leg-pair 8 and 9), hs = haplosegments, mc = mandibular cardo, ms = mandibular stipes, oz = ozopore, pl = pleurite, pr = preanal ring, st = sternite, te = tergite, v = stigmatic plate.

However, developmental studies show that there is no strict correspondence between the dorsal and ventral elements of these units in non-ring-forming millipedes, causing problems and controversy when defining segments (Brena 2015). Walking-legs adhere ventrally to the body-rings/segments and their podomeres are traditionally termed (from proximal to distal) coxa, prefemur, femur, postfemur, tibia and tarsus. In some taxa an additional ring, called trochanter, lacking intrinsic muscles, intercalates between coxa and prefemur (Verhoeff 1903). In mature males single or several leg-pairs can be modified, like the posterior chelae-like telopods of the Pentazonia (Glomeridesmida, Glomerida, Sphaerotheriida), or the gonopods (one to four modified leg-pairs of body-rings seven and eight) of Helminthomorpha. The latter are used for indirect sperm transfer between the gonopores of males and females, which are located behind the second leg pair (Verhoeff 1928) (Fig. 3A). These highly specialized appendages are in most taxa of taxonomic value and their structure can be specific for species, genera or even families (Akkari et al. 2013). The posterior most leg-less unit of the body is termed telson and consists of a preanal sclerite or ring, which surrounds the anal valves (paraproct), and sometimes a ventral sclerite called subanal plate or hypoproct (Verhoeff 1928) (Fig. 3B). In some millipede taxa the posterior dorsal tip of the preanal ring (the epiproct) carries spinnerets, which produce threads (Adis et al. 2000; Shear 2008). The architecture and shape of the collum, body-rings and legs often correspond to certain habits of the millipedes and so called eco-morphotypes can be distinguished, including bulldozers, wedge types, borers, rollers and bark dweller (Golovatch & Kime 2009).

The head of the millipedes, which is studied in detail in chapters 2, 3, 4 and 6, carries the mouthparts (mandibles, gnathochilarium) and the main sensory organs (eyes, antennae, Tömösváry organ), and is therefore vital for feeding and the perception of the environment. The shape and structure of the head can greatly vary between the different Diplopoda taxa but follows a common pattern (Fig. 4). The head is formed by a dorsal head capsule, carrying the antennae, ommatidia and Tömösváry organs, laterally visible mandibles, and a gnathochilarium, which closes the head ventrally (Fig. 4A). In contrast to insects, the head capsule of the Diplopoda lacks sutures, which clearly delimit certain areas (Verhoeff 1928). Therefore, the division into areas mainly relies on their relative position to each other, to cephalic appendages (antennae, mandibles, gnathochilarium), to the eyes (ommatidia) and Tömösváry organ, and to the incisura lateralis, which incises the head capsule laterally (Fig. 4A).

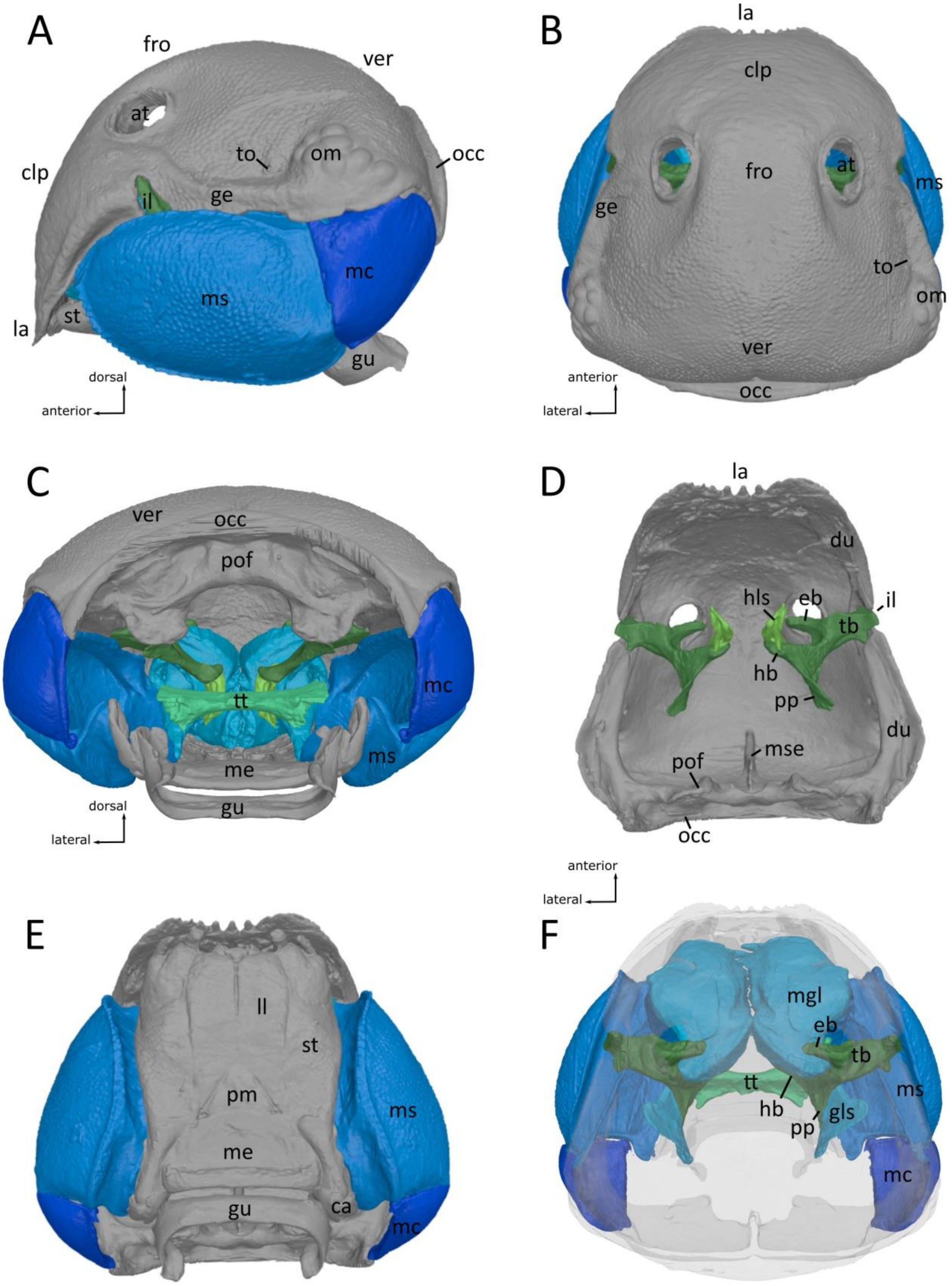

Figure 4. Exemplary structure of the head of a Diplopoda, here *Striaria* sp. (Chordeumatida; ZFMK-MYR02017). **A.** Lateral view. **B.** Dorsal view. **C.** Posterior view. **D.** ventral view, mandibles and gnathochilarium removed. **E.** Ventral view. **F.** Dorsal view, head capsule and gnathochilarium transparent. Not to scale. **Abbreviations:** at = insertion of antenna, ca = gnathochilarial cardine, clp = clypeus, du = ventral duplicature of the head capsule, eb = tentorial epipharyngeal bar, fro = frons, ge =

genae, gls = gnathal lobe sclerite, gu = gula, hb = tentorial hypopharyngeal bar, hls = hypopharyngeal lateral sclerite, il = incisura lateralis, la = labrum, ll = gnathochilarial lamella lingualis, mc = mandibular cardo, me = gnathochilarial mentum, mgl = mandibular gnathal lobe, ms = mandibular stipes, mse = median septum, occ = occiput, om = ommatidia, pm = gnathochilarial promentum, pof = postoccipital flange, pp = tentorial posterior process, st = gnathochilarial stipes, tb = transverse bar, to = Tömösváry organ, tt = transverse mandibular tendon, ver = vertex.

Typically, the millipede head is divided into a forehead with the anterior upper lip (labrum), carrying teeth, and the clypeus extending posteriorly to the antennal socket and incisura lateralis. This area is followed by the frons extending between the antennal sockets and ommatidia, and the epicranium, comprising the vertex and the occiput (Fig. 4B, C). The epicranium gives internally rise to a median septum (Fig. 4D), which serves as muscle attachment site. Posteriorly the head capsule bends inwards, forming a postoccipital flange, which gives rise to a so-called neck phragma (Fig. 4C) in some groups and serves for muscle attachment. The area lateral of the antennae is called genae. The head capsule forms a more or less pronounced duplicature at its ventral margin, which faces the mandibles and forms the underside of the capsule (Verhoeff 1928; Manton 1964; Koch 2015) (Fig. 4D). The shape of the head capsule can greatly vary between different millipede taxa, being large and spherical in most taxa, flattened and drawn out laterally in some Pentazonia, or small and conical in some Colobognatha (Silvestri 1903; Verhoff 1928) (Fig. 5). On its underside, the forehead extends into a membranous wall, the epipharynx, which extends from the labrum to the pharyngeal opening and forms the dorsal roof of the so called preoral chamber. The preoral chamber is a cavity anterior of the actual mouth opening, in which food is physically processed by the mandibles before ingestion (Fechter 1961; Köhler & Alberti 1990). The preoral chamber is closed ventrally by the hypopharynx and the underlip, called gnathochilarium, preventing food from falling out of the preoral cavity (Fechter 1961) (Fig 4E). The gnathochilarium consist of several externally visible sclerites (mentum, cardines, stipites, lamellae lingualis), which can be fused or reduced to varying degrees (Verhoeff 1928).

The mandibles of the Diplopoda insert laterally between head capsule and gnathochilarium and always consist of at least two parts: a usually laterally visible base, and a separately movable gnathal lobe, extending into the preoral cavity (Figs 4A, 5). The base can be further subdivided in some taxa into a cardo and a stipes (not to be confused with the cardo and stipes of the insect maxillae), which are jointed to each other (Koch 2015). The base is articulated to the gnathal lobe, which is typically divided into different areas, fulfilling certain functions during feeding.

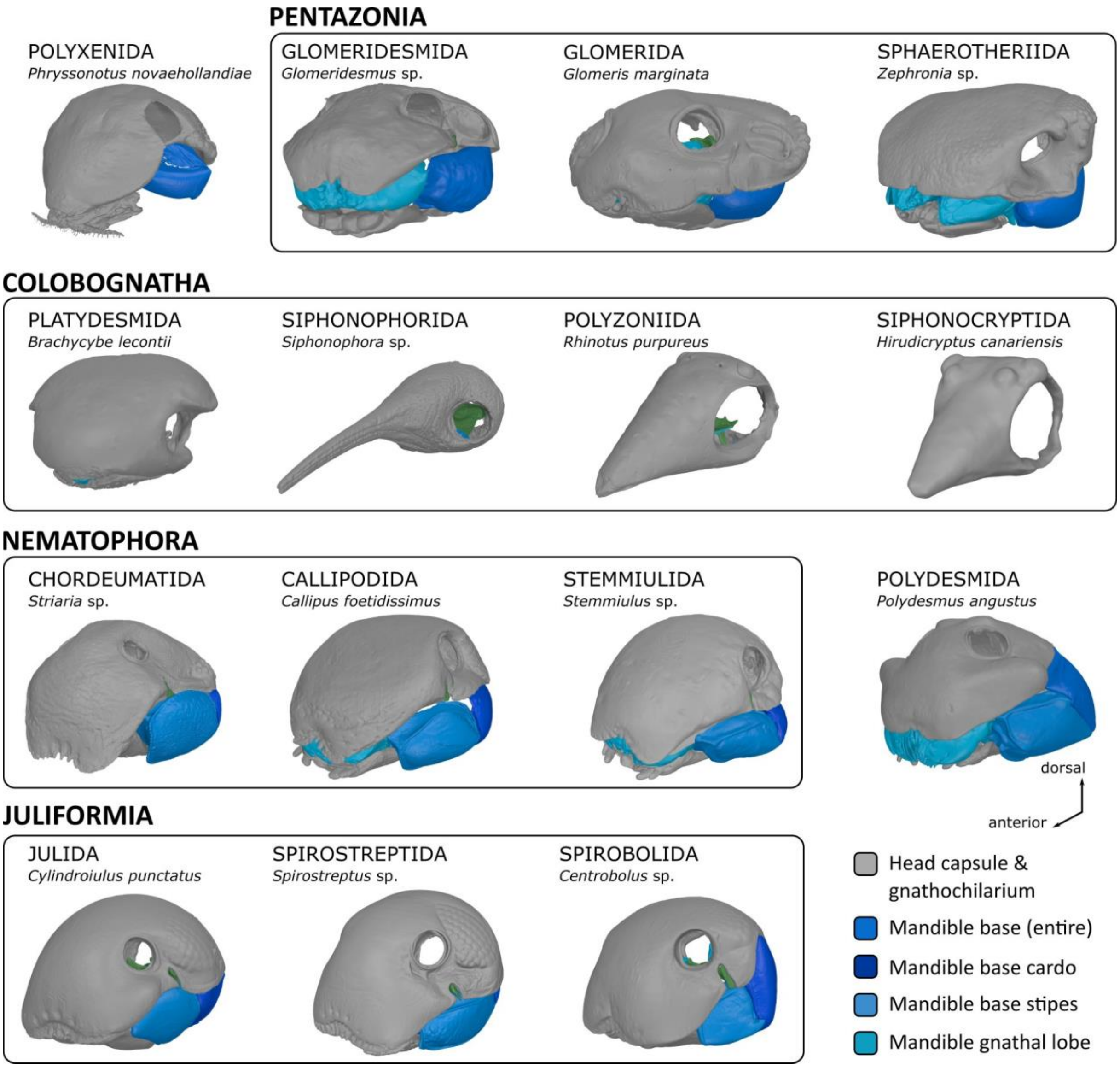

Figure 5. Variation of the head within the Diplopoda, fronto-lateral view. Boxes indicate the classically recognized higher taxa (e.g. Enghoff et al. 2015). Segmentation of the head capsule, gnathochilarium and mandibles based on partly unpublished µCT data, as described in Moritz et al. 2021 (chapter 2). Note in Colobognatha the mandibles are internalized and not visible externally.

The gnathal lobe can carry apical teeth, used for cutting large pieces, comb-like pectinate lamellae, used to crush and further shred food, and a large molar plate (Fig. 6), used to grind food and to squeeze liquids out of the food (Köhler & Alberti 1990; Semenyuk et al. 2011). The structure of the gnathal lobe and its modifications can be diagnostic for taxa and might correlate to certain feeding preferences (Semenyuk et al. 2011). For example, in the colobognathan taxa certain areas are reduced in size or completely absent (Ishii & Tamura1995), and in some hydrophilous Julida the pectinate lamellae are hypertrophied and function as filter (Enghoff 1985). While the mandibular gnathal lobe received a lot of attention from a taxonomic, as well as functional perspective (e.g. Enghoff 1979; Enghoff 1985; Köhler & Alberti 1990; Ishii & Tamura 1995; Semenyuk et al. 2011; Oeyen & Wesener 2018), the structure and function of the mandibular base has only been studied for few taxa in detail (e.g. Silvestri 1903; Fechter 1961; Manton 1964).

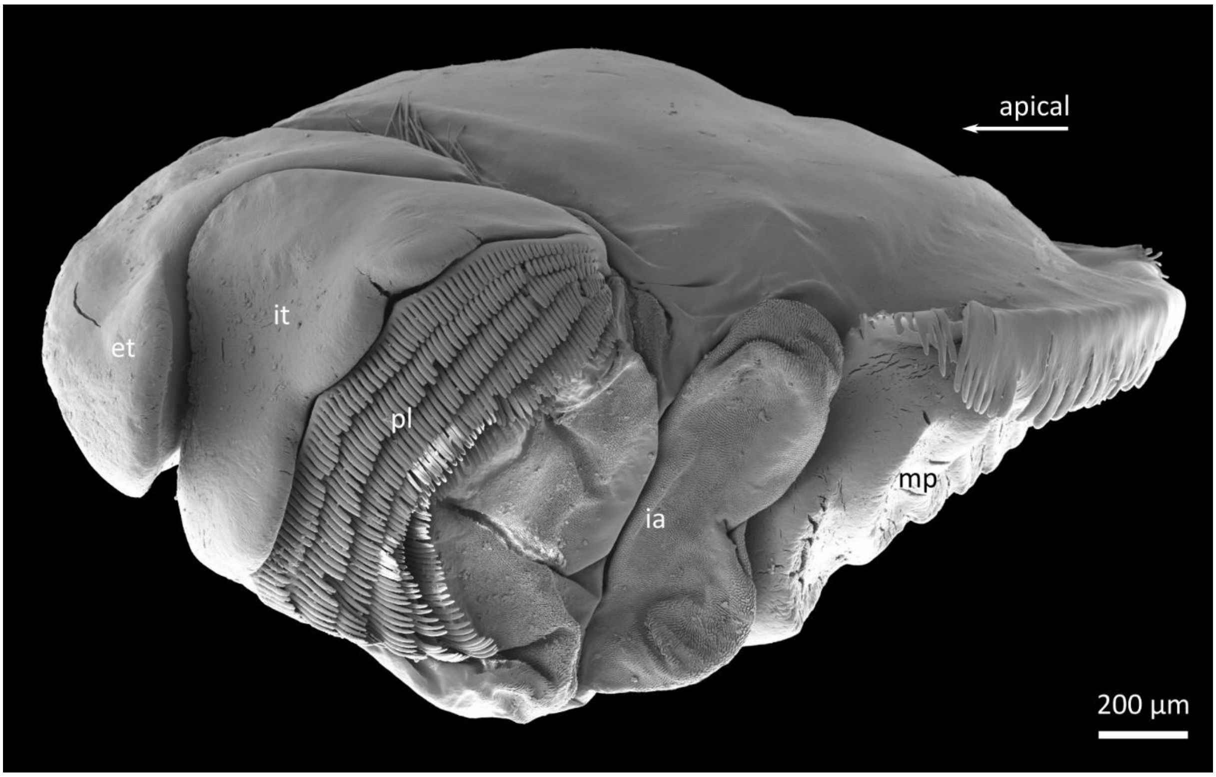

Figure 6. The mandibular gnathal lobe of the biting-chewing flat-backed millipede *Coromus vittatus* (Cook, 1896) (Diplopoda: Polydesmida). Abbreviations: et = external tooth, ia = intermediate area, it = internal tooth, mp = molar plate, pl = pectinate lamellae.

Internally, the wall of the preoral chamber is supported by the tentorial complex (Fig. 4F), a strongly sclerotized cuticular structure, which extends partially into the head lumen and serves as muscle attachment site for antennal, mandibular, gnathochilarial and pharyngeal muscles (Fechter 1961; Manton 1964; Wilson 2002; Koch 2003). Based on few eugnathan species studied (Silvestri 1903; Fechter 1961; Manton 1964; Wilson 2002; Naumann et al. 2020), the tentorial complex and its movement are assumed to be involved in the abduction of the gnathochilarium and mandibles, which lack, contrary to insects (e.g. Weihmann & Wipfler 2019), direct abductor muscles. It is assumed that the tentorium can be moved forward and downward by the tentorial muscles. This movement is translated onto the gnathochilarium, which is lowered, and onto the gnathal lobes, which are moved apart (Fechter 1961). Only a single muscle, the mandibular adductor muscle, inserts directly on the gnathal lobe and is responsible for closing it, while a large number of muscles inserts on the mandibular base. The main attachment site for the muscles of the mandibular base is formed by the tentorial complex and the transverse mandibular tendon, a collagenous endoskeleton. Our knowledge of the internal morphology of the millipede head and its skeletomuscular system is mainly based on the study of few highly derived eugnathan taxa (Silvestri 1903; Fechter 1961; Manton 1964;

Wilson 2002; Naumann et al. 2020). Most millipedes follow this pattern and feed on solid food with biting-chewing mouthparts, moved with the assumed mechanism described above. However, the internal morphology of the heads of less diverse, and smaller bodied taxa (e.g. Polyxenida, Pentazonia, Colobognatha, Siphoniulida) remains largely unknown. It has been suggested that in some groups special feeding mechanisms evolved. For example the Colobognatha, studied in chapters 2 and 3, have long been assumed to feed suctorial on rather liquid food (Cook & Loomis 1928; Brandt 1834; Manton 1961; Dunger 1993) based on their external morphology and the structure of the partly reduced and internalized mandibles. Yet they have not been observed feeding and the internal morphology of their feeding apparatus remained unknown. It has even been suggested that the Siphonophorida, which have a long beak, can bore into trees to feed on tree sap (Verhoeff 1941). Knowledge of these lesser known taxa's internal morphology, studied in chapters 2 – 4, is essential for our understanding of the feeding mechanism and evolution of the Diplopoda and certain character systems.

1.3 Diplopoda phylogeny

Phylogenetics is the study of the evolution and relationship of taxa and aims to infer their position within a phylogenetic tree (Wägele 2005). To understand how certain groups and characters evolved, these have to be studied in a phylogenetic context. Recent phylogenetic hypothesis based on molecular and morphological data place the Diplopoda within the Arthropod subphylum Myriapoda (Fig. 7A), which also includes the centipedes (Chilopoda), garden centipedes (Symphyla) and Pauropoda (Regier et al. 2005, 2010; Miyazawa et al. 2014; Szucsich et al. 2020) (Fig. 1). However, several previous analyses recovered a paraphyletic Myriapoda, branching paraphyletic to Hexapoda (Kraus & Kraus 1994; Kraus 2001) or Chelicerata (Negrisolo et al. 2004). Myriapod monophyly is only supported by few morphological characters (Manton 1964), which mainly include negative characters, like the absence of median eyes, the absence of a crystalline cone in the ommatidia, the absence of a perforatorium in the sperm, and the absence of scolopidial mechanoreceptors (Dohle 1988; Edgecombe 2004). Other putative autapomorphies of the Myriapoda are the bipartite mandible, divided into base and gnathal lobe, and the so called 'swinging tentorium' (Manton 1964; Koch 2003; Edgecombe 2004). The latter refers to the cephalic endoskeleton, the tentorial complex, which supports the preoral chamber and serves in the abduction of the mandibles, via its unique swinging movement (Koch 2003) outlined above. However, the tentorial complex has only been studied in few taxa, and its structure varies across Diplopoda as is demonstrated in chapters 2 – 4 and 6. Regardless of Myriapoda monophyly, there is molecular (Fernández et al. 2018) and morphological (Kraus & Kraus 1994; Ax 1999; Kraus 2001, Edgecombe 2004) support for a monophyletic Progoneata (Symphyla + Pauropoda +

Diplopoda) and Dignatha (Diplopoda + Pauropoda) (Fig. 7A, B). However, recent molecular studies (Szucsich et al. 2020; Wang et al. 2021; Benavides et al. 2022) support a monophyletic Edafopoda (Pauropoda + Symphyla), and a monophyletic Pectinopoda (Diplopoda + Chilopoda), rejecting the Dignatha and Progoneata (Fig. 7C).

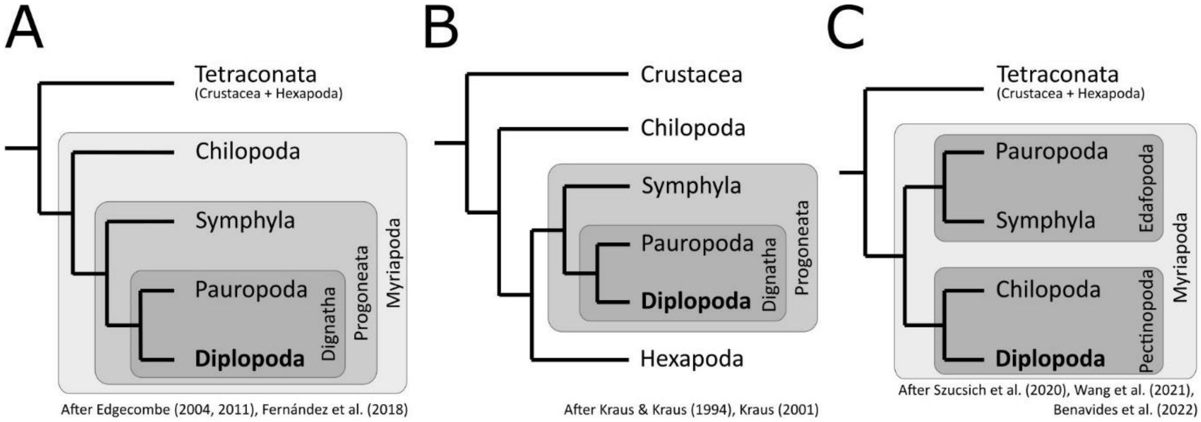

Figure 7. Some of the competing phylogenetic hypotheses of the Myriapoda. **A.** Relationship after Edgecombe (2004, 2011) and Fernández et al. (2018). **B.** Relationship after Kraus & Kraus (1994) and Kraus (2001). **C.** Relationship after Szucsich et al. (2020), Wang et al. (2021) and Benavides et al. (2022).

Monophyly of the Diplopoda is well supported by molecular (Regier et al. 2005; Miyazawa et al. 2014; Rodriguez et al. 2018; Benavides et al. 2022) and morphological (Koch 2003; Edgecombe 2004, 2011) data. However, it remains ambiguous how the millipede taxa are related, and several competing hypotheses have been proposed. In all phylogenetic analyses the Polyxenida are retrieved as sister group to the remaining millipedes, the Chilognatha. Within Chilognatha, the Pentazonia (Glomeridesmida, Glomerida, Sphaerotheriida) are retrieved as sister to Helminthomorpha, in which the Colobognatha (Platydesmida, Polyzoniida, Siphonocryptida, Siphonophorida) are retrieved as sister to the Eugnatha (Enghoff 1984; Enghoff et al. 1993; Sierwald et al. 2003; Regier et al. 2005; Sierwald & Bond 2007; Blanke & Wesener 2014; Miyazawa et al. 2014; Rodriguez et al. 2018; Benavides et al. 2022). Although most phylogenetic hypotheses agree on these higher-ranking clades, the relationships within these groups are controversial and groupings are often only supported by single or few apomorphies (e.g. Sierwald & Bond 2007; Blanke & Wesener 2014).

In Pentazonia, the Glomerida either group with Glomeridesmida based on molecular and total evidence analyses (Regier et al. 2005; Sierwald & Bond 2007; Rodriguez et al. 2018; Benavides et al. 2022) or with Sphaerotheriida based on morphological data (Sierwald et al. 2003; Blanke & Wesener 2014). The resolution of the Pentazonia has consequences for the interpretation how volvation evolved in Glomerida and Sphaerotheriida, as is discussed in chapter 4. Among the pentazonian taxa, only for the Sphaerotheriida apomorphic characters have been well worked out with 39 potential apomorphies (Wesener 2016), while for the other two taxa, especially Glomeridesmida, only few apomorphies (often reductive traits) are known as discussed in chapter 5.

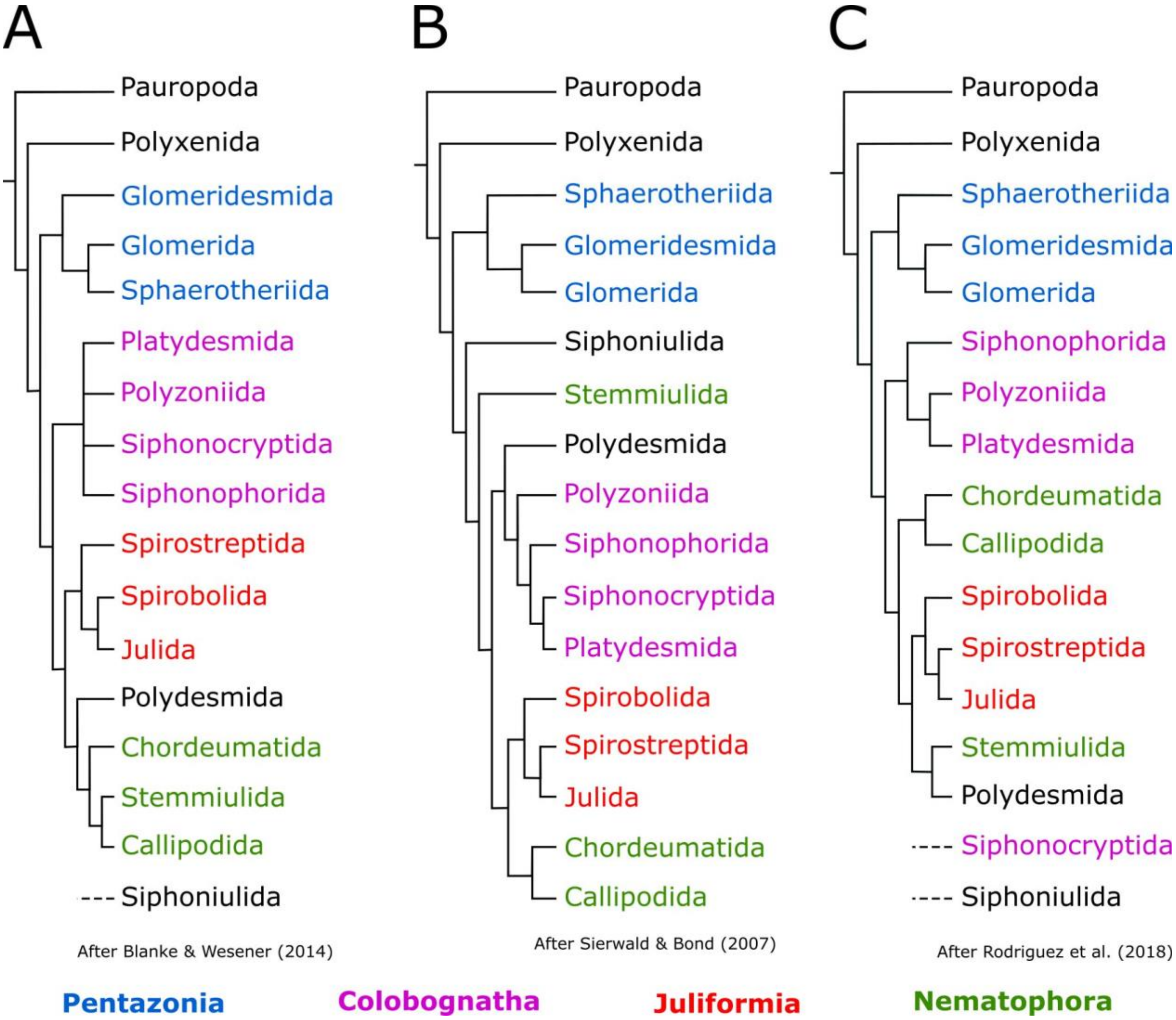

Figure 8. Competing hypotheses on the phylogeny of the Diplopoda orders. **A.** Phylogenetic relationship of 15 orders (not including Siphoniulida) based on 64 morphological characters, after Blanke & Wesener (2014). **B.** Phylogenetic relationship of 16 orders based on a total evidence analyses of 41 morphological characters and 1459 amino acid residues (molecular data for Siphonocryptida and Siphoniulida lacking), after Sierwald & Bond (2007). **C.** Phylogenetic relationship of 14 orders (not including Siphoniulida and Siphonocryptida) based on transcriptomic data, after Rodriguez et al. (2018). Dotted lines indicate taxa not included in study; colors represent the traditionally recognized higher taxa (e.g. Enghoff et al. 2015).

Within Colobognatha the relationship is either unresolved (Sierwald & Bond 2007; Blanke & Wesener 2014), or Platydesmida group either with Polyzoniida (Regier et al. 2005; Rodriguez et al. 2018; Benavides et al. 2022), Siphonophorida (Sierwald et al. 2003; Miyazawa et al. 2014), or Siphonocryptida (Sierwald & Bond 2007). The latter is a special case as the ordinal status of Siphonocryptida, which has been proposed by Shelley (2002) as the 16th millipede order, is highly questionable, and Siphonocryptida might rather need to be included within

Polyzoniida, as traditionally done (e.g. Enghoff & Golovatch 1995). The main problem in resolving Colobognatha is the limited knowledge we have of the morphology and biology of these millipedes, and the limited taxon sampling in molecular studies with no data for the Siphonocryptida. This is mainly due to the generally low interest in the group by taxonomists (Brewer et al. 2012a), their minute size, which complicates examination, and the paucity of material, as many groups show a limited relictual distribution and have not been collected since their initial discovery. However, chapter 2 and 3 examine in detail the head morphology of all colobognathan taxa and discuss these characters in a phylogenetic and functional context.

Within Eugnatha commonly the taxa Juliformia (Julida, Spirobolida, Spirostreptida) and Nematophora (Callipodida, Chordeumatida, Stemmiulida) have been retrieved based on morphological data (Sierwald et al. 2003; Blanke & Wesener 2014). However, analyses of molecular data retrieve a polyphyletic Nematophora with the Stemmiulida at varying positions (Regier et al. 2005; Sierwald & Bond 2007; Rodriguez et al. 2018; Benavides et al. 2022). Furthermore, the Polydesmida either group with Colobognatha (Regier et al. 2005; Sierwald & Bond 2007), a monophyletic Nematophora (Sierwald et al. 2003; Blanke & Wesener 2014) or Stemmiulida (Rodriguez et al. 2018; Benavides et al. 2022). The position of the poorly known Siphoniulida, which show a mix of morphological characters otherwise only found in Colobognatha, Juliformia, or Nematophora, remains unknown (Sierwald et al. 2003).

1.4 Diplopoda fossil record

Today living species represent only a fraction of the overall diversity, which existed over time. Therefore, the present-day biota does not necessarily reflect the whole morphological and ecological diversity of a taxon and can be misleading when analyzing the origin of a group. The fossil record offers windows into the past to study how the diversity, ecology and morphology of a taxon changed over time and therefore, is essential to understand trends in the evolution of certain characters and groups in space and time (Jablonski & Shubin 2015). However, the fossil record is usually incomplete (Gingerich 1985), and its quality largely differs over time and groups. It is affected by various filters, which determine whether fossil remains are preserved until today. These filters concern the anatomy and biology of the organism itself, as well as the environmental conditions during and after fossilization (Benton & Harper 2009). This is also the case for the Myriapoda, which show large differences in the extant of the fossil record between timespans as well as between taxonomic groups (Shear & Edgecombe 2010).

The fossil record of the Diplopoda dates back at least to the Devonian ca. 414 million years ago (ma) with the fossils of *Pneumodesmus newmani* Wilson & Anderson, 2004 and *Cowiedesmus eroticopodus* Wilson & Anderson, 2004 (Wilson & Anderson 2004; Suarez et al.

2017; Brookfield et al. 2021). *Cowiedesmus eroticopodus* already had gonopods, which are characteristic for the derived Helminthomorpha (Wilson & Anderson 2004). If these gonopods are homologous to those of Helminthomorpha, it can be assumed that the origin of the Diplopoda dates back far before. Analyses of molecular data, time-calibrated with fossils, suggest that the Diplopoda diversified in the Ordovician more than 450 ma (Fernández et al. 2018; Rodriguez et al. 2018; Benavides et al. 2022), which coincides with fossils of Myriapod-like trackways (Johnson et al. 1994). Thus, millipedes might have been among the first fully terrestrial animals. The Paleozoic (541 – 251.9 ma) Diplopoda fauna is characterized by today extinct taxa, like the extinct Arthropleuridea, Amynilyspedida, Archypolipoda, Pleurojulida and Xyloiulidea. Not a single extant order is known from the Paleozoic. In contrast the Chilopoda are already in the Paleozoic represented by the two extant orders Scutigeromorpha and Scolopendromorpha, and the single extinct order Devonobiomorpha (Shear & Edgecombe 2010).

These Paleozoic taxa mostly disappeared by the end of the era, with only few Mesozoic (251.9 – 66 ma) representatives known from the Triassic (Shear et al. 2009; Dzik 1981; Shear & Edgecombe 2010). Furthermore, there are a few specimens of uncertain placement known from the Mesozoic, which are referred to as *incerta sedis* (Jell 1983; Shear & Edgecombe 2010). Only a handful of millipede fossils, mainly from the Cretaceous, have been described from the Mesozoic, and could be placed in extant orders (Cockerell 1917; Dzik 1975; Nguyen Duy-Jacquemin & Azar 2004; Liu et al. 2017). Therefore, it can be assumed, that a faunal shift from the Paleozoic towards modern taxa took place at the transition from the Paleozoic to the Mesozoic. In contrast, from the Cenozoic (66 ma – present) a large number of millipede fossils, belonging to extant families, are known (Bachofen von Echt 1942; Pierce 1946, 1951, 1957; Shear 1981; Santiago-Blay & Poinar 1992; Donovan & Veltkamp 1994; Duncan 1998).

This means, that the Mesozoic, reaching from 251.9 ma to 66 ma, is largely a 185.9 million years long gap in our knowledge on millipede diversity and evolution, with only few scattered fossils. However, knowledge of the Diplopoda fauna of this era is essential to understand when, where and how the extant orders observed today diversified and evolved.

One of the most promising Mesozoic deposits, which might help to bridge this gap to some extent, is the Cretaceous Burmese amber from Myanmar, which dates back ca. 99 ma (Shi et al. 2012). This deposit yielded many exceptionally well preserved inclusions, which greatly contributed to our understanding of the evolution of certain taxa and especially Arthropods (Bai et al. 2016, 2018; Wang et al. 2018; Jouault et al. 2021; Pohl et al. 2021). This also included several millipedes (Cockerell 1917; Nguyen Duy-Jacquemin & Azar 2004; Liu et al. 2017), Chilopoda and Symphyla, of which some have been described and documented during this study (chapters 7 & 8). The high quality of three-dimensional preservation allows a detailed study of these inclusions and a comparison to extant representatives, as the external morphology of the fossils can be described almost in as much detail as that of extant species. This is enhanced even further by the combination of classical examination via light-microscopy with modern imaging techniques like µCT (e.g. Dierick 2007; Liu et al. 2017) and confocal laser scanning microscopy (e.g. Cai & Huang 2014; Fu et al. 2021).

1.5 Aims and scope

The series of studies presented here aims to contribute to our knowledge and understanding of the morphology of the Diplopoda in an evolutionary and functional context. Thus, I anticipate to close some of the gaps in our knowledge of the Diplopoda regarding the morphology of neglected taxa and character systems, as well as their fossil record. For our understanding of the evolution of the Diplopoda and their morphology, it is essential to study the whole morphological diversity of the group in detail, including the colobognathan and pentazonian taxa (chapters 2 – 5), which received little attention so far. Furthermore, character systems previously employed are restudied in detail (chapter 6) as conflict remains within the literature on their distribution and structure across Diplopoda. Extinct taxa (chapter 7 & 8) need to be considered when discussing the morphology and evolution of the group, as the millipede diversity observed today only represents a fraction of their overall diversity. This thesis aims to elucidate how, why, and when certain morphological features, especially regarding the head and feeding apparatus, arose within Diplopoda.

Chapter 2 – First steps toward suctorial feeding in millipedes: Comparative morphology of the head of the Platydesmida (Diplopoda: Colobognatha)

2.1 Bibliography

Leif Moritz, Alexander Blanke, Jörg U. Hammel & Thomas Wesener (2021) First steps toward suctorial feeding in millipedes: Comparative morphology of the head of the Platydesmida (Diplopoda: Colobognatha). *Invertebrate Biology* 140: e12312.

https://doi.org/10.1111/ivb.12312

2.2 Summary

Most millipedes (Diplopoda) feed with biting-chewing mouthparts on dead plant material (Manton 1964, 1977). However, the Colobognatha (Platydesmida, Polyzoniida, Siphonocryptida, Siphonophorida) are supposed to feed on softer and/or liquid food with their partly reduced mouthparts (Verhoeff 1914; Cook & Loomis 1928; Brandt 1834; Manton 1961; see also chapter 3). The phylogeny of the Colobognatha remains controversial. Almost all possible combinations within Colobognatha (Sierwald et al. 2003; Sierwald & Bond 2007; Rodriguez et al. 2018) as well as polyphyly (Hoffman 1982) have been proposed. The Platydesmida are a key taxon to understand the evolution of the colobognathan head and mouthparts, as these millipedes, among Colobognatha closest resemble the condition observed in biting-chewing millipedes (Verhoeff 1914). However, the platydesmidan head and especially its internal morphology received only little attention (Sinclair 1905; Blanke & Wesener 2014), impeding further understanding of mouthpart evolution in millipedes. Thus, the skeletomuscular system of the head has only been described in detail for few eugnathan species (Silvestri 1903; Fechter 1961; Manton 1964; Wilson 2002; Naumann et al. 2020).

In this study the morphology of the head and its skeletomuscular system was studied for six platydesmidan species in the four genera *Brachycybe* Wood, 1864, *Dolistenus* Fanzago, 1874, *Gosodesmus* Chamberlin, 1922 and *Pseudodesmus* Pocock, 1887, to better understand the evolution of the colobognathan mouthparts and to investigate characters, which can help to resolve the phylogeny of the Diplopoda. Therefore, SR-µCT and µCT data was obtained, and the skeletomuscular system was 3D-segmented. Furthermore, the potential opening angles of the mandibles were measured to infer a possible feeding mechanism.

This study shows that the skeletomuscular system of the Platydesmida head differs markedly from that of eugnathan millipedes, while the head within Platydesmida appears largely homogeneous in its structure. The head of the Platydesmida is pyriform, widens posteriorly, overlapping the collum, and its genae, lateral of the antennae, are largely bulged to accommodate the internalized mandibles (entognathy). Variation of the head shape mainly concerns the epicranium and genae within Platydesmida. The former may extend laterally above the antennae as is the case in *Brachycybe*, *Gosodesmus* and *Pseudodesmus*, while the latter varies in its curvature. Compared to biting chewing millipedes (Fechter 1961; Manton 1964; Wilson 2002; Naumann et al. 2020) the mandibular musculature and its attachments sites are reduced in Platydesmida. A median septum is absent and the collagenous transverse mandibular tendon, which is the main attachment site for mandibular muscles in non-colobognathan millipedes, is reduced in size. It forms a small bridge spanning between the tentorial posterior processes. The muscles typically associated to the transverse mandibular tendon are absent and all mandibular muscles originate from the tentorial complex and the head capsule. The narrow and elongated mandibular base consists of two separate elements (cardo and stipes), as is the case in the Eugnatha, while the gnathal lobe and its armature is partially reduced compared to non-colobognathan millipedes.

Based on the structure of the mandibles, their musculature, and the measured opening angle of the gnathal lobes, we suggest that the platydesmidan mandibles do not perform a pronounced biting chewing movement as is typical for non-colobognathan millipedes. We suggest that the mandibles are rather used for scraping as suggested already by Verhoeff (1914). Previously it has been suggested that the shape of the foregut (with invaginations) might enable the Platydesmida to suctorial feeding (Wong et al. 2020). However, the shape of the foregut of the Platydesmida does not differ markedly from that of other millipede taxa (Fontanetti et al. 2015). We suggest that negative pressure is mainly generated by the pharyngeal dilator muscles, although a real sucking pump, as in other suctorial arthropods, is absent in Platydesmida. Thus, the term slurping ("Schlürfen" sensu Verhoeff 1914: 353) might best describe the feeding mode of the Platydesmida.

The Platydesmida share several characters separating them from other Diplopoda, like tergites with a dorsal suture, fused pleurotergites, an epicranium overlapping the collum, partially internalized mandibles, a transverse mandibular tendon reduced in size, and the absence of an incisura lateralis. As the position of the Platydesmida remains controversial a phylogenetic analysis of the Diplopoda including all Colobognatha, and detailed examinations of the head morphology of the remaining Colobognatha (see chapter 3) are needed, to clarify whether these characters are autapomorphies of Platydesmida or Colobognatha, or plesiomorphic characters. Some of these characters might represent an intermediate state towards the strong modifications seen in the remaining Colobognatha, which might enable suctorial feeding.

The published article is the result of a collaboration of Leif Moritz with Thomas Wesener (ZFMK/LIB), Alexander Blanke (IEZ) and Jörg U. Hammel (DESY, Hereon). LM prepared the specimens. Measurements were performed by LM and JUH at the DESY. For *Brachycybe lecontii* µCT data from Blanke & Wesener (2014) was used. LM evaluated the data and performed 3D-segmentation. All authors were involved in the interpretation of the data. Figure plates and the first draft of the manuscript were prepared by LM, and subsequently edited by all authors.

Chapter 3 – A previously unknown feeding mode in millipedes and the convergence of fluid feeding across arthropods

3.1 Bibliography of published article

Leif Moritz, Elena Borisova, Jörg U. Hammel, Alexander Blanke & Thomas Wesener (2022) A previously unknown feeding mode in millipedes and the convergence of fluid feeding across arthropods. *Science Advances* 8: eabm0577.

https://doi.org/10.1126/sciadv.abm0577

3.2 Summary

Fluid-feeding is widespread among arthropods, and present in arachnids, which externally digest their prey (Snodgrass 1948), some parasitic crustaceans (Günther 1931), and fluid-feeding insects, which contribute to half of the insect diversity (Krenn 2019). The majority of millipedes (Diplopoda) possess prominent biting-chewing mandibles, which are used to fragmentate dead plant-material (Manton 1964). However, for a small group of millipedes, the Colobognatha (ca. 250 species (Enghoff et al. 2015)), fluid-feeding has been proposed based on their outer morphology (Brandt 1834; Cook & Loomis 1928; Manton 1961), but feeding has not been observed directly and their internal morphology remained largely unknown. Besides the Platydesmida (see chapter 2), the Colobognatha comprise the three orders Polyzoniida, Siphonocryptida and Siphonophorida. These three taxa share more or less acuminate heads, which are large and anteriorly tapering or drawn out into a beak in the Siphonophorida, while small and conical in the Polyzoniida and Siphonocryptida (Enghoff et al. 2015). The relationship of the colobognathan taxa is controversial (Sierwald et al. 2003; Sierwald & Bond 2007; Blanke & Wesener 2014; Rodriguez et al. 2018), not at least because their internal morphology largely remained terra incognita. The main limitations, which hindered the detailed study of the group, are the paucity of material, the minute size of the species with often less than 1 mm in diameter, and the poorly worked out taxonomy of the group.

In this study the external and internal structure of the head of representatives of the six currently recognized families within the Polyzoniida (Hirudisomatidae, Polyzoniidae; Siphonotidae), Siphonocryptida (Siphonocryptidae) and Siphonophorida (Siphonophoridae, Siphonorhinidae) were studied in detail, to understand the feeding apparatus and document several characters of these millipedes for the first time. The external structure was studied using scanning electron microscopy (SEM). The internal structure was studied using SR-µCT, 3D-segmentations, and histology. Furthermore, a literature review was conducted to compare their morphology to that of fluid feeding arthropods.

This study shows that the head of the studied Polyzoniida, Siphonocryptida and Siphonophorida possess (i) externally opening salivary glands, (ii) mandibles that move in an anterior-posterior direction, and (iii) a pumping chamber similar to that of suctorial insects. These characters have not been reported previously and are unique within Diplopoda.

(i) While in non-colobognathan millipedes (Polyxenida, Pentazonia, Eugnatha) the salivary glands open into the pre-oral chamber, a chamber formed anterior of the actual mouth-opening (El-Hifnawi & Seifert 1973), we show that in Polyzoniida, Siphonocryptida and Siphonorhinidae (Siphonophorida) the salivary glands open via multiple cuticular ducts externally on and above the labrum and on the gnathochilarium. Similar ducts could also be observed in µCT data of Platydesmida from Blanke & Wesener (2014) and Moritz et al. (2021a). These externally opening salivary glands are probably an autapomorphic character of the Colobognatha and might function in lubrification, suspension or external digestion of food. The latter has already been proposed by Lewis (1984). (ii) The largely internalized mandibles of the Polyzoniida, Siphonocryptida and Siphonophorida consist of three parts: a small cardo, a long stipes, and a variable shaped gnathal lobe. We suggest that the mandibles perform a protrusion-retraction movement, similar to the maxillae of hexapods (François et al. 1992; Blanke 2019), rather than a transverse biting movement. This is based on the arrangement of the mandibular elements and the musculature associated to it, which spans from the mandibular stipes to the probably immobile tentorial complex, and from the stipes and gnathal lobe posteriorly to the head capsule. Thus, the mandibles might be used to penetrate surfaces or to detach particles. (iii) Within the head of Polyzoniida, Siphonocryptida and Siphonophorida a pumping chamber is formed by the preoral chamber and the anterior region of the pharynx. The u-shaped floor of this chamber is formed by the mesally fused and rigid tentorial complex, while the roof is formed by a thin, supposedly flexible cuticle, to which large dilator muscles adhere. Furthermore, dorsal compressor muscles and a posterior sphincter muscle are present in some groups. The structure of this pumping chamber shows remarkable similarities to that of suctorial insects, like Diptera (Snodgrass 1944), Hemiptera (Forbes 1964, 1977) and Lepidoptera (Eberhard & Krenn 2005; Krenn 2019), and we suggest that the sucking pump of millipedes functions similarly and evolved convergently to these. As the relationship of the Colobognatha remains unresolved it is not clear whether the pumping chamber evolved in a common ancestor of Polyzoniida, Siphonocryptida and Siphonophorida, or multiple times convergently within Colobognatha.

We suggest that the externally opening salivary glands, the protractible mandibles and the sucking pump enable the Polyzoniida, Siphonocryptida and Siphonophorida to exploit more or less liquid food. This might be further facilitated by capillary forces at the minute opening of the preoral chamber as calculated for the long-beaked Siphonophoridae. The characteristic morphology of the colobognathan head could already be observed in the Cretaceous ca. 99 million years ago (see chapter 7 & 8). While in insects, fluid feeding might have led to a drastic diversification (Kornev & Adler 2019), it remains uncertain why the fluid-feeding colobognathan millipedes (ca. 250 species) are comparatively species poor today. This might be related to their poor dispersal ability and the sensitivity to environmental changes.

The published article is the result of a collaboration of Leif Moritz with Thomas Wesener (ZFMK/LIB), Alexander Blanke (IEZ), Elena Borisova (PSI) and Jörg U. Hammel (DESY, Hereon). LM and AB prepared the specimens. Measurements were performed by LM, EB, JUH, and AB at the DESY, PSI and Sping8. SEM images were obtained by LM and TW. The histological sections were made by Juliane Vehof (ZFMK/LIB). LM evaluated the data and performed 3D-segmentation. All authors were involved in the interpretation of the data. Figure plates and the manuscript were prepared by LM and subsequently edited by all authors.

Chapter 4 – An apparently non-swinging tentorium in the Diplopoda (Myriapoda): Comparative morphology of the tentorial complex in giant pill-millipedes (Sphaerotheriida)

4.1 Bibliography of published article

Leif Moritz, Thomas Wesener & Markus Koch (2018) An apparently non-swinging tentorium in the Diplopoda (Myriapoda): comparative morphology of the tentorial complex in giant pill-millipedes (Sphaerotheriida). *ZooKeys* 741: 77–91.

https://doi.org/10.3897/zookeys.741.21909

4.2 Summary

Molecular studies consistently retrieve a monophyletic Myriapoda including the four taxa Chilopoda, Diplopoda, Pauropoda and Symphyla (Gai et al. 2006; Regier et al. 2010, Miyazawa et al. 2014; Fernández et al. 2016; Szucsich et al. 2020). However, only few morphological characters support the monophyly of Myriapoda. Most of these characters are assumed to be either plesiomorphic or reductive (Koch 2003; Edgecombe 2004). The few supposedly apomorphic characters of the Myriapoda mainly concern the mouthparts and feeding mechanism, as Myriapoda share bipartite mandibles with separately movable gnathal lobes, and a 'swinging tentorium', which is involved in the movement of the mouthparts (Edgecombe & Giribet 2002; Koch 2003; Edgecombe 2004). The paired tentorial complex is a mobile skeletal structure within the head of myriapods, which supports the membranous wall of the preoral chamber, serves as muscle attachment site, and is fundamental for the abduction of the mandible gnathal lobe, which lacks direct abductor muscles (Fechter 1961; Manton 1964; Koch 2015). However, the exact mandibular mechanism remains unclear (Fechter 1961; Manton 1964). In Diplopoda the tentorial complex is composed of four parts: the exoskeletal hypopharyngeal bar, epipharyngeal bar and transverse bar, and the endoskeletal posterior process. A hypopharyngeal lateral sclerite ("Nebententorium" sensu Attems 1926) is associated to the tentorial hypopharyngeal bar. The structure of the tentorial complex and its musculature has mainly been described for eugnathan millipedes (vom Rath 1886a; Silvestri 1903; Voges 1916; Attems 1926; Verhoeff 1928; Seifert 1932; Snodgrass 1950; Fechter 1961; Manton 1964; Naumann et al. 2020). For the giant pill millipedes (Sphaerotheriida) there are only few descriptions of the tentorial complex (vom Rath 1886a; Silvestri 1903; Attems 1926; Verhoeff 1928), indicating that it differs markedly from the pattern observed in other millipedes.

To understand the structure and function of the tentorial complex of the Sphaerotheriida more comprehensively, we studied the tentorial complex of the three genera *Arthrosphaera* Pocock, 1895, *Sphaeromimus* de Saussure & Zehntner, 1902 and *Zoosphaerium* Pocock, 1895 comparatively using µCT.

In the studied Sphaerotheriida the tentorial complex consists of the exoskeletal epipharyngeal bar and hypopharyngeal bar, and an endoskeletal posterior process. However, a transverse bar is absent despite the presence of an incisura lateralis. The epipharyngeal bar, which passes over into the transverse bar in other millipedes, ends in a broad plate within the epipharyngeal wall in Sphaerotheriida. In other millipedes the transverse bar acts as connection of the tentorial complex to the head capsule and articulates to the latter laterally via the incisura lateralis (Fechter 1961; Manton 1964). The epipharyngeal bar of the Sphaerotheriida serves as attachment site for antennal muscles (m.) and dorsally spanning tentorial m., including muscles associated to the transverse bar in other millipedes. The posterior process acts as attachments site for a pharyngeal m. and for a posterior spanning tentorial muscle. The hypopharyngeal bar is devoid of muscles, while gnathochilarial muscles are associated to the pharyngeal lateral sclerite. Variation of the tentorium between species mainly concerns the shape and relative size of its components. Each gnathal lobe gives rise to a strong, ventral condylus, which projects between the tentorial epipharyngeal bar and a socket-shaped projection from the incisura lateralis of the head capsule.

While the general structure of the tentorial complex appears to be highly conserved within Sphaerotheriida, it largely differs from that of the remaining Diplopoda (vom Rath 1886a; Silvestri 1903; Voges 1916; Attems 1926; Verhoeff 1928; Seifert 1932; Snodgrass 1950; Fechter 1961; Manton 1964; Koch 2003; Blanke & Wesener 2014; Koch 2015; Moritz et al. 2021a, 2022) by the absence of a transverse bar and the shape of the epipharyngeal bar. As the general function of the transverse bar in millipedes is the connection of the tentorial complex to the head capsule, around which the tentorium performs its characteristic swinging movement (Manton 1964), its absence must have severe consequences on the tentorial mobility. We suggest that the tentorial mechanism in Sphaerotheriida varies greatly from that described for Juliformia (Fechter 1961; Manton 1964; Naumann et al. 2020). However, the tentorial complex has probably retained mobility as the muscles considered to be essential for its movement in other millipedes (Fechter 1961; Manton 1964; Wilson 2002; Naumann et al. 2020) are present in the giant pill millipedes. We suggest that, when moving forward, the tentorial epipharyngeal bar exerts force on the gnathal lobe's condylus and forces it against the socket-shaped incisura lateralis, allowing the abduction of the mandible. The shape of the tentorium might contribute more characters to the list of head modifications, related to volvation in giant pill millipedes (Golovatch 2003; Blanke & Wesener 2014; Tuf et al. 2016). In volvatory millipedes the head lumen and endoskeletal structures are often reduced in size (Koch 2015). Thus, the loss of the transverse bar in Sphaerotheriida might be related to a size reduction of the endoskeleton. In the also volvatory Glomerida a transverse bar is present, but the posterior process is absent (Voges 1916; Dohle 1964). These different modification in both volvatory groups, might indicate that the ability to roll into a ball evolved independently in Sphaerotheriida and Glomerida, and characters used to argue for a monophyletic Oniscomorpha (Sphaerotheriida + Glomerida) might be convergences. However, the structure of the tentorial complex of the other pentazonian taxa Glomeridesmida remains unknown.

This study is the result of a collaboration of Leif Moritz, Thomas Wesener (ZFMK/LIB) and Markus Koch (IEZ). LM obtained and analyzed the µCT data and performed the 3D segmentation. MK performed dissections and obtained photographs. All authors were involved in the interpretation of the data. LM wrote the first draft of the manuscript, which was subsequently edited by all authors.

Chapter 5 – A tarsal spinning organ in glomeridesmid millipedes (Diplopoda: Pentazonia: Glomeridesmida)

5.1 Bibliography of published article

Leif Moritz & Thomas Wesener (2021) A tarsal spinning organ in glomeridesmid millipedes (Diplopoda: Pentazonia: Glomeridesmida). *Arthropod Systematics & Phylogeny* 79: 555–567.

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5.2 Summary

Within Diplopoda several orders are morphologically not well characterized and only supported by few supposedly apomorphic characters. One of these taxa is the order Glomeridesmida, which is one of the less diverse millipede taxa with 36 described species (Wesener et al. 2021). These small and sluggish millipedes occur in South-East Asia, India, South and Central America, and Oceania, and are classified in two families, the Glomeridesmidae and the Termitodesmidae (Shelley 2011; Jeekel 2003; Enghoff et al. 2015). The latter are only known from termite nests. Among extant taxa the Glomeridesmida have been suggested to be most similar to the groundplan of the chilognathan millipedes, with a mix of plesiomorphic and reduced characters (Enghoff 1990). However, there are only a few apomorphic characters known for this group, e.g. a strongly elongated membranous fringe on the mandible molar plate, the presence of 20 tergites, and modifications of the penultimate and ultimate leg pairs in females (Oeyen & Wesener 2018). In contrast, the morphology of the two more diverse and larger pentazonian taxa, the Holarctic pill millipedes (Glomerida) and the giant pill millipedes (Sphaerotheriida), are well studied (Wesener 2016; Oeyen & Wesener 2018) and only recently 39 potential apomorphies have been listed for the Sphaerotheriida (Wesener 2016). So far no spinning organs, thread-producing organs or fiber-producing organs have been reported from the Pentazonia. However, thread-producing organs are widespread among Myriapoda and known from Lithobiomorpha (Chilopoda) (Rosenberg & Müller 2009; Kenning et al. 2019) and Symphyla (Szucsich & Scheller 2011), and within Diplopoda from Polyxenida (Schömann 1956), Polydesmida (Adis et al. 2000; Shear 2008), Nematophora (Shear 2008; Enghoff & Akkari 2011; Enghoff & Reboleira 2013), and potentially Siphoniulida (Sierwald et al. 2003) and Siphonophorida (Marek & Bond 2006; Read & Enghoff 2009; Marek et al. 2012, 2016). In these taxa the excreted threads serve as defense against predation or during molting or are involved in mating or egg-protection.

In this study scanning electron microscopy, light-microscopy, and histology were combined to examine the external and internal morphology of a previously unknown exocrine gland on the legs of Glomeridesmida. Therefore, we studied the legs of 21 morphospecies (including several undescribed species), covering both families (Glomeridesmidae, Termitodesmidae) and the geographic distribution of the group, as well as mature males and females, and different postembryonic stadia.

This study shows that all investigated representatives of the Glomeridesmida carry a field of 20–30 pores (hereafter spinning field) with a diameter of 0.35 µm on the tarsi of each walking leg. These spinning fields were present in both sexes and in juveniles. The pores are the external openings of cuticular conducting canals, that run as a bundle through the podomeres to an exocrine gland located in the walking leg's femur. Fibers could be observed to be ejected from the spinning fields in several specimens. These fibers either fused to a bundle or coiled up separately. Particles stuck to some of the fibers.

We suggest that Glomeridesmida in general possess a tarsal spinning gland or thread producing organ and that this character is apomorphic for the group, as it occurs in Glomeridesmidae and Termitodesmidae but is unknown from the other well studied Pentazonia. Although the legs of the Glomeridesmida have been studied and depicted previously (Silvestri 1903; Carl 1942; Iniesta et al. 2012; Wesener et al. 2014; Mauriès 2020), these pores have not been reported. This is mainly due to the small size of the spinning field (3–5 µm in diameter) and its position in a depression on the outer side of the tarsus, as it is not clearly visible in anterior or posterior views, which are typically depicted in taxonomic description. As the material studied here is from museum collections and was fixed and stored in ethanol, ultrastructural investigations via transmission-electron-microscopy and detailed histological investigations were not possible. However, it can be assumed that the spinning gland of the Glomeridesmida is an aggregated gland consisting of several secretory units, with cells clustered in the femur, opening via separate conducting canals on the tarsus. Superficially similar exocrine gland openings on walking legs are known among Diplopoda from Polyxenida, Chordeumatida, Polydesmida and some Julida. However, in these groups the glands are restricted to single legs of mature males (Verhoeff 1928; Schömann & Schaller 1954). Furthermore, telsonian spinnerets are present in Nematophora and Polydesmida (Adis et al. 2000; Shear 2008) and possibly in Siphoniulida (Sierwald et al. 2003). Therefore, the tarsal spinning organs of the Glomeridesmida are unique within Diplopoda, in their position, arrangement and structure and can be considered as apomorphic. The actual function of the exocrine glands remains unknown because living specimen of the Glomeridesmida have only seldom been observed alive and spinning activity has never been reported (Escherisch 1911; Hirst 1913; Iniesta et al. 2012; Wesener et al. 2021). We suggest that the secretion product might be used for defense against predation as in Lithobiomorpha (Kenning et al. 2019) and Symphyla (Betz & Kölsch 2004), for protection during molting as in Nematophora (Enghoff &

Akkari 2011), or for securing the walls of tunnels as is the case in web-spinners (Embioptera) (Büsse et al. 2015) and some ants (Formicidae) (Billen & Peeters 2020), which possess similar organs on their tarsi. We suggest that in Glomeridesmida the threads are not exclusively used for mating or brood care, as both sexes and all studied postembryonic stadia possess these organs.

The published article is the result of a collaboration of Leif Moritz and Thomas Wesener (ZFMK/LIB). LM prepared the specimens, performed light-microscopic and SEM examinations, and evaluated the histological sections. Histological sections were obtained by Juliane Vehof (ZFMK/LIB). Both authors were involved in the interpretation of the data. Figures and the first draft of the manuscript were prepared by LM, and subsequently edited by both authors.

Chapter 6 – No Tömösváry organ in flat backed millipedes (Diplopoda, Polydesmida)

6.1 Bibliography of published article

Leif Moritz & Markus Koch (2020) No Tömösváry organ in flat backed millipedes (Diplopoda, Polydesmida). *ZooKeys* 930: 103–115.

https://doi.org/10.3897/zookeys.930.48438

6.2 Summary

Within Diplopoda the grouping of taxa is often only supported by single or few supposedly apomorphic characters. Several morphological characters, commonly used in taxonomy, systematics, and phylogenetic analyses, are studied in detail only for single or few species. However, these characters are considered as apomorphic for a whole assemblage of taxa. Such characters are often taken from the older literature, without reexamination of the structures. This literature is a treasure trove for morphological data but should be taken with caution. One such character is the so called Tömösváry organ, a cephalic sensory organ, which is known from several Myriapoda taxa. The function of the organ is unknown, but it might be involved in the perception of vibration (Meske 1961), olfaction (Bedini & Mirolli 1967), gravitation (Krishnan 1968) or humidity (Bedini & Mirolli 1967). The Tömösváry organ can vary in Diplopoda if present, in its size, shape and position. Thus, the states of the Tömösváry organ are commonly used in cladistics analyses of the group (Sierwald & Bond 2007; Blanke & Wesener 2014), and it is supposedly present in Polyxenida, Pentazonia, Callipodida, Chordeumatida and Polydesmida, while reduced in Colobognatha, Stemmiulida and Juliformia (Blanke & Wesener 2014).

For the flat-backed millipedes (Polydesmida), the most diverse millipede order, including 5,000 of the 12,000 described species (Enghoff et al. 2015), a Tömösváry organ has been reported already by Attems (1899, 1926, 1937). The first and only detailed description of this organ in Polydesmida has been given in 1906 by Hennings. Since then, this peculiar organ has not been reexamined in detail for the group, but repeatedly stated to be present in Polydesmida (Verhoeff 1928; Snodgrass 1952; Enghoff et al. 2015; Müller & Sombke 2015) and used as taxonomic and phylogenetic character (Sierwald & Bond 2007; Blanke & Wesener 2014; Bouzan et al. 2017a, b). However, Attems (1926) questioned the presence of the Tömösváry organ in some genera of Polydesmida, and Seifert (1932) stated it to be generally absent in Polydesmida, without a discussion. Apparently, its absence in Polydesmida is accepted among some taxonomists (W. A. Shear and P. E. Marek, pers. comm. 12th May 2017). To clarify the state of the Tömösváry organ in Polydesmida we examined the morphology of the head of four species using µCT and histology. Furthermore, we reviewed the existing literature on the Tömösváry organ in flat-backed millipedes.

We demonstrate that a Tömösváry organ is absent in the studied Polydesmida. Apparently, the structure typically described and depicted as Tömösváry organ in Polydesmida (e.g. Hennings 1906; Bouzan et al. 2017a, b) demarcates the distal roundish expansion of the incisura lateralis and is the attachment site of the tentorial complex to the head capsule. At this point the tip of the tentorial transverse bar projects through the head capsule. Externally the transverse bar appears oval in shape and is surrounded by a rim of the cephalic cuticle. The general structure of the tentorial complex of the studied species corresponds to the state described by Seifert (1932) for *Strongylosoma stigmatosum* (Eichwald, 1830). A structure resembling the general appearance of the Tömösváry organ in millipedes, with a sensory cavity lined by a thin porous cuticle and a peg-like epithelium (Müller & Sombke 2015) is not present in the Polydesmida.

The observed structure of the attachment of the tentorial complex to the head capsule resembles the description of the alleged Tömösváry organ of the Polydesmida, which is described as a pear-shaped or oval pit covered by a membrane with a median hard swelling or central disc (Hennings 1906; Snodgrass 1952). Therefore, we suggest that a Tömösváry organ is generally absent in Polydesmida, and all previous descriptions of it (e.g. Attems 1899; Hennings 1906; Verhoeff 1928) unambiguously refer to a structure we identified as the projection of the tentorial transverse bar through the head capsule; i.e. the connection of the tentorial transverse bar to the head capsule was misidentified as the Tömösváry organ in Polydesmida. This is congruent with previous doubt on the presence of a nervus tömösváry in Polydesmida. In other millipedes this nerve innervates the Tömösváry organ (Sombke & Rosenberg 2015).

The absence of a Tömösváry organ in Polydesmida is a character state shared with Colobognatha, Stemmiulida, Juliformia and Siphoniulida (Sierwald & Bond 2007; Blanke & Wesener 2014). It is unknown how often the Tömösváry organ was reduced independently within Diplopoda, as varying positions have been retrieved for Polydesmida. Analyses based on morphological data retrieve Polydesmida as sister to Nematophora (Sierwald et al. 2003; Blanke & Wesener 2014), while molecular and total evidence analyses retrieve Polydesmida either as sister to Juliformia (Cong et al. 2009), Stemmiulida (Rodriguez et al. 2018), or Colobognatha (Sierwald & Bond 2007), which all lack a Tömösváry organ (Blanke & Wesener 2014). The correction of the character states of the Tömösváry organ in Polydesmida in the analysis provided by Blanke & Wesener (2014, their characters 6 and 7) does not alter the topology of their phylogenetic hypothesis. However, the resolution of the chilognathan orders in this analysis, questions the homology of the Tömösváry organ across millipedes, because its presence in some Nematophora optimizes most parsimoniously as a reversal. This study shows, that although the older morphological works are a valuable source of information, characters have to be taken with caution and restudied to clarify their presence and homology. This is also the case for well-known taxa like the Polydesmida and often employed characters like the Tömösváry organ.

The published article is the result of a collaboration of Leif Moritz and Markus Koch (IEZ). The µCT data has been obtained and analyzed by LM. The histological sections of *Polydesmus angustus* analyzed here, were obtained by LM during his bachelor thesis, in which first doubts on the presence of a Tömösváry organ in *P. angustus* were raised (Moritz 2015). Both authors were involved in the interpretation of the data. The manuscript and figures have been prepared by LM and subsequently edited by LM and MK.

Chapter 7 – Checklist of the Myriapoda in Cretaceous Burmese amber and a correction of the Myriapoda identified by Zhang (2017)

7.1 Bibliography of published article

Thomas Wesener & Leif Moritz (2018) Checklist of the Myriapoda in Cretaceous Burmese amber and a correction of the Myriapoda identified by Zhang (2017). *Check List* 14(6): 1131–1140.

https://doi.org/10.15560/14.6.1131

7.2 Summary

Extant species only represent a fraction of the biodiversity, which has been present through time on the planet. Therefore, knowledge on the fossil record and past diversity is essential to understand the evolution, biogeography, and present diversity of a taxon. Millipedes probably have been among the first fully terrestrial animals, with a fossil record dating back to the Silurian more than 400 million year ago (ma) (Wilson & Anderson 2004). All Paleozoic fossils of millipedes are placed in extinct orders, while most Mesozoic fossils can be placed in extant orders, which are still present today (Shear 1997; Shear & Edgecombe 2010). However, there are large gaps in the fossil record regarding certain geological epochs as well as certain taxa. Until now only a handful of fossils were known from the entire Mesozoic (251.9 – 66 ma) (Cockerell 1917; Dzik 1975, 1981; Jell 1983; Shear 1997; Nguyen Duy-Jacquemin& Azar 2004; Shear & Edgecombe 2010), and for many extant orders none or only few fossils were known. In contrast centipedes (Chilopoda) are already represented in the Paleozoic by members of extant orders and only a single extinct order (Shear & Edgecombe 2010). In recent decades Burmese amber proofed to be a rich source of arthropod fossils, which shed light on the evolution of several groups (Ross 2019). Burmese amber dates back to the mid-Cretaceous ca. 99 ma (Shi et al. 2012) and is mainly mined in the Hukawng Valley of northern Myanmar (Zherikhin & Ross 2000; Grimaldi et al. 2002; Cruickshank & Ko 2003).

Here we studied the diverse myriapod fauna of the Cretaceous Myanmar trapped in Burmese amber. We studied 460 inclusions of Diplopoda, Symphyla and Chilopoda using light-microscopy and identified the specimens at least to order level. Furthermore, we reviewed the present literature and corrected previous erroneous identifications of Myriapoda in Burmese amber.

All Myriapoda known from Burmese amber can be placed in extant orders and not a single representative of an extinct order could be identified. We record representatives of 13 of the 16 extant Diplopoda orders (Polyxenida, Glomeridesmida, Glomerida, Siphonophorida, Polyzoniida, Platydesmida, Siphoniulida, Chordeumatida, Polydesmida, Stemmiulida, Callipodida, Spirostreptida, Spirobolida). This includes the first fossils of the order Platydesmida, as well as the oldest records of the seven orders Polyxenida, Glomeridesmida, Glomerida, Siphonophorida, Polyzoniida, Siphoniulida, and Spirostreptida. Representatives of the three orders Julida, Sphaerotheriida and Siphonocryptida were absent. The most abundant millipede taxon in Burmese amber is the order Polydesmida with 161 of 527 specimens (including literature records). Furthermore, the Colobognatha are common with 102 Polyzoniida, 72 Siphonophorida and eight Platydesmida specimens. Several specimens can be placed in extant families (e.g. Andrognathidae, Cambalidae, Heterochordeumatidae, Glomeridesmidae, Polyxenidae, Siphoniulidae, Siphonophoridae, Siphonorhinidae, Siphonotidae, Synxenidae). Centipedes (Chilopoda) are represented by 30 specimens in total, of which 22 are new records. In Burmese amber the four extant orders Geophilomorpha, Lithobiomorpha, Scolopendromorpha and Scutigeromorpha were preserved, with Geophilomorpha being the most abundant one. Symphyla are represented in Burmese amber by three specimens with the oldest records for both symphylan families Scolopendrellidae and Scutigerellidae.

For Diplopoda extent representatives of only eight orders (Glomerida, Sphaerotheriida, Siphonophorida, Chordeumatida, Polydesmida, Julida, Spirostreptida, Spirobolida) are known from Myanmar (Likhitrakarn et al. 2017), while we record 13 orders from the Cretaceous Myanmar. Of these 13 orders only six are also known from the present-day Myanmar (Glomerida, Siphonophorida, Chordeumatida, Polydesmida, Spirostreptida, Spirobolida), while Polyxenida, Glomeridesmida, Polyzoniida, Platydesmida, Siphoniulida, Stemmiulida and Callipodida are only known from Cretaceous Myanmar. The Sphaerotheriida and Julida are known from the extant Myanmar but not from Burmese amber. We suggest that these differences are mainly due to an undersampling of the extant millipede fauna of Myanmar and a bias towards preservation of certain taxa in amber. Given the distribution pattern of these groups in Southeast Asia (Shelley & Golovatch 2011) it can be expected that the taxa found in Burmese amber also occur in present day Myanmar.

All studied specimens could be placed in extant orders or even families and closely resemble extant representative in their external morphology. This means that the major lineages observed today, with their characteristic morphology were already established in the Cretaceous almost 100 ma and their external morphology only changed slightly since then. Today the Colobognatha, which are highly specialized towards fluid feeding (Moritz et al. 2021a, 2022; see also chapters 2 & 3), are one of the least diverse groups with 250 extant species (Enghoff et al. 2015) and show a low abundance in tropical forests. However, this group was one of the most abundant taxa found in Cretaceous amber with 33.5% of all millipede inclusions. We suggest that this is either due to a sampling bias, as the Colobognatha might have lived on the amber producing trees, or that the extant Colobognatha represent only a relict of a once greater diversity of fluid feeding millipedes.

The published article is the result of a collaboration of Leif Moritz and Thomas Wesener (ZFMK/LIB). The millipedes were identified by LM and TW. All authors were involved in the interpretation of the data. The first draft of this manuscript was written by TW and subsequently edited by LM and TW. The figures were prepared by LM.

Chapter 8 – The first known fossils of the Platydesmida — an extant American genus in Cretaceous amber from Myanmar (Diplopoda: Platydesmida: Andrognathidae)

8.1 Bibliography of published article

Leif Moritz & Thomas Wesener (2019) The first known fossils of the Platydesmida—an extant American genus in Cretaceous amber from Myanmar (Diplopoda: Platydesmida: Andrognathidae). *Organisms Diversity & Evolution* 19: 423–433.

https://doi.org/10.1007/s13127-019-00408-0

8.2 Summary

The Diplopoda have been inhabiting terrestrial ecosystem for more than 400 million years (Wilson & Anderson 2004) and show a great morphological diversity related to certain lifestyles (Golovatch & Kime 2009). To understand the timing and pattern of millipede evolution and how certain characters evolved, a detailed study of their fossil record is essential. Burmese amber, dating back ca. 99 ma (Shi et al. 2012), offers an insight into the rich millipede fauna of the Cretaceous Myanmar (chapter 7). Modern techniques like µCT allow to study the morphology of these millipedes in detail for comparisons to extant species. Among millipedes the colobognathan taxa (Platydesmida, Polyzoniida, Siphonocryptida, Siphonophorida) are unusual in many aspects. Among others they have highly derived mouthparts, which enable them to feed on liquids (Moritz et al. 2021a, 2022; see also chapter 2 & 3). The fungivorous Platydesmida (Macias et al. 2019) are thought to be a key taxon to understand the evolution of the group, as they show the least derived mouthparts among Colobognatha (Verhoeff 1914). The Platydesmida are species-poor with 63 extant species in 14 genera. Of these, 37 species and 12 genera are placed within the family Andrognathidae Cope, 1869 (Enghoff et al. 2015). Some species are known to form aggregations and show paternal brood care (Wong et al. 2020). Only recently the first fossils of the group have been recorded from Burmese amber (Wesener & Moritz 2018; see also chapter 7), which are here described in detail.

We studied 15 specimens combining classical light-microscopy and line drawings with modern µCT and 3D reconstructions to exploit a variety of characters in their three-dimensionality and to compare those to extant Platydesmida.

The studied specimens can be placed in the family Andrognathidae Cope, 1869, based on the narrow sternites and the coxae, which are almost in touch but separated by a knob-like sternal extension. Furthermore, the long and slender habitus of the specimens is commonly observed in representatives of the family Andrognathidae. The specimens belong to the genus *Andrognathus* Cope, 1869, which includes three extant species (*A. corticarius* Cope, 1869, *A.*

grubbsi Shorter, Hennen & Marek, 2018, *A. hoffmani* Shear & Marek 2009), based on the following character combination, which is diagnostic for the genus (Cook & Loomis 1928; Gardner 1974; Shear & Marek 2009; Shorter et al. 2018): Tergite 5 enlarged with anterior pointing paranota; tergites with small round tubercles; prominent coxal sacs from leg-pair 3 onwards; hypoproct absent. All studied specimens represent the species *Andrognathus burmiticus* Moritz & Wesener 2019, which is new to science. This species differs from all extant *Andrognathus* by the presence of ventral pleurotergal lobes lateral of the legs (especially well developed laterally of the gonopods on body-rings 7), the presence of numerous dorsal rows of tubercles on the metazonites, and the absence of a transverse tergite division. *Andrognathus burmiticus* is distinct from *A. corticarius* by the presence of a single lobe on the praranota of tergite 5, as is the case in *A. grubbsi* and *A. hoffmani*. The species *A. burmiticus* shows intraspecific variation in the number of body-rings (30–54) and in the number of rows of tubercles on the tergites (8–12). The majority of specimens were fully developed mature individuals and only a single juvenile specimen was found. All specimens were found in separate amber pieces and never in groups.

Previously there has not been a single unambiguous fossil record of Platydesmida. *Andrognathus burmiticus* represents the first described fossil, which can be clearly placed in the order. The somatic characters and their detailed examination via µCT allow to place this fossil in the genus *Andrognathus,* which is today represented by 3 extant species, all distributed in North America (Shorter et al. 2018). Therefore, we suggest that the genus originated more than 99 ma. This is in congruence with estimates based on the analyses of genomic data, which retrieved a median age of 75.8 ma with a large 95% confidence interval of 36.84 – 128.55 ma, for the split of *Andrognathus corticarius* and *Brachycybe lecontii* + *Platydesmus* sp. (Rodriguez et al. 2018). Therefore, the genus might has originated between 98.79±0.62 ma (based on the age of the fossils (Shi et al. 2012)) and 128.55 ma (based on molecular data (Rodriguez et al. 2018)). Furthermore, the fossils show that the genus was more widespread in the past and extinction events occurred in Asia. Representatives must have migrated between Asia and North America, which might have happened via Beringia or a North Atlantic land bridge. A similar scenario has been proposed for the genus *Brachycybe* Wood, 1864, which has an extant distribution in North America and Asia and might has originated ca. 50 ma, with migration between North America and Asia ca. 20 ma (Brewer et al. 2012b). This study shows that diversification of extant taxa took place early in the evolutionary history of the Diplopoda. The specialized morphology observed in extant taxa, like the fluid feeding Colobognatha, already evolved more than 100 ma, and the few species observed today (ca. 250 species in Colobognatha, ca. 60 species in Platydesmida) might only be the remnant of a once greater and wider distributed diversity.

The published article is the result of a collaboration of Leif Moritz and Thomas Wesener (ZFMK/LIB). The study was designed by LM and TW. Data was acquired and analyzed by LM. Both authors were involved in the interpretation of the data. The manuscript was written by LM and subsequently edited by LM and TW.

Chapter 9 – Summary of the research results and advance in knowledge

The presented studies contribute to our knowledge of the morphological diversity of millipedes (Diplopoda) through time and help to better understand the morphology of the group in a functional and evolutionary context. The here presented studies show for the first time detailed data on the internal structures of millipedes of the subclasses and orders Colobognatha, Glomeridesmida and Sphaerotheriida. Previously unknown (chapters 2 – 5) or misinterpreted character states (chapter 6), such as the presence of a sucking-pump to take up liquid food, are documented and discussed. Furthermore, the first or oldest fossil data is presented for half of the 16 extant millipede orders, with implications for the timing of their evolution (chapters 7 & 8). However, these studies also indicate open questions and outline future directions to better understand the evolution of one of the oldest land-living taxa and a notoriously understudied group, the Diplopoda.

9.1 New insight into the structure and function of the feeding apparatus in millipedes

Our knowledge regarding the biology and morphology of the Diplopoda is largely biased towards the biting-chewing Eugnatha (e.g. Silvestri 1903; Verhoeff 1928; Seifert 1932; Wilson 2002), which include with more than 11,000 species the majority (>90%) of the ca. 12,000 described extant millipede species (Brewer et al. 2012a). However, the Eugnatha cover only half of the Diplopoda higher-level diversity, excluding eight morphologically distinct orders (Blanke & Wesener 2014). This bias also concerns the knowledge of the feeding apparatus and mechanism (Silvestri 1903; Fechter 1961; Manton 1964; Naumann et al. 2020). Morphological diversity can be found in the less-diverse taxa, like the highly derived Colobognatha, Pentazonia and Polyxenida. For the first time the structure of the head's skeletomuscular system of representatives of the Colobognatha (chapters 2 & 3) and Sphaerotheriida (chapter 4) has been studied in detail comparatively to understand the evolution and function of their feeding apparatus.

Here it could be shown that, similar to insects (Krenn 2019), modifications of common elements of the feeding apparatus (e.g. mandibular sclerites and tentorial complex) and deviations from a common bauplan can lead to fundamentally different structures and functions within Diplopoda. These modifications can be assumed to have tremendous effects on the feeding mechanism and ecology of these groups. Such modifications concern, on a higher level, the division of the mandibular base into cardo and stipes in the common ancestor of Helminthomorpha (see below), as well as further modifications of these elements in lower ranking taxa. Compared to the eugnathan millipedes (chapter 6), in Colobognatha a stepwise internalization and elongation of the mandibles, fusion of the tentorial complex and reduction of muscles could be observed. These modifications lead to a different movement of the mouthparts and the formation of a sucking apparatus. One of the four groups of the Colobognatha, the Platydesmida, show with a reduced mandibular musculature and muscle attachment sites, a mobile tentorium and internalized but transversely moving mandibles, an intermediate state between the biting-chewing Eugnatha and the remaining suctorial Colobognatha (chapter 2). In the other three Colobognatha groups, the Polyzoniida, Siphonocryptida and Siphonophorida, the tentorial complex loses its mobility, while the movement of the mandibles is restricted to a longitudinal axis, resulting in a protrusion-retraction mechanism (chapter 3). Although direct observations of the feeding process were not possible, comparisons to the morphology of the well-studied feeding mechanisms of various insects (e.g. Eberhard & Krenn 2005; Kornev & Adler 2019; Krenn 2019) allowed the inference of the function of the modified mouthparts from its morphology, and suctorial feeding on more or less fluid food appears most probable. However, this discovery needs to be verified experimentally in the future. X-ray videography of living animals might offer the opportunity to observe the movement of the mandibles and sucking apparatus, as has been done for suctorial (Kim et al. 2011; Lee et al. 2014) and biting insects (Betz et al. 2008; Wang et al. 2020). In contrast to the Colobognatha, in the biting-chewing giant pill millipedes (Sphaerotheriida) the decoupling of the tentorial transverse bar from the head capsule, and the condylus on the mandibular gnathal lobe, probably lead to a higher mobility of the tentorial complex, while still allowing a transverse biting-chewing movement (chapter 4). In the Sphaerotheriida the shape of the tentorial complex and head might not only be related to the feeding itself, but also to the ability of the animal to roll into a perfect ball (Wesener 2016). Volvation requires a reduction in the size of the head, which is shorter and wider than in other Diplopoda, and therefore modifications of structures within the head like the tentorial complex. Thus, in Colobognatha and Sphaerotheriida a swinging tentorium, one of the apomorphies of the Myriapoda (Koch 2003), is secondarily lost.

9.2 Phylogenetic implications

With the development of faster and cheaper methods to generate and analyze large molecular data sets in recent decades, morphological traits became less popular for reconstructing phylogenetic relationships. However, phenotypic data remains indispensable to understand the evolution and relationship of taxa, as it provides independent data to test the plausibility of molecular phylogenies and allows the inclusion of fossil representatives in a phylogenetic framework (Wiens 2004; Giribet 2015; Lee & Palci 2015). The latter is especially important for calculating the rate of evolution and dating splits (divergence times) in phylogenetic hypotheses (Marshall 1990). Present cladistic analyses of the Diplopoda based on morphological data include a relative low number of characters, with a maximum of 64 characters (Blanke & Wesener 2014), compared to 189 characters for Hexapoda (Giribet et al. 2004), 236 characters for the Hymenoptera (Vilhelmsen 2001), and 365 characters for Holometabola (Beutel et al. 2011). Furthermore, the cladistic analyses of millipedes (Enghoff et al. 1993; Sierwald et al. 2003; Blanke & Wesener 2014) use higher taxa (orders) as terminals relying on a groundplan approach instead of an exemplary approach, and use a mix of characters observed in different species mainly extracted from the literature, which is burdened by several a priori assumptions. In the presented studies we provided for the first time a detailed description of several character states in little known taxa on species level, covering the morphological diversity within the groups (chapters 2 – 6). In the future this might help to resolve the internal phylogenetic relationship of problematic taxa such as the Pentazonia and Colobognatha, and to test the plausibility of phylogenetic hypothesis based on molecular data. For Colobognatha, which have been retrieved as monophyletic in all previous phylogenetic reconstructions (Sierwald & Bond 2007; Wesener et al. 2014; Rodriguez et al. 2018; Benavides et al. 2022), it is shown that all taxa share externally opening salivary glands and the absence of mandibular muscles originating from the transverse mandibular tendon (chapters 2 & 3). Siphonophorida, Polyzoniida and Siphonocryptida share a pumping chamber, which is formed by a mesally fused tentorial complex, protractible mouthparts, and the absence of a transverse mandibular tendon. Polyzoniida and Siphonocryptida share small triangular heads (chapter 3) and a displacement of the anterior salivary glands and brain from the head into the anterior body-rings (supplement chapter 3). Although it is intriguing to assume the relationship of the Colobognatha as Platydesmida + (Siphonophorida + (Polyzoniida + Siphonocryptida))) based on the morphological data, this (1) has to be tested in a cladistic analyses, and (2) it has to be considered that these characters are strongly related to their feeding mechanism and diet, and therefore might have evolved convergently, as is the case for various insect taxa (chapter 3). Therefore, more detailed ultrastructural and developmental investigations, as well as the study of the internal morphology of fossil representative are needed to assess the homology of these structures. Based on the morphological data presented here, a monophyletic taxon including Polyzoniida and Siphonocryptida is well supported. Previously Siphonocryptidae has been considered as a family within Polyzoniida (e.g. Enghoff & Golovatch 1995) but it was elevated to the 16th millipede order based on an unpublished phylogenetic reconstruction, which retrieved Siphonocryptida as a sistergroup of Platydesmida, and without providing compelling evidence (Shelley 2002). For the volvatory (rolling-up) Oniscomorpha (Glomerida + Sphaerotheriida), it could be demonstrated that a size reduction of the tentorial complex, which might be related to volvation, is realized in different ways. In Sphaerotheriida the tentorial transverse bar is reduced (chapter 4), while in Glomerida the tentorial posterior process is reduced (Voges 1916; Dohle 1964). Thus, convergent evolution of volvation in both taxa is probable, which is further supported by molecular data retrieving a paraphyletic Oniscomorpha (Regier et al. 2005; Sierwald & Bond 2007; Rodriguez et al. 2018; Benavides et al. 2022). Furthermore, we provide support for the monophyly of the pentazonian taxon Glomeridesmida, with Glomeridesmidae + Termitodesmidae, by the presence of unique tarsal spinning organs, previously unknown for Diplopoda (chapter 5). The Glomeridesmida were considered to closely represent the chilognathan ground plan, and mainly characterized by plesiomorphic and reductive traits, like the absence of eyes, the absence of a mid-dorsal suture, and the absence of defense glands (Enghoff 1990). Finally, for the well-studied flat-backed millipedes (Polydesmida), to which more than 40% of the described Diplopoda species belong, it is shown that the Tömösváry organ, which is often used as character in cladistic analyses (Sierwald et al. 2003; Blanke & Wesener et al. 2014), is absent. In Polydesmida the open incisura lateralis was erroneously interpreted as Tömösváry organ (chapter 6).

The data from the studies presented in the chapters 1 – 6 can be used to add new characters and to adjust character states in the most recent phylogenetic analyses conducted by Blanke & Wesener (2014). This concerns the mandibular base of the Colobognatha, which consists of two articles (their character 13: state 0 changed to 1 (see chapters 2 & 3)), and the Tömösváry organ in Polydesmida, which is absent (their character 6: state 1 changed to 0; character 7: state 0 changed to – (see chapter 6)). The phylogenetic analyses of the modified matrix, using the same method and parameters as Blanke & Wesener (2014), resulted in 5 shortest trees with a length of 97 steps. The consensus tree has an identical topology as the phylogenetic hypothesis presented by Blanke & Wesener (2014). Bootstrap values (random number seed: 3453; replicates: 1000; collapse nodes with less than: 50%) and jackknife support showed only minor differences to those retrieved by Blanke & Wesener (2014) (Fig. 9). The new coding of characters also has implication for the interpretation of their evolution. The divided mandibular base evolved in the last common ancestor of Helminthomorpha (Colobognatha + Eugnatha) and not within the last common ancestor of Eugnatha as suggested by Blanke & Wesener (2014). The most parsimonious explanation for the distribution of the Tömösváry organ across the phylogeny is a loss of the organ in the last common ancestor of Helminthomorpha, and an independent gain in Chordeumatida and Callipodida, questioning its homology across Diplopoda. These examples show that the morphological characters employed for the Diplopoda, and their homology are only poorly understood and must be critically reevaluated. Furthermore, the data presented here can be used to clarify certain character statements in future analyses, like “standard diplopodan”, “colobognathan type” and “siphoniulid type” mouthparts (Sierwald et al. (2003) character 5), which are burdened with various assumptions and neglect the diversity of the mouthparts within the different groups.

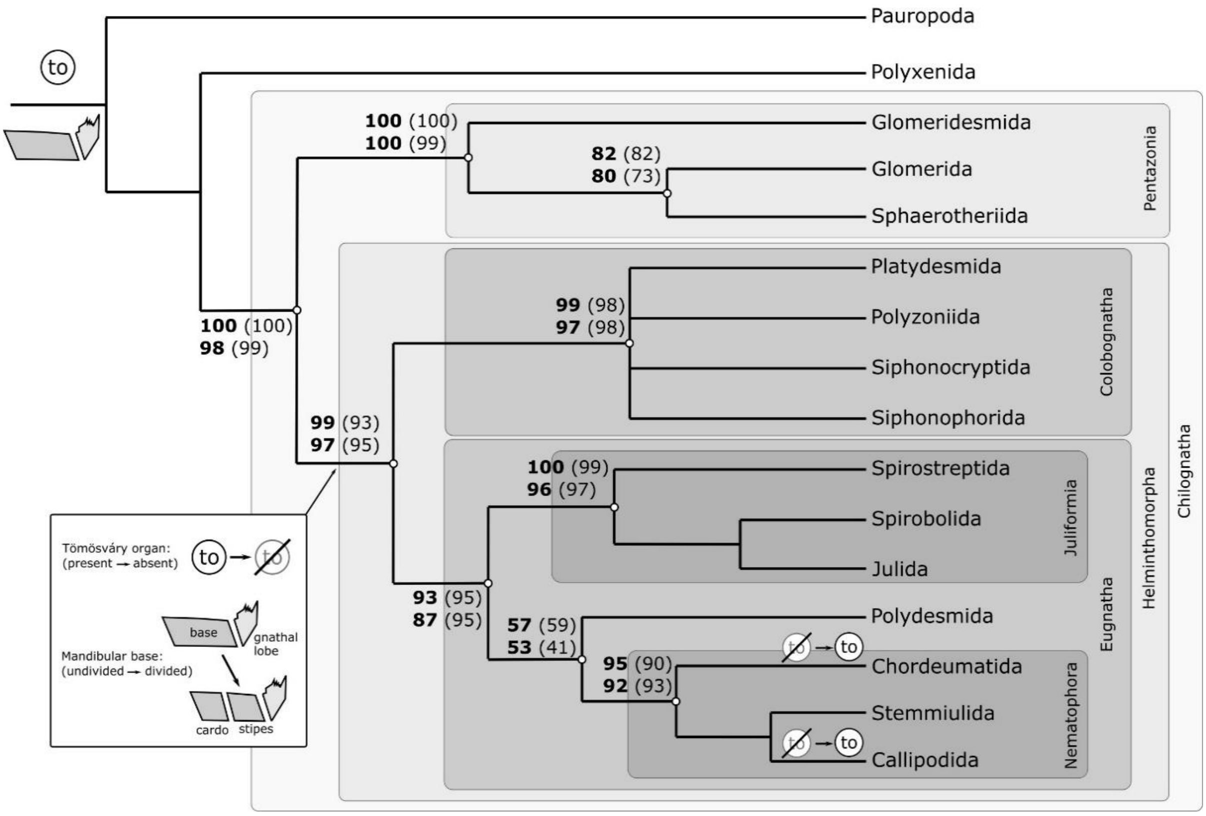

Figure 9. Consensus tree with 98 steps of the five most parsimonious trees derived from maximum parsimony analysis, based on the altered data set of Blanke & Wesener (2014). Following changes to the original dataset have been made: For Polydesmida the Tömösváry organ has been coded as absent (character state 6: 0) and its shape has been coded as inapplicable (character state 7: -). For Platydesmida, Polyzoniida, Siphonocryptida and Siphonophorida the mandibular base has been coded as divided (character state 13: 1). Upper number on nodes indicates bootstrap value, lower number indicates Jackknife value (bold = this study; in parentheses () = from Blanke & Wesener (2014)). Transformations of the corrected characters are mapped on the branches.

9.3 Diversification and morphological evolution through time

The Diplopoda originated more than 400 ma with the earliest fossils being derived representatives of the Helminthomorpha (Wilson & Anderson 2004). Some estimates based on molecular data even suggest an origin in the Cambrian, 495 ma, and divergence of Penicillata (Polyxenida) and Chilognatha in the Ordovician 450 ma (Benavides et al. 2012). Here it is demonstrated that already more than 99 ma most extant orders (13 of 16), and even some extant millipede families and genera, were present in the Cretaceous Myanmar (chapters 7 & 8). This means that the extant millipede taxa diversified already before the Cretaceous and appear almost unchanged in their external morphology since at least 99 million years, while the Paleozoic groups disappeared by the beginning of the Mesozoic. These fossils are the first and/or oldest record for the Symphyla and for eight of 16 millipede orders, including

Platydesmida, Siphonophorida, Polyzoniida, and Glomeridesmida, giving the minimum age of these taxa. Molecular clock approaches attempted to date splits on molecular phylogenies to infer the age of clades. These molecular clocks require calibration with fossils, which can clearly be assigned to a taxon (Fernández et al. 2018; Benavides et al. 2022). Recently, the myriapod fossils from Burmese amber, of which some have been described here (chapters 7 & 8; Wesener & Moritz 2018; Moritz & Wesener 2018, 2021b; Stoev et al. 2019), have been used to calibrate the molecular clock to study the diversification of Myriapoda (Benavides et al. 2022). These analyses result in relatively large time spans of more than 200 million years for the probable origin (95% highest posterior density) of some groups, and in gaps between the first appearance in the fossil record and molecular estimated divergence times (Fernández et al. 2018; Benavides et al. 2022). However, the incorporation of the Cretaceous millipede fossils from Burmese amber already led to a better match of fossil data and molecular estimated divergence times (Benavides et al. 2022) compared to earlier studies (Fernández et al. 2018). Based on these results, it can be assumed that the discovery and incorporation of more fossils will lead to further improvements.

Despite their ancient origin, early diversification, and morphological diversity, the Diplopoda count only ca. 12,000 described extant species (Enghoff et al. 2015) and are estimated to include a total of 13,000 – 20,000 species (Brewer et al. 2012a). The often quoted 80,000 species suggested by Hoffman (1980) are not supported by data (Brewer et al. 2012a). Thus, millipede diversity is low compared to crustaceans with more than 30,000 described species (Schram 2013), arachnids with more than 90,000 described species (Adis & Harvey 2000) and insects with almost one million described species (Grimaldi & Engel 2005). Within the Diplopoda, especially the Colobognatha show a low diversity with ca. 250 described species, contributing to only ca. 2% of the described extant Diplopoda species (Enghoff et al. 2015). There are several factors, that have been assumed to affect the diversity of higher taxa (Mayhew 2007; Stadler et al. 2014): The age of a group might be relevant, as older clades had more time to accumulate species; the rate of evolution might influence how fast a clade diversifies; ecological factors and occupied niches might limit the diversity of taxa. However, the diversity of higher taxa is also largely influenced by how these taxa are defined, i.e. how many species are attributed to these arbitrary groups.

For insects it has been suggested that certain morphological, ecological, and behavioral changes had an influence on their diversification. The diversity of insects might have been enhanced among others by the evolution of wings and wing-folding, as this increased their dispersal ability (Mayhew et al. 2007). Therefore, the comparatively low diversity of millipedes, and especially Colobognatha, might be linked to their low dispersal ability, as they cannot move over large distances to escape from unfavorable conditions, like climatic changes.

Furthermore, mouthpart diversity might have played a role in insect diversification (Labandeira 1997), although niche specialization can increase the extinction risk compared to generalists (Mayhew et al. 2007). Thus, the low diversity within Colobognatha might also be related to their potentially specialized diet. However, the exact diet of many taxa remains unclear and has only been studied in detail for the single Platydesmida species *Brachycybe lecontii*. These results showed that *B. lecontii* feeds on a great diversity of different fungi from at least nine different orders (Macias et al. 2019). Therefore, it can be assumed that, although the feeding apparatus of the Colobognatha is highly derived and specialized towards fluid feeding and possibly fungivory, these millipedes might not be specialized towards specific prey species and are rather generalists. Based on the biogeography (Shelley & Golovatch 2011) and fossil record (chapters 7 & 8) of the group it appears most probable that the extant species of the Colobognatha are the relict of a once greater and more widespread diversity. Furthermore, it can be assumed that our view of millipede diversity is largely biased towards certain groups with clear diagnostic characters, like species-specific gonopods (Brewer et al. 2012a). I suggest that the actual diversity of Colobognatha is much higher than the number of described species, as in recent years new species have been described from various little studied areas like Australia (Marek et al. 2021), New Zealand (Moritz et al. 2021b) and India (Anilkumar et al. 2022), and several species from these areas still await their description (Marek et al. 2021; Moritz et al. 2021b).

9.4 Micro-computed tomography in Myriapodology

As shown in the presented studies µCT and SR-µCT can help to generate detailed 3D-data on the internal anatomy of a wide millipede taxon sampling, including very small and rare specimens, as well as fossil inclusions, non-destructively in a relatively short time. These techniques are widely used to study insects (Wipfler et al. 2016) but are rarely employed in Myriapodology. However, they hold the potential to greatly advance the field (Akkari et al. 2015). The µCT investigations of millipedes show that their strongly sclerotized and calcified cuticle results in a clear contrast between cuticular structures and soft tissue, which eases segmentation compared to insects or spiders. However, this calcification makes histological investigations difficult and more time demanding than in other arthropods. The data acquisition can be highly accelerated with µCT, but the time-demanding analysis and interpretation of the data remains the bottleneck. In the course of this project, high-resolution µCT data has been acquired for more than 50 species, covering 15 of the 16 Diplopoda orders, of which only a subset could be analyzed in the presented work. The remaining data will be analyzed in the future and made available to the scientific community. It becomes evident that an integrative approach, combining modern µCT techniques with classical histology and microscopy, is inevitable to fully understand the morphology of an organism. Therefore, µCT cannot replace but rather complements classical techniques in the field. For example, the externally opening salivary glands and the nature of the pumping apparatus of the Colobognatha (chapter 3), the tarsal glands of the Glomeridesmida (chapter 5), and the true identity of the supposed Tömösváry organ in Polydesmida (chapter 6) could only be identified with histological sections or SEM. The same is true for amber inclusions, where µCT is a valuable tool to study the morphology of an organism in 3D, but fails to resolve small details visible in light-microscopy, such as setae and surface structures (chapter 8). Therefore, as for any other technique, the key to exploit the full potential of µCT is to know its limitations, and to combine it with other adequate techniques. In future the fast and non-destructive generation of 3D-data for small specimens via µCT and SR-µCT will allow a large taxon sampling with many specimens, enabling large scale cladistic and geometric morphometric studies in Myriapoda, as have already been done for spiders (Kallal & Wood 2022) and some vertebrates (e.g. Felice et al. 2021).

Supplementary Material

Chapter 2: All data acquired in this study is deposited on Morphobank (O'Leary & Kaufman 2011) under project number 3745 (doi: 10.7934/P3745) and can be accessed via the following link: http://morphobank.org/permalink/?P3745.

Chapter 3: Supplementary material can be found under https://www.science.org/doi/10.1126/sciadvabm0577. All histological and µCT data used in this study is deposited on Zenodo (doi: 10.5281/zenodo.5215894) and can be accessed via the following link: https://zenodo.org/record/5215894#YysVsbTP2Uk.

Chapter 4: µCT data for this study is deposited on Morphobank (O'Leary & Kaufman 2011) under project number 2795 (doi: 10.7934/P2795) and can be accessed via the following link: http://morphobank.org/permalink/?P2795.

Chapter 5: Supplementary figures can be found under https://arthropod-systematics.arphahub.com/article/70002/element/5/31//.

Chapter 6: All histological and µCT data is deposited on Morphobank (O'Leary & Kaufman 2011) under project number 3582 (doi: 10.7934/P3582) and can be accessed via the following link: http://morphobank.org/permalink/?P3582.

Chapter 8: µCT data for this study is deposited on Morphobank (O'Leary & Kaufman 2011) under project number 3277 (doi: 10.7934/P3277) and can be accessed via the following link: http://morphobank.org/permalink/?P3277.

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Appendix

Appendix Chapter 2

Leif Moritz, Alexander Blanke, Jörg U. Hammel & Thomas Wesener (2021) First steps toward suctorial feeding in millipedes: Comparative morphology of the head of the Platydesmida (Diplopoda: Colobognatha). *Invertebrate Biology* 140: e12312.

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ORIGINAL ARTICLE

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First steps toward suctorial feeding in millipedes: Comparative morphology of the head of the Platydesmida (Diplopoda: Colobognatha)

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Abstract

Although most millipedes (Diplopoda) show a biting–chewing feeding mode, representatives of the so-called Colobognatha (Platydesmida, Polyzoniida, Siphonocryptida, Siphonophorida), with their more or less reduced mouthparts, are assumed to have evolved a suctorial feeding mode, which enables them to exploit food sources like algal film and fungi hyphae. The Platydesmida are viewed as the key taxon for the understanding of mouthpart adaptations in Colobognatha, as their mouthpart elements still closely resemble those of biting–chewing millipedes. Studies of the skeletomuscular system of the Platydesmida are rare, impeding further understanding of the mouthpart evolution in millipedes. To overcome this impediment in the understanding of millipede evolution, we study the internal morphology of the head in specimens from four genera of Platydesmida: *Brachycybe lecontii*, *Dolistenus* spp., *Gosodesmus claremontus*, and *Pseudodesmus* spp. Although all Platydesmida show similarly structured skeletal elements and accompanying musculature, they are distinct from all other Diplopoda. Their head lacks eyes and organs of Tömösváry, and is pyriform and posteriorly bulging, overlapping the collum. The narrow mandibles are hidden underneath the broad genae. A previously described gnathochilarial postmentum is absent. Muscle attachment sites, such as the transverse mandibular tendon and the median septum, are reduced. The strong modifications of the head capsule, mandibles, and musculature limit the movability of the mandible for biting–chewing motions based on simulations of possible gape angles. Platydesmida likely show a scraping and slurping feeding mode, which is facilitated by the comparably well-developed pharyngeal dilator muscles. We suggest that the Platydesmida show an intermediate state between the biting–chewing millipedes and the other suctorially feeding Colobognatha.

KEYWORDS
functional morphology, mandible, micro-CT, musculature, tentorium

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1 | INTRODUCTION

As decomposers and soil-forming organisms, millipedes (Diplopoda) are of great importance for terrestrial ecosystems (Dunger, 1983). They show various morphological adaptations to different habitats and trophic niches (Golovatch & Kime, 2009; Kime & Golovatch, 2000; Köhler & Alberti, 1990; Manton, 1954, 1961, 1977). Unlike the biting–chewing mouthparts of the large majority of the Diplopoda species, the mouthparts of the predominantly fungivorous Colobognatha (Platydesmida, Polyzoniida, Siphonocryptida, Siphonophorida) are adapted for a scraping–sucking feeding habit (Koch, 2015; Manton, 1961; Verhoeff, 1926–1928; Wong et al., 2020). Within Colobognatha, Platydesmida are a small order with slightly more than 60 described extant species, and a disjunct distribution in North and Central America, Southeast Asia, the European Mediterranean, and the Caucasus (Enghoff et al., 2015). They reach a length of 7–60 mm, and are characterized by a small pyriform head with partially internalized mandibles, absent eyes, and a distinct dorsal suture on their pleurotergites, which often bear large paranota (Enghoff et al., 2015). These morphological characters were present in extinct representatives dating back to the Cretaceous ca. 99 mya (Moritz & Wesener, 2019; Wesener & Moritz, 2018). The Platydesmida often occur in large aggregations (Lewis, 1984; Shorter et al., 2018; Wong et al., 2020). They are fungivorous (Gardner, 1975; Lewis, 1984; Macias et al., 2019) and assumed to show a suctorial liquid-feeding mode (Lewis, 1984; Macias et al., 2019; Wong et al., 2020). Platydesmida are among the few arthropods that exhibit male brood care (Kudo et al., 2009; Wong et al., 2020), which is also a unique behavior within myriapods. There are two distinct body types present among the Platydesmida (Enghoff et al., 2015): very slender and elongated, such as in *Dolistenus* (Figure 1A); and with very wide paranota, such as in *Brachycybe*, *Gosodesmus*, and *Pseudodesmus* (Figure 1B–D). The partially internalized and reduced mouthparts are characters shared with the other colobognathan taxa, whose monophyly and relationships to each other are, however, debated (Blanke & Wesener, 2014; Hoffman, 1977, 1982; Sierwald & Bond, 2007).

The external morphology of the Platydesmida has been described for various taxa (Attems, 1951; Gardner, 1975; Mauriès, 2015; Recuero & Rodríguez-Flores, 2020; Shorter et al., 2018; Wong et al.,

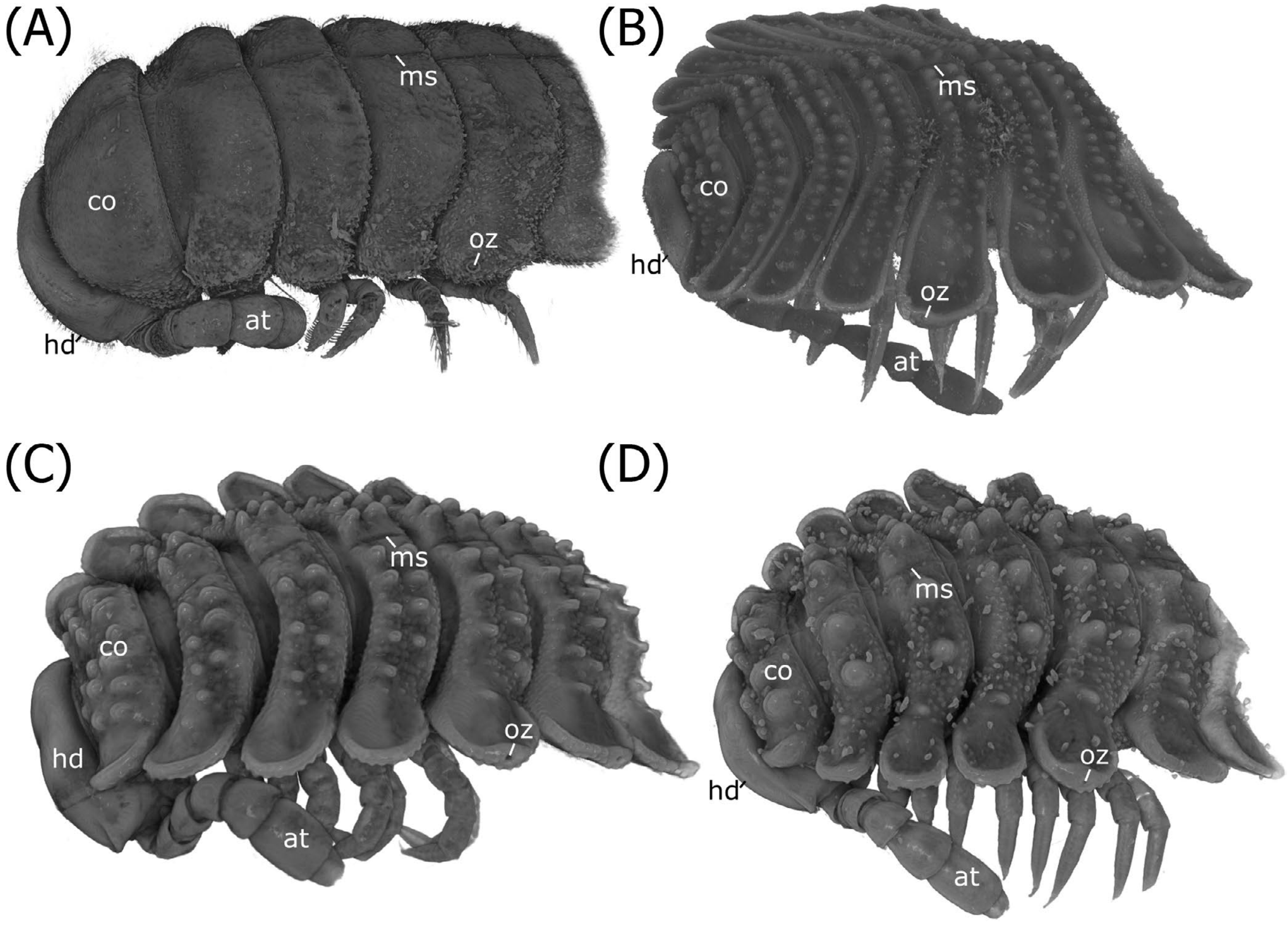


FIGURE 1 The anterior body of the Platydesmida. A. *Dolistenus* sp. from Spain (ZFMK-MYR06615). B. *Brachycybe lecontii* (µCT data from Blanke & Wesener, 2014). C. *Gosodesmus claremontus* (ZFMK-MYRZ02011). D. *Pseudodesmus camptotrichus* (071/01). Not to scale. at, antennae; co, collum; hd, head; ms, median suture; oz, ozopores

2020). The internal morphology and the muscular system have so far been described for the body rings and legs of *Dolistenus savii* Fanzago 1876 by Manton (1961, 1977), who also illustrated some muscles of the head. In addition, Blanke and Wesener (2014) studied the internal morphology of the head and anterior body rings of *Brachycybe lecontii* Wood 1864. The internal cuticular structures of the head (head capsule, gnathochilarium, mandible, tentorial complex) have been described mainly in historic works for *Plutodesmus typhlus* (Daday 1889) (Silvestri, 1903; Verhoeff, 1911–1914), *Platydesmus* sp., and *Pseudodesmus tuberculatus* Silvestri 1899 (Silvestri, 1903), and for *Platydesmus mexicanus* Humbert & De Saussure 1869 (Sinclair, 1905). The latter also described the nervous system of *P. mexicanus*. Thus far there has not been a detailed investigation of the head musculature of the Platydesmida nor a comparison between different platydesmidan species.

Here we review the existing literature and comparatively study the head morphology and skeletomuscular system of two platydesmidan species, *Dolistenus* sp. and *B. lecontii*, as well as the skeletal system of *Gosodesmus claremontus* Chamberlin 1922 and *Pseudodesmus* spp. These species span the morphological diversity and geographical breadth of the Platydesmida. Such data will hopefully provide a base for future comparisons with the morphology of the more strongly modified heads of the other colobognathan taxa, Polyzoniida, Siphonocryptida, and Siphonophorida.

2 | METHODS

2.1 | Specimens and data deposition

Six specimens belonging to different species of four different genera (*Dolistenus*, *Brachycybe*, *Gosodesmus*, and *Pseudodesmus*) of the Platydesmida were studied. One specimen each of *Dolistenus* sp. from Spain (ZFMK-MYR06615: Spain, Malaga, Tolox, 36°40′53″N 04°55′10″W, humid pine forest under stones) and *Dolistenus* sp. from Italy (ZFMK-MYR04718: Italy, Piemonte, Ormea, 44°09′14.81″N 07°54′24.36″E, *Fagus* forest in leaf litter) from the collection of the Zoological Research Museum A. Koenig (ZFMK, Bonn, Germany), which were preserved in 96% EtOH, were studied using synchrotron micro-computed tomography (SR-µCT). For *B. lecontii*, the SR-µCT data provided by Blanke and Wesener (2014), which were obtained at the Deutsches Elektronen-Synchrotron (DESY, Hamburg, Germany), were analyzed. In addition, one specimen each of *G. claremontus* (ZFMK-MYR02011: United States of America, California, Redwood NP, 41°18′56.46″N 124°01′38.57″W, redwood forest under dead wood), *Pseudodesmus* sp. (ZFMK-MYR10034: Thailand, Khlong Sok, Amphoe Phanom, 08°52′43.9″N 98°40′25.1″E, humid forest on tree stem), and *Pseudodesmus camptotrichus* (Attems 1938) (071/01, stored at the Senckenberg-Museum Frankfurt a. M.: Laos, Khammouane, Ban Nong Ping, Xe Bang Fai,17°22′19.8″N 105°50′16.6″E, French Khammouane Expedition 2007) were studied using conventional µCT. All data acquired were uploaded to Morphobank (O'Leary & Kaufman, 2011) under project number 3745 (doi: 10.7934/P3745) and can be accessed via the following link: http://morphobank.org/permalink/?P3745.

2.2 | Micro-computed tomography

For synchrotron micro-computed tomography (SR-µCT), the specimens of *Dolistenus* spp. (ZFMK-MYR04718, ZFMK-MYR06615) were stained with 3% iodine for 24 hr and critical point dried with a Leica EM CPD 300. SR-µCT data were acquired at the Imaging Beamline P05 (IBL; Greving et al., 2014; Haibel et al., 2010; Wilde et al., 2016) operated by the Helmholtz-Zentrum-Geesthacht at the storage ring PETRA III (Deutsches Elektronen-Synchrotron—DESY, Hamburg, Germany). A photon energy of 14 keV and a sample-to-detector distance of 25 mm were used to image the specimens. Projections were recorded using a custom-developed 20 MP CMOS camera system (Lytaev et al., 2014) with an effective pixel size of 0.305 µm. For each tomographic scan, 2401 projections at equal intervals between 0 and π were recorded. Tomographic reconstruction was done using filtered back-projection implemented in a custom reconstruction pipeline (Moosmann et al., 2014) using Matlab (Math-Works) and the Astra Toolbox (Palenstijn et al., 2011; van Aarle et al., 2015, 2016). For further processing, raw projections were binned four times resulting in an effective pixel size of the reconstructed volume of 1.22 µm. For conventional micro-computed tomography (µCT), the specimens were scanned in 95% ethanol with a Skyscan 1272 (Brucker microCT) and reconstructed in NRecon Version 1.7.0.4 (Brucker microCT). For scanning and reconstruction parameters, see Morphobank.

2.3 | Image processing, 3D visualization, and measurements

Image stacks were modified in Fiji ImageJ version 1.50e (Schindelin et al., 2012; www.imagej.net). Segmentation was performed in ITK-SNAP version 3.6.0 (Yushkevich et al., 2006; www.itksnap.org). Segmented structures were smoothed and rendered in Meshlab version 2016.12 (Cignoni et al., 2008; www.meshlab.org) and Blender version 2.77 (www.blender.org). Head distances and the potential opening range of the mandible were measured in Blender version 2.77 (Figure 2). For Scenario 1, the minimal opening angle, the labrum was assumed to be rigid and served as a limiting border for mandible movement. For Scenario 2, the maximal opening angle, the labrum was assumed to be flexible, and the border between labrum and clypeus was used as limit of mandible movement. Volume renders were calculated in Drishti version 2.6.3 (Limaye, 2012). Images were edited in GIMP version 2.10.6 (https://www.gimp.org) and Inkscape version 1.0 (http://www.inkscape.org). All software used are distributed under the GPL/GNU.

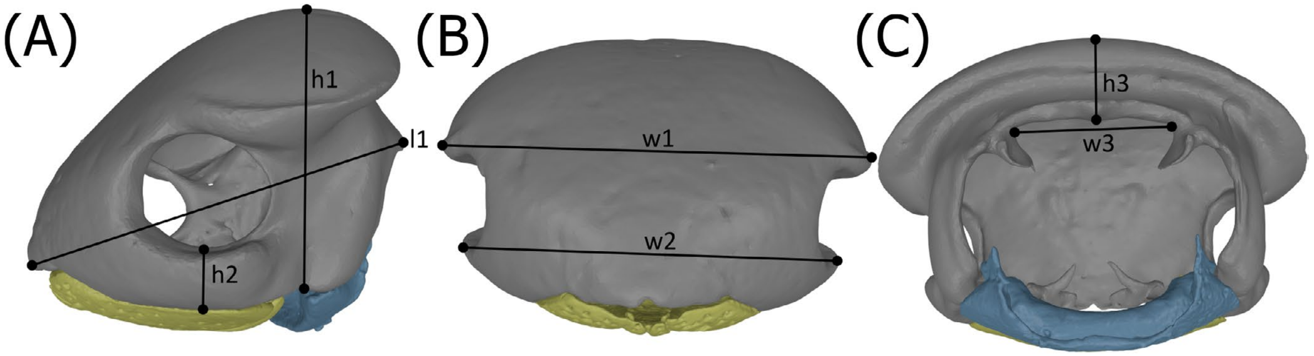


FIGURE 2 Distances measured in Blender. *Brachycybe lecontii*. Segmentation based on µCT data. A. Lateral view. B. Frontal view. C. Posterior view. h1, height at epicranium; h2, height of gena; h3, height of postoccipital flange; l1, length from labrum to mesial tip of postoccipital flange; w1, width of epicranium; w2, width of genae; w3, width between postoccipital processes

2.4 | Terminology

The terminology follows Koch (2015) and Wilson (2002), if applicable. Muscles differing from Wilson's description are named according to their insertion and relative position within the head. Abbreviations of muscles are numbered continuously and do not necessarily correspond to abbreviations used for homologous muscles by Wilson (2002).

3 | RESULTS

Despite differences in the general appearance, the studied specimens of Platydesmida were similar to each other in the structure of their skeletomuscular system. For the specimens of *G. claremontus* (ZFMK-MYR02011), *Pseudodesmus camptotrichus* (071/01), and *Pseudodesmus* sp. (ZFMK-MYR10034), only the skeletal elements could be analyzed.

3.1 | Skeletal elements

3.1.1 | Head capsule

The head capsule of the studied specimens of Platydesmida was small and pyriform and showed the same principal structure and shape in all studied genera. Variations within the Platydesmida could be observed in their proportions regarding the ratio of length, width and height, and the shape of the lateral extension of the epicranium above the antennal base (Figure 3; Tables 1 and 2). The head capsule did not bear any sutures that would divide it into different areas. The terms applied to areas of the head capsule in the following only serve for communication but do not necessarily imply homology. The width of the head ranged 365–850 µm and its length ranged 268–704 µm. The head was longer than wide in *Dolistenus* spp. and *G. claremontus*, and wider than long in *B. lecontii* and *Pseudodesmus* spp. (Table 1). The toothless labrum consisted of a thinner cuticle than the remaining head capsule (Figure 4A). A pair of bars (Epipharynxhalter *sensu* Verhoeff, 1926–1928) arose from the inner surface of the labrum, which extended posteriorly within the epipharynx and arched above the mandibular gnathal lobe (Figure 4B). In *B. lecontii*, fibers of uncertain composition and function were observed; these were associated with the labrum and ran posteriorly to the epipharynx. The short clypeus, which was delimited by the anterior margin of the antennal sockets, was followed by a larger frons located between both antennae (Figure 5A). The antennal sockets were, relative to the head, larger in *B. lecontii* and *Pseudodesmus* sp., occupying more than a third of the head's lateral side, whereas they were smaller in *G. claremontus*, *Dolistenus* spp., and *P. camptotrichus* (Figure 3, Table 2). The epicranium posterior to the antennae was widened and formed a bulge, which partly overlapped the collum (Figures 4A, 5A, and 6A–E). The epicranium was as wide as or wider than the genae (Table 1). In *G. claremontus*, the epicranium showed a pair of medial dents posterior to the antennal sockets (Figure 6C), which corresponded to the origin of the pharyngeal dilator muscle p2. Whereas the epicranium was rounded laterally in *Dolistenus* spp., it extended laterally and arched above the antennal base in *B. lecontii*, *Pseudodesmus* spp., and *G. claremontus* (Figure 3, Table 2). Posteriorly, the epicranium gave rise to the inward-projecting postoccipital flange and the neck phragma (Figures 4A and 5C). The postoccipital flange possessed a pair of postoccipital processes, which arose from its ventral margin and projected medially to serve as muscle attachment (Figure 4B). The distance between the postoccipital processes ranged 120–280 µm (Table 1). In *Dolistenus* spp. and *G. claremontus*, the postoccipital projections were knoblike and round in cross section, whereas they were flat and platelike in *B. lecontii* and *Pseudodesmus* spp. (Figure 3). In *P. camptotrichus*, a cuticular pointed projection arose mesially from the posterior epicranium and extended into the head lumen (Figure 6D), whereas in *G. claremontus* (Figure 6C) and *Pseudodesmus* sp. (Figure 6E), a weak cuticular swelling was located at the same position, and no trace of such a structure was found in *Dolistenus* spp. (Figure 6A) and *B. lecontii* (Figure 6B, Table 2, Figures S1 and S2). If present, such a process or swelling served as attachment site for the median antennal muscle (a1) and the posterior pharyngeal muscle (p3), which otherwise originated directly

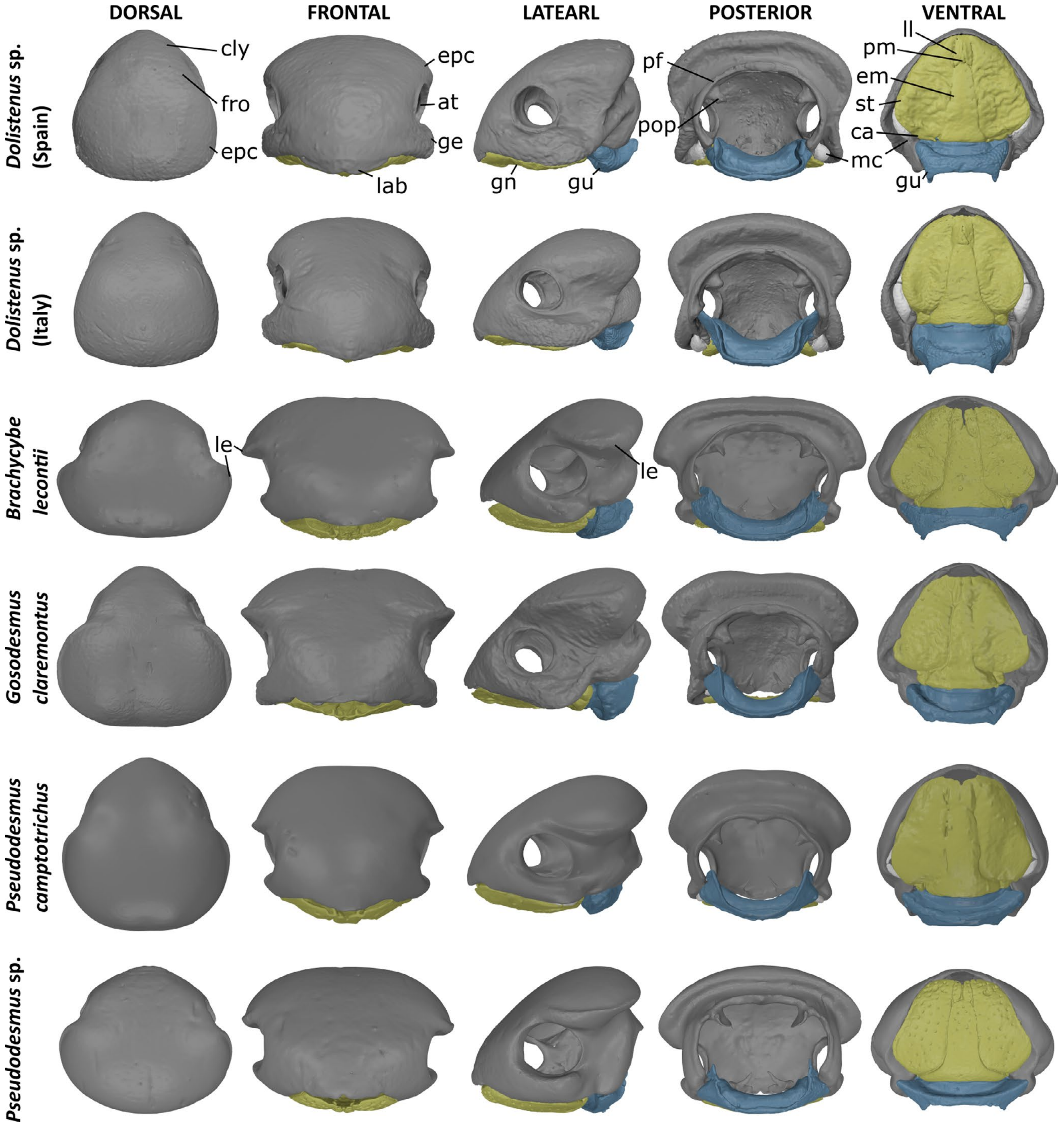


FIGURE 3 Comparative head morphology of the studied Platydesmida, showing the head capsule (dark gray), mandibular base (light gray), gnathochilarium (yellow), and gula (blue). Segmentation based on µCT data. Not to scale. at, insertion of antennae; ca, cardines; cly, clypeus; em, eumentum; epc, epicranium; fro, frons; ge, gena; gn, gnathochilarium; gu, gula; lab, labrum; le, lateral extension of the epicranium; ll, lamella lingualis; mc, mandibular cardo; pf, postoccipital flange; pm, promentum; pop, postoccipital process; st, gnathochilarial stipes

from the postoccipital flange. The genae, lateral of the antennae, were broad and convex or inflated. In *B. lecontii* and *Pseudodesmus* spp., the genae were ventrally rounded, whereas they were straight in *Dolistenus* and *Gosodesmus* (Figure 3). The ventral margin of the genae formed an inward-projecting duplicature, which faced the mandible base (Figure 6F–J). The incisura lateralis, eyes, and organ of Tömösváry were absent (Figure 3).

3.1.2 | Gnathochilarium

All studied species shared a gnathochilarium consisting of a mentum, paired stipites, paired lamellae linguales, and paired cardines. The mentum widened posteriorly and narrowed anteriorly. The mentum in *B. lecontii* (Figure S1), *G. claremontus*, and *Pseudodesmus* spp. was entire. The mentum in *Dolistenus* spp. was bipartite (eumentum + promentum;

TABLE 1 Measurements of the head capsule and mandibular base of the Platydesmida

Distance (µm) or ratio	*Brachycybe lecontii*	*Dolistenus* sp. (Spain)	*Dolistenus* sp.(Italy)	*Gosodesmus claremontus*	*Pseudodesmus camptotrichus*	*Pseudodesmus* sp.
h1	203	227	290	256	396	482
h2	38	60	82	64	54	104
h3	44	55	80	72	106	133
l1	268	391	472	503	558	704
w1	365	367	421	442	576	850
w2	295	361	415	430	454	727
w3	120	156	166	178	215	280
lc	40	85	101	60	67	85
ls	85	132	152	144	177	220
lc/ls	0.47	0.64	0.66	0.42	0.38	0.39
w1/w2	1.24	1.02	1.01	1.03	1.27	1.17
l1/w1	0.73	1.07	1.12	1.14	0.97	0.83

h1, height at epicranium; h2, height of gena; h3, height from medial postoccipital flange to epicranium; l1, length from labrum to mesial tip of postoccipital flange; lc, length of mandibular cardo; ls = length of mandibular stipes; w1, width of epicranium; w2, width of gena; w3, width between postoccipital processes.

TABLE 2 Morphological differences in the heads of the studied Platydesmida

Characters	*Brachycybe lecontii*	*Dolistenus* sp. (Spain)	*Dolistenus* sp. (Italy)	*Gosodesmus claremontus*	*Pseudodesmus camptotrichus*	*Pseudodesmus* sp.
Head capsule						
Head shape	Width > length	Length > width	Length > width	Length > width	Width > length	Width > length
Antennal sockets size	Large	Small	Small	Small	Small	Large
Epicranium, shape laterally	Extending laterally above antennal base	Rounded	Rounded	Extending laterally above antennal base	Extending laterally above antennal base	Extending laterally above antennal base
Postoccipital projections	Platelike	Knoblike	Knoblike	Knoblike	Platelike	Platelike
Posterior epicranum, attachment for a1	At postoccipital flange	At postoccipital flange	At postoccipital flange	Cuticular swelling	Pointed projection	Cuticular swelling
Posterior epicranum, attachment for p3	At postoccipital flange	At postoccipital flange	At postoccipital flange	Cuticular swelling	Pointed projection	Cuticular swelling
Genae	Ventrally rounded	Straight	Straight	Straight	Ventrally rounded	Ventrally rounded
Gnathochilarium						
Mentum	Entire	Bipartite	Bipartite	Entire	Entire	Entire
Mandible						
Cardo in ventral view	Hidden	Visible	Visible	Hidden	Visible	Hidden

Figures 3 and 5D; Table 2). The promentum (if present) lay anterior to the eumentum and was bordered laterally by the paired lamellae linguales, which met anterior to the promentum or mentum, respectively. Laterally, large gnathochilarial stipites extended along the whole length of the gnathochilarium. Posterior of the stipites and lateral to the mentum/eumentum lay the small paired cardines, which were hidden underneath the mentum and stipites in ventral view (Figures 3 and 4B). As in all Colobognatha (Blanke & Wesener, 2014; Enghoff et al., 2015; Hoffman, 1982), gnathochilarial palps were absent. The ducts of the paired maxillary nephridia opened between the lamellae linguales and stipites. Posteriorly, the gnathochilarium was bordered by a broad gula. The gula showed a transverse fold so that the proximal part of the gula overlapped its distal portion (Figure 6F–J).

3.1.3 | Mandible

The mandibles showed the same structure in all studied Platydesmida. The mandible was internalized (entognathous) and consisted of a bipartite narrow base (cardo + stipes) and a gnathal lobe (Figures 4C,

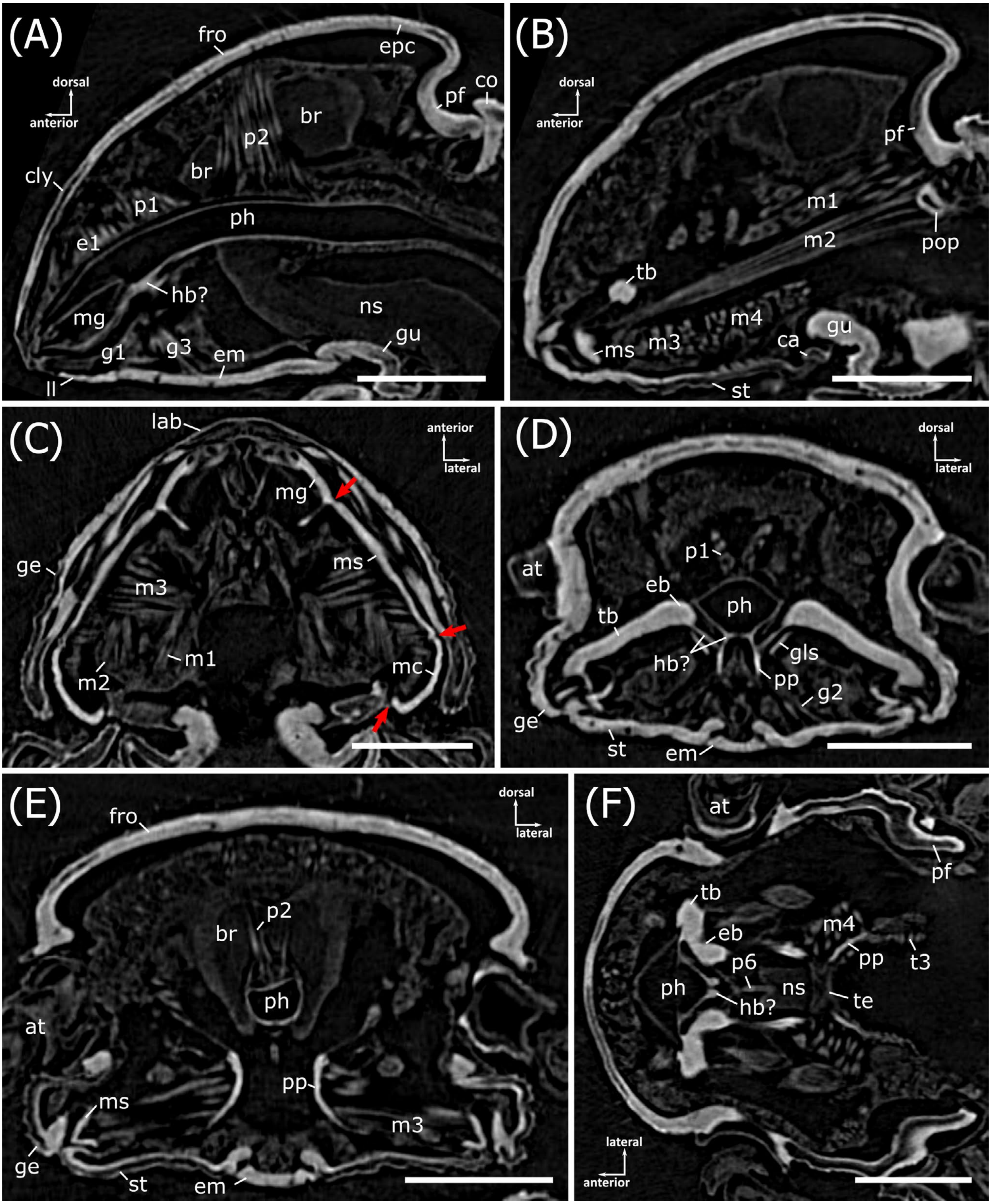


FIGURE 4 *Dolistenus* sp. (ZFMK-MYR06615), virtual sections based on SR-µCT data. A. Medial sagittal sections. B. Sagittal section through mandibular base. C. Transverse section through mandible. D. Horizontal section through transverse bar of tentorial complex. E. Horizontal section through posterior process of tentorial complex. F. Transverse section through tentorial complex. Scale = 100 µm. at, antennae; br, brain; ca, gnathochilarial cardines; cly, clypeus; co, collum; e#, epipharyngeal muscle; eb, tentorial epipharyngeal bar; em, gnathochilarial eumentum; epc, epicranium; fro, frons; g#, gnathochilarial muscle; ge, gena; gls, gnathal lobe sclerite; gu, gula; hb?, possible tentorial hypopharyngeal bar; lab, labrum; ll, gnathochilarial lamella lingualis; m#, mandibular muscle; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; ns, nervous system; p#, pharyngeal muscle; pf, postoccipital flange; ph, pharynx; pop, postoccipital process; pp, tentorial posterior process; st, gnathochilarial stipes; t#, tentorial muscle; tb, tentorial transverse bar; te, transverse mandibular tendon

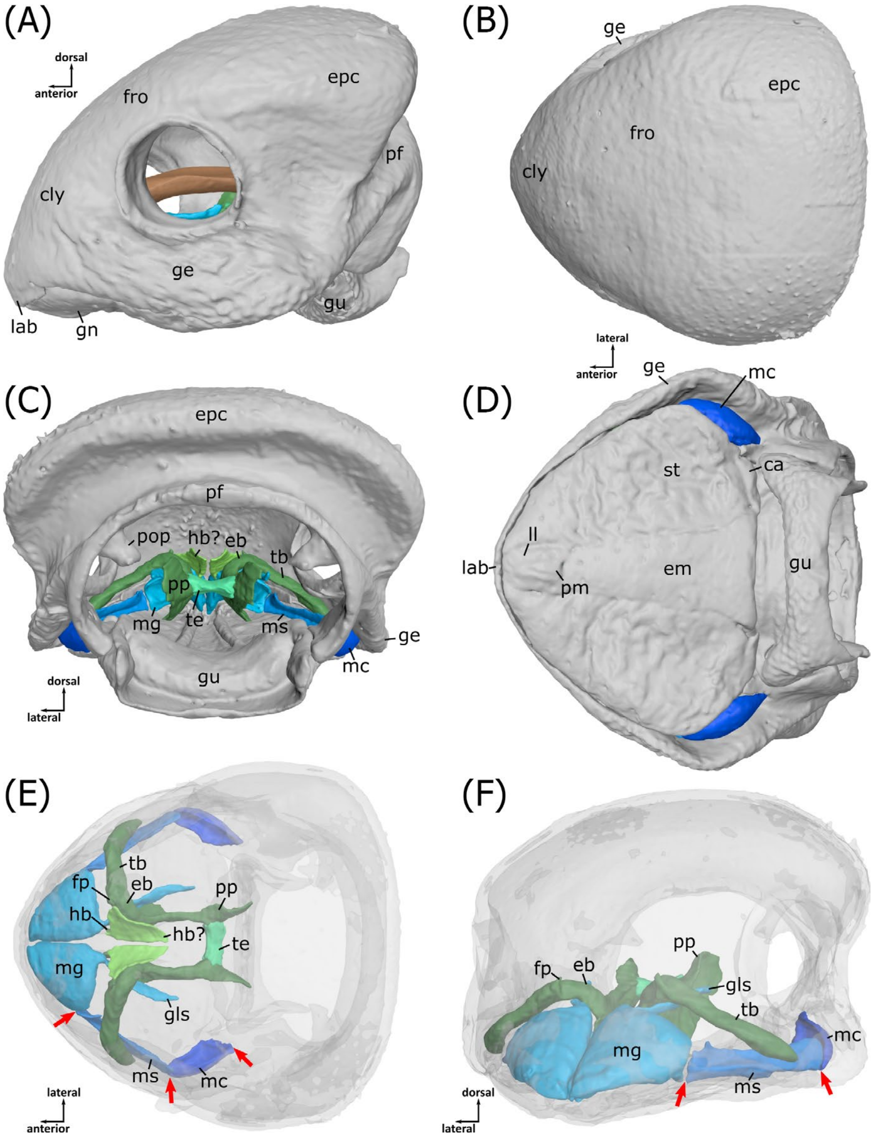


FIGURE 5 *Dolistenus* sp. (ZFMK-MYR06615), skeletal elements and 3-D segmentation. A. External morphology, lateral view. B. External morphology, dorsal view. C. Posterior view. D. Gnathochilarium, ventral view. E. Mandibles and tentorial complex, dorsal view. F. Mandibles and tentorial complex, fronto-lateral view. Arrows indicate articulation of the mandible. Not to scale. ca, gnathochilarial cardines; cly, clypeus; eb, tentorial epipharyngeal bar; em, gnathochilarial eumentum; epc, epicranium; fp, frontal projection from tentorial transverse bar; fro, frons; ge, gena; gls, gnathal lobe sclerite: gn, gnathochilarium; gu, gula; hb, tentorial hypopharyngeal bar; lab, labrum; ll, gnathochilarial lamella lingualis; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; pf, postoccipital flange; pm, gnathochilarial promentum; pop, postoccipital process; pp, tentorial posterior process; st, gnathochilarial stipes; tb, tentorial transverse bar; te, transverse mandibular tendon

5E and 6F–J). The mandibular cardo was approximately half as long as the mandibular stipes and approximately twice as long as wide. The mandibular stipes was elongated and approximately four times as long as wide (Table 1). The cardo is articulated posterior to the gena of the head capsule and partially visible from the outside. In *Dolistenus* spp. and *Pseudodesmus camptotrichus*, the cardo was well visible in ventral view, whereas in *B. lecontii*, *Pseudodesmus* sp., and *G. claremontus*, it was hidden underneath the gnathochilarium (Figure 3, Table 2). Cardo and stipes were articulated via a syndesis, an elastic membrane connecting both sclerites. The gnathal lobe was at its lateral face articulated to the stipes via a dorsal ball-and-socket joint (Figure 4C). The mandibular gnathal lobe was triangular, as long as the mandibular cardo, and bore apical teeth. A molar plate and pectinate lamellae could not be identified on the CT data. A slender, platelike apodeme—the gnathal lobe sclerite—extended posteriorly from the medial margin of the gnathal lobe, parallel to the tentorial posterior process and the pharynx, into the head capsule (Figure 5E).

3.1.4 | Tentorial complex, pharynx, and transverse mandibular tendon

There was no variation in the principal structure of the tentorial complex among the studied species. The robust paired tentorial

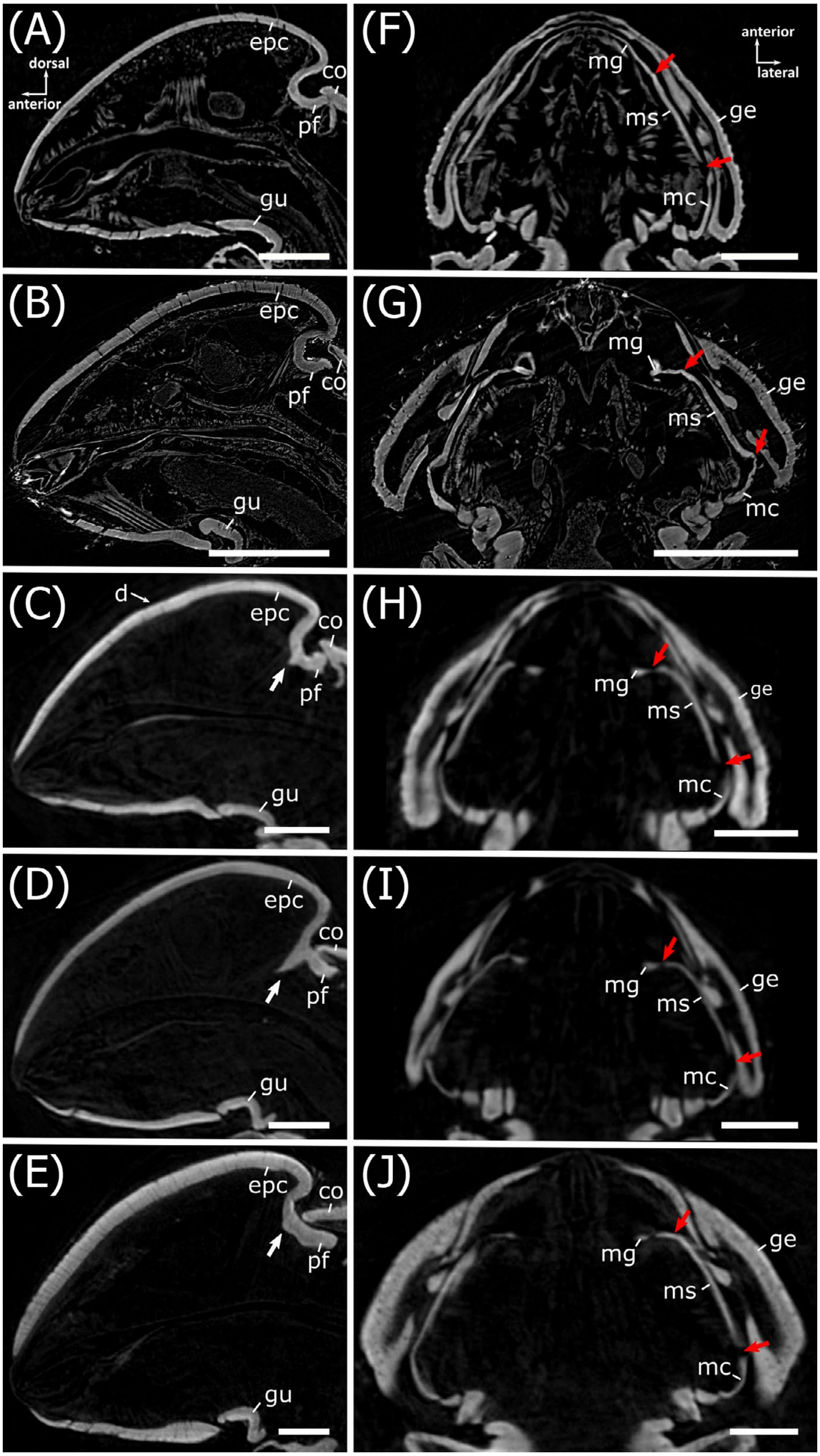


FIGURE 6 Comparative head morphology of Platydesmida: *Dolistenus* sp. (ZFMK-MYR04718) (A, F), *Brachycybe lecontii* (µCT data from Blanke & Wesener, 2014) (B, G), *Gosodesmus claremontus* (ZFMK-MYR02011) (C, H), *Pseudodesmus camptotrichus* (071/01)(D,I), and *Pseudodesmus* sp. (ZFMK-MYR10034) (E,J). A–E. Medial sagittal section through head; white arrows indicate medial process from the head capsule. F–J. Transverse section through mandibles, red arrows indicate articulations. Scale = 100 µm. co, collum; d, dent (depression); epc, epicranium; ge, gena; gu, gula; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; pf, postoccipital flange

complex consisted of (a) a transverse bar with a small anterior projection, (b) an epipharyngeal bar, (c) a hypopharyngeal bar, and (d) a posterior process (Figure 5E). The transverse bar was connected to the head capsule at the ventral margin of the gena, arched above the mandibular stipes, and extended to the midline, where it met the epipharyngeal bar (Figure 4D). The transverse bar gave rise to a small anterior projection (Figures 5E and 7C), which might be homologous to the frontal process of Polyxenida (compare Koch, 2003) or the distal portion of the epipharyngeal bar in other millipedes, and served as attachment site for tentorial muscles. Mesially, the epipharyngeal bar ran within the epipharyngeal wall posterior to the mandibular gnathal lobe, where it sent off a short hypopharyngeal bar. Mesially, the hypopharyngeal bar bent posteriorly and ran along the ventral wall of the pharynx. It was not clear whether this part of the tentorial complex is homologous to the hypopharyngeal bar or the hypopharyngeal lateral sclerite (Nebententorium *sensu* Verhoeff, 1911–1914, 1926–1928; Figures 4D,F and 5E). Posteriorly, the tentorial complex extended

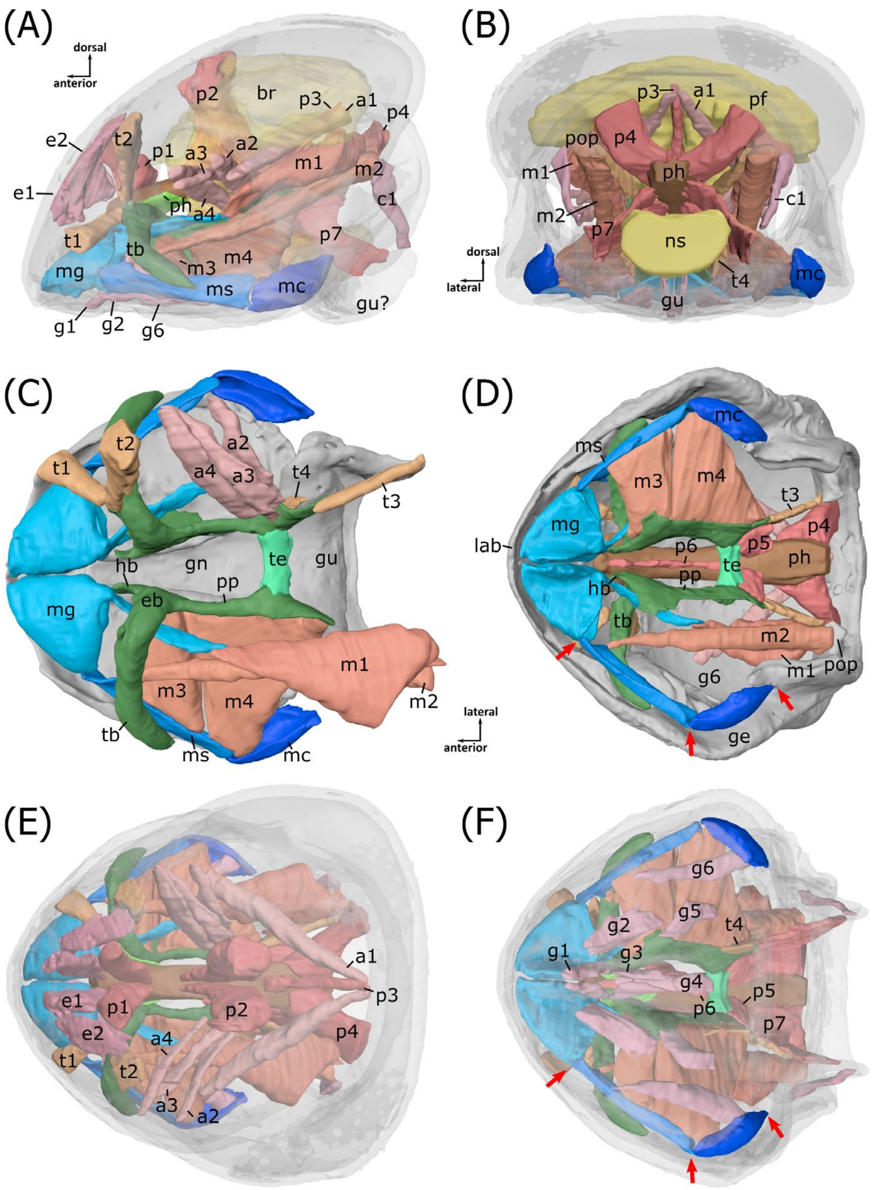


FIGURE 7 *Dolistenus* sp. (ZFMK-MYR06615), musculature of the head and 3-D segmentation. A. Internal morphology and musculature, lateral view. B. Internal morphology and musculature, posterior view. C. Mandibular and antennal musculature, dorsal view, head capsule removed. D. Mandibular and antennal musculature, ventral view, gnathochilarium removed. E. Tentorial and pharyngeal musculature, dorsal view. F. Gnathochilarial musculature, ventral view. Arrows indicate articulation of the mandible. Not to scale. a#, antennal musculature; br, brain; c1, posterior gula muscle; e#, epipharyngeal musculature; eb, tentorial epipharyngeal bar; g#, gnathochilarial musculature; ge, gena; gn, gnathochilarium; gu, gula; hb, tentorial hypopharyngeal bar; lab, labrum; m#, mandibular musculature; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; ns, nervous system; p#, pharyngeal musculature; ph, pharynx; pop, postoccipital process; pp, tentorial posterior process; t#, tentorial musculature; tb, tentorial transverse bar; te, transverse mandibular tendon

into a long platelike posterior process, which ran parallel to the pharynx and the gnathal lobe sclerite (Figure S1).

The anterior part of the pharynx was rhomboid in cross section (Figure 4D,F). Posteriorly, the pharynx, which was lined by cuticle (see Discussion), was elliptical in cross section (Figure 4E). The paired tentoria were interconnected via a collagenous transverse mandibular tendon, which extended as a simple, unbranched bridge between the posterior processes ventral to the pharynx (Figures 4F and 5E).

3.2 | Musculature

The terminology follows Wilson (2002), when applicable. The numbering of muscles is continuous and differs from the scheme used by Wilson (2002). All muscles described were present in *Dolistenus* spp. (ZFMK-MYR06615, ZFMK-MYR04710) and in *B. lecontii* (Video S1, Figure S2; Table 3).

3.3 | Potential opening angle of the mandible

Assuming a rotation of the mandibular gnathal lobe around a single axis, the movement of the mandibular gnathal lobe was limited anterolaterally by the labrum and the epipharyngeal sclerites, and posteromedially by the tentorial complex. The latter mainly restricted the movement of the gnathal lobe apodeme. The minimal opening angle of the gnathal lobe under the most conservative assumption (Scenario 1; see Methods) ranged 1.6–5.7°. Only in *G. claremontus* were opening angles higher, at ~15°. Under Scenario 2 the maximal opening angle of the mandibles ranged 5.1–15°. Again the specimen of *G. claremontus* differed considerably, with an opening angle of up to 50°. The resulting apical distance between the mandibular gnathal lobes ranged 5.9–24.6 µm (under Scenario 1) and ranged 10.9–62.2 µm (under Scenario 2), and distances were considerably higher under both scenarios in *G. claremontus* (Table 4).

4 | DISCUSSION

To understand the phenotypic evolution within Diplopoda, information is needed about the variation of characters among different diplopodan taxa. The Platydesmida are the subgroup of the Colobognatha which show the least modified mouthparts and are often referred to as an intermediate form between the Eugnatha and the other Colobognatha (e.g., Verhoeff, 1912–1914, 1926–1928). Therefore, information about head and mouthpart character states in the Platydesmida might be highly relevant for the understanding of the evolutionary history of millipedes. Head and mouthpart characters were proven to be phylogenetically informative in many cases (Brozek, 2014; Brozek & Bourgoin, 2013; Edgecombe, 2004; Ganske et al., 2018; Krenn, 2007). In previous studies, mainly single species were studied and the observed characters were assumed to be typical for the whole Platydesmida (e.g., Blanke & Wesener, 2014; Verhoeff, 1911–1914). Here, we studied for the first time the variation of the internal morphology of different Platydesmida species and compared it to the characters reported for other taxa in the literature.

4.1 | Variation in the head shape in the Platydesmida

The general appearance of the head is homogenous throughout the Platydesmida. In all studied species, the head is pyriform and widened posteriorly, overlaps the collum, and shows bulged genae, covering the mandibular bases. Considerable variation can be observed in the shape of the epicranium and genae. The lateral margin of the epicranium overlaps the antennal base to various degrees, which might serve as protection for the antennal base. The gena varies in its curvature. Further variation can be seen in the relative length of the head in relation to its width (Figure 3; Table 1). The shape of the head is sometimes used as a taxonomic character in the Platydesmida (e.g., Gardner, 1975) and might provide a greater variety of characters which can be used in taxonomic as well as in phylogenetic studies.

4.2 | The gnathochilarium in the Platydesmida

The gnathochilarium of all studied Platydesmida follows the typical diplopodan structure, consisting of a complete or divided mentum (promentum + eumentum), as well as paired stipites, paired lamellae linguales, and paired cardines, and therefore differs largely from the reduced gnathochilarium of the remaining Colobognatha (Koch, 2015). Our observations mainly correspond to earlier descriptions of the Platydesmida (Koch, 2015; Verhoeff, 1911–1914). There is variation within the Platydesmida regarding the presence of an entire or composite mentum (promentum + eumentum). An entire mentum is, for example, present in *Brachycybe* (this study), *Platydesmus* (Attems, 1951; therein as *nomen nudum Raphonotum*), *Andrognathus* (Shorter et al., 2018), *Gosodesmus* (this study), and *Pseudodesmus* (this study). A divided mentum is present in *Dolistenus* (Attems, 1951; Mauriès, 2015; this study) and *Ebenostenus* (Mauriès, 2015). This state does not only vary within the Platydesmida but also across the eugnathan millipedes (Koch, 2015). As discussed by Koch (2015), the origin of the promentum is unclear. The cardines of the Platydesmida are barely visible in ventral view in the studied taxa but lie below the stipites and mentum. Furthermore, for some Platydesmida, including *Brachycybe*, a postmentum (Praebasilare *sensu* Attems, 1926, 1951; Silvestri, 1903) was reported to be positioned between mentum and gula (Attems, 1951; Hoffman, 1990; Silvestri, 1903), but could not be observed in this study. This interpretation might be the result of the folded shape of the gula, with its proximal portion overlapping the apical part. In ventral view it appears as if there is an additional broad plate between the mentum and the gula, although the CT data show that in all studied Platydesmida this plate is part of the gula

TABLE 3 Cephalic musculature of the Platydesmida *Dolistenus* spp. and *Brachycybe lecontii*. m. = muscle

Muscle	Origin	Insertion	Figures
Median septum antennal m. (a1)	Medial postoccipital flange, lateral to p3	Posterior margin of the antennal base	7A,B,E; 8
Median antennal m. (a2)	Tentorial posterior process, posterior to a3	Posterior margin of the antennal base, dorsal to a1	7A,C,E; 8A
Lateral antennal m. (a3)	Tentorial posterior process, dorsal to a2 and a4	Anterior margin of the antennal base, lateral to a4	7A,C,E, 8A
Anterior antennal m. (a4)	Tentorial posterior process, anterior and ventral to a3	Anterior margin of the antennal base, medial to a3	7A,C,E, 8A
Median epipharyngeal m. (e1)	Clypeus, medial to e2	Epipharynx, medial to e2	7A,E, 8B
Lateral epipharyngeal m. (e2)	Clypeus, lateral to e1	Epipharynx, lateral to e1	7A,E, 8B
Lamella lingualis m. (g1)	Hypopharyngeal lateral sclerite/ hypopharyngeal bar medial to g2	Lamella lingualis	7F, 8B
Anterior gnathochilarial stipes m. (g2)	Hypopharyngeal lateral sclerite / hypopharyngeal bar, lateral to g1	Gnathochilarial stipes	7F, 8B
Lateral mentum m. (g3)	Hypopharyngeal lateral sclerite/ hypopharyngeal bar, posterior to g1	Anterior portion of mentum	7F, 8B
Median mentum m. (g4)	Hypopharynx, medial to g3	Gnathochilarial mentum	7F, 8B
Posterior gnathochilarial stipes m. (g5)	Tentorial posterior process	Stipes, posterior to g2	7F, 8B
Lateral gnathochilarial stipes m. (g6)	Lateral head capsule behind articulation of mandibular cardo	Lateral margin of gnathochilarial stipes	7C,D,F, 8B
Gnathal lobe sclerite m. (m1)	Postoccipital process, dorsally to m2	Gnathal lobe sclerite	7C, 8A
Dorsal mandibular stipes m. (m2)	Postoccipital process, ventrally to m1	Anterior part of mandibular stipes, dorsally to m3	7C,D, 8A
Mandibular stipes m. (m3)	Tentorial posterior process, anterior to m4	Mandibular stipes, anterior to m4	7C,D; 8A
Mandibular cardo m. (m4)	Tentorial posterior process, posterior to m3	Posterior part of mandibular stipes and anterior part of mandibular cardo	7C,D, 8A
Precerebral pharyngeal dilator m. (p1)	Border of clypeus and frons, posterior to e1 and e2	Anterior of brain to dorsal pharyngeal wall, anterior to p2	7E, 8
Intercerebral pharyngeal dilator m. (p2)	Frons between p1 and p3	Through brain to dorsal pharyngeal wall, between p1 and p3	7E, 8
Posterior pharyngeal dilator m. (p3)	Medial postoccipital flange, medial to a1	Posterior of brain to dorsal pharyngeal wall, posterior to p2	7B,E; 8
Dorsal pharyngeal dilator m. (p4)	Postoccipital flange, medial of postoccipital process	Dorsal pharyngeal wall, posterior to p3	7B,D,E, 8
Tentorial pharyngeal dilator m. (p5)	Medial surface of tentorial posterior process	Ventral pharyngeal wall, anterior to p7	7D, 8
Transverse mandibular tendon pharyngeal dilator m. (p6)	Transverse mandibular tendon	Anterior on ventral pharyngeal wall	7D, 8
Ventral postoccipital flange pharyngeal dilator m. (p7)	Gula	Ventral pharyngeal wall posterior to p5	7F, 8
Anterior tentorial m. (t1)	Anterior lateral portion of clypeus	Anterior projection of tentorial transverse bar, lateral to t2	7C, 8
Dorsal tentorial m. (t2)	Clypeus lateral to e2	anterior projection of tentorial transverse bar, medial to t1	7C, 8
Posterior tentorial m. (t3)	Postoccipital process medial to m2	Posterior on posterior process	7C, 8
Ventral tentorial m. (t4)	Anterior margin of Gula	Ventral margin of posterior process	7F, 8
Posterior gula m. (c1)	Base of postoccipital process	Posterior margin of gula	7B, 9A
c2	Median on collum	Posoccipital process	9A,C
c3	Anterior margin of 2nd tergite	Ventrally on postoccipital flange	9B,C

(Continues)

TABLE 3 (Continued)

Muscle	Origin	Insertion	Figures
c4	First tracheal apodeme	Dorsally on Postoccipital flange	9A
c5	Collum, posterior to c2	Posterior margin of gula	9A,C
c6	First tracheal apodeme	Anterior on gula	9B
c7	First tracheal apodeme	Posterior margin of gula	9C
c8	Apically on first tracheal apodeme	Lateral margin of postoccipital flange	9A

TABLE 4 Hypothetical opening angle of mandibles measured in Blender version 2.77

	Scenario 1				Scenario 2			
Species	Angle of left mandible (L)	Angle of right mandible (R)	Angle between L & R	Distance between L & R (µm)	Angle of left mandible (L)	Angle of right mandible (R)	Angle between L & R	Distance between L & R (µm)
Brachycybe lecontii	3.3°	3.8°	3.8°	5.9	4.78°	5.1°	9.4°	10.9
Dolistenus sp. (Spain)	5.3°	2.4°	7.51°	19.8	12.4°	8.4°	20.4°	34.0
Dolistenus sp. (Italy)	2.9°	1.6°	4.31°	8.0	11.5°	9.6°	16.2°	32.6
Gosodesmus claremontus	15.9°	15.2°	29.1°	56.0	23.3°	26.7°	48.1°	113.0
Pseudodesmus camptotrichus	4.1°	6.7°	11.0°	24.6	13.0°	15.0°	25.0°	62.2
Pseudodesmus sp.	5.7°	3.1°	8.7°	24.5	11.0°	7.5°	19.0°	54.0

In Scenario 1, the minimum opening angle, the labrum was assumed to be rigid and a limiting border for mandible movement. In Scenario 2, the maximum opening angle, the labrum was assumed to be flexible, and the border between labrum and clypeus was used as limit of mandible movement.

and not delimited by a suture (Figure 6A–E). We therefore doubt the general presence of a postmentum in Platydesmida.

4.3 | The tentorial complex and pharynx in the Platydesmida

The tentorial complex shows the same principal structure among all studied Platydesmida. It consists of the typical components found in eugnathan millipedes and the Myriapoda in general: A transverse bar, an epipharyngeal bar, a hypopharyngeal bar, and a posterior process. A structure homologous to either the hypopharyngeal lateral sclerite (Nebententorium *sensu* Verhoeff, 1911–1914, 1926–1928) or a part of the hypopharyngeal bar extends backward and lines the ventral pharyngeal wall. This structure might correspond to the broadened sclerite interpreted as the hypopharyngeal lateral sclerite by Verhoeff (1911–1914, 1926–1928), which occupies, according to the author, the hypopharynx (see his figure 474) of the Platydesmida. In our CT data, we could not find any trace of a suture separating this sclerite from the remaining tentorial complex, but the gnathochilarial muscles associated with this sclerite (g1–g3) correspond to muscles which are associated with the hypopharyngeal lateral sclerite in the Juliformia (e.g., Wilson, 2002). Therefore, this structure might be the product of a fused hypopharyngeal bar and hypopharyngeal lateral sclerite. We suggest that the anterior portion of the pharynx, which is partially lined by the tentorial complex, is part of the preoral chamber in the Platydesmida and not of the foregut as in other millipedes (i.e., the ventral wall is formed by the hypopharynx), because the hypopharyngeal lateral sclerite as well as the hypopharyngeal bar are restricted to the hypopharynx in other millipedes (e.g., Verhoeff, 1926–1928). This means that the functional mouth opening, which corresponds in other millipedes to the opening of the foregut (Fontanetti et al., 2015), is positioned more anteriorly and formed within the preoral chamber in the Platydesmida. This view is further supported by the absence of the ring muscles and the thick epithelium usually associated with the foregut behind the mouth opening in other millipedes (Fontanetti et al., 2015; Seifert, 1932; Verhoeff, 1928–1932) and by the presence of strongly developed cuticle. The typical shape of the pharynx (i.e., foregut), with columnar cells forming inwards projecting folds (plicae *sensu* Fontanetti et al., 2015), can only be found more posteriorly.

4.4 | Comparison of the head musculature in the Platydesmida and Eugnatha

So far, the head musculature of the Diplopoda has only been described in detail for the Juliformia, in which the musculature is largely the same across the different species studied; see Silvestri (1903) for *Plusioporus salvadorii* SILVESTRI 1895; see Fechter (1961)

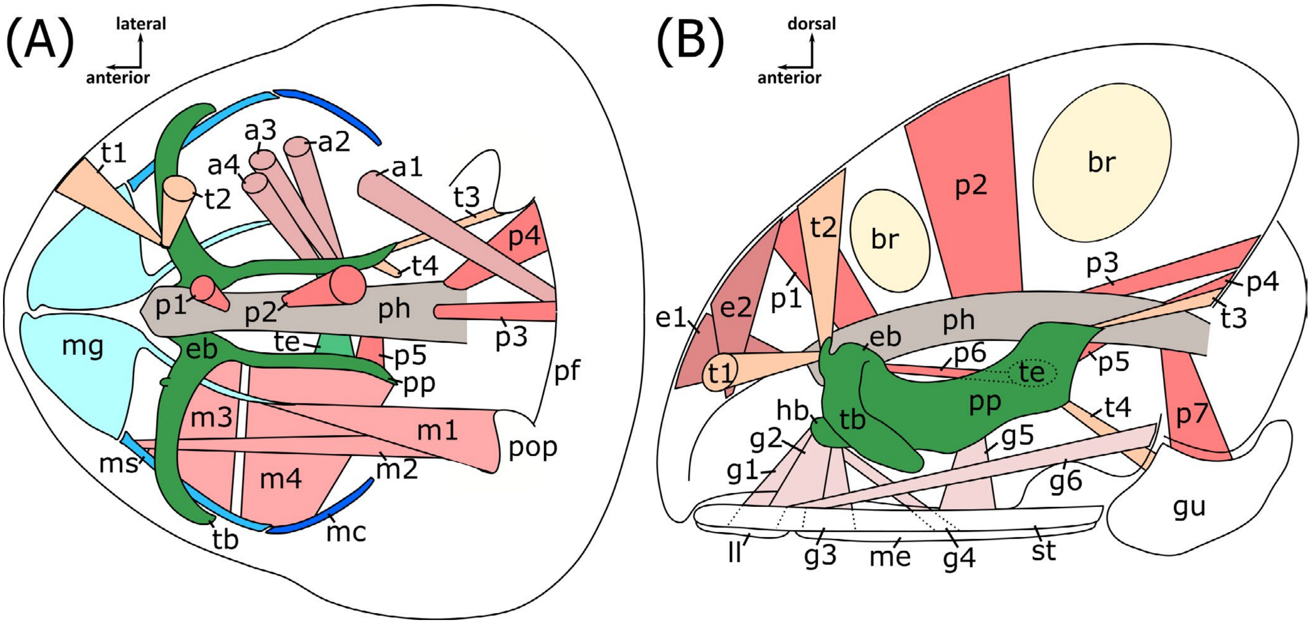


FIGURE 8 Schematic drawing of the generalized musculature of the Platydesmida, based on the SR-µCT data from individuals of *Dolistenus* spp. and *Brachycybe lecontii*. A. Dorsal view. B. Lateral view. a#, antennal musculature; br, brain; e#, epipharyngeal musculature; eb, tentorial epipharyngeal bar; g#, gnathochilarial musculature; gu, gula; hb, tentorial hypopharyngeal bar; ll, gnathochilarial lamella lingualis; m#, mandibular musculature; mc, mandibular cardo; me, gnathochilarial mentum; mg, mandibular gnathal lobe; ms, mandibular stipes; p#, pharyngeal musculature; pf, postoccipital flange; ph, pharynx; pop, postoccipital process; pp, tentorial posterior process; st, gnathochilarial stipes; t#, tentorial musculature; tb, tentorial transverse bar; te, transverse mandibular tendon

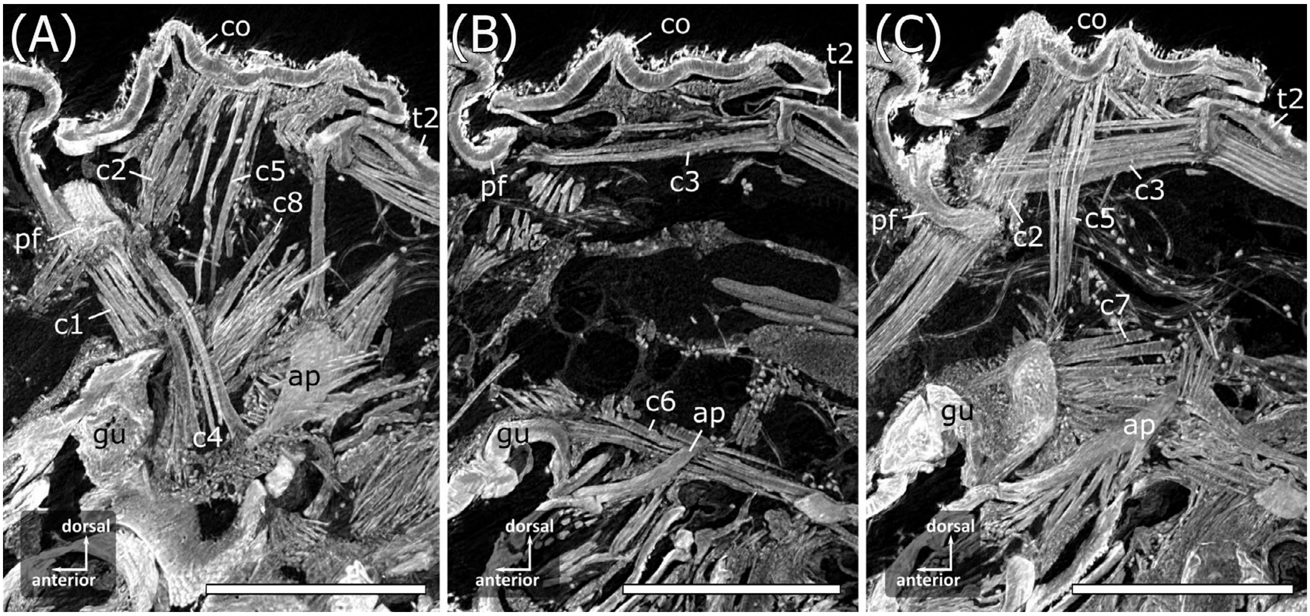


FIGURE 9 *Brachycybe lecontii*, body musculature associated with the head; Z-projections based on SR-µCT data from Blanke and Wesener (2014). A. Sagittal section through lateral region of postoccipital flange and collum. B. Sagittal section through mesial region of postoccipital flange and collum. C. Sagittal section through lateral region of postoccipital flange and collum. Scale = 100 µm. ap, apodeme; c#, collum musculature; co, collum; gu, gula; pf, postoccipital flange; t2, tentorial muscle 2

for *Cylindroiulus caeruleocinctus* (Wood 1864), as *C. teutonicus*; see Manton (1964) for *Poratophilus punctatus* Attems 1928; see Wilson (2002) for *Phyllogonostreptus nigrolabiatus* (Newport 1844); see Nauman et al. (2020) for *Ommatoiulus avatar* Akkari & Enghoff 2015.

The musculature in the Platydesmida, which was previously known only from a partial description by Manton (1961) for *D. savii*, is the same in *Dolistenus* spp. and *B. lecontii*. However, the head musculature in these species differs from that in Juliformia mainly because of the reduction of the mandibular muscles associated with the transverse mandibular tendon (Figure 10).

The species of Platydesmida we studied are also consistent with Juliformia in antennal musculature. The antennal musculature (a1–a4) corresponds in composition, insertion, and origin to that described in the Juliformia (Fechter, 1961; Naumann et al., 2020; Wilson, 2002).

Whereas in the Juliformia a set of four muscles inserts on the epipharynx, in the Platydesmida only two epipharyngeal muscles (e1, e2) of uncertain homology are present. Manton (1961: figure 28) illustrates a single epipharyngeal muscle for *D. savii* (Manton, 1961: figure 28). The reduction or fusion of some epipharyngeal muscles might be related to the small size of the epipharynx and the preoral chamber in platydesmidan millipedes compared to non-colobognathan millipedes.

The gnathochilarium in the Platydesmida is associated with six pairs of muscles, compared to up to nine muscles in most Juliformia (e.g., Naumann et al., 2020). The fewer muscle pairs in Platydesmida might be related to the limited movability of the gnathochilarium, which is tightly appressed to the genae.

The mandibular muscles (m3, m4) originate in the Platydesmida from the tentorial posterior process, and are probably homologous to the anterior mandibular cardo muscle and the posterior mandibular stipes muscle of Wilson (2002), which in the Juliformia also originate from the tentorial posterior process (Fechter, 1961; Manton, 1964, 1977; Naumann et al., 2020; Silvestri, 1903; Wilson, 2002). In eugnathan millipedes, the transverse mandibular tendon, which is reduced in size in the studied Platydesmida, is the main attachment site for the mandibular muscles (Fechter, 1961; Manton, 1964, 1977; Naumann et al., 2020; Snodgrass, 1952; Wilson, 2002). All mandibular muscles originally arising from the transverse mandibular tendon in the Juliformia are absent in the Platydesmida; they did not shift onto the tentorial complex. In the Platydesmida, the remaining mandibular muscles are the mandibular adductor muscle (m1), which was also shown by Manton (1961: figure 28b) as adductor mandibulae from the gnathal lobe in *D. savii*, and the dorsal mandibular stipes muscle (m2), which arises from the postoccipital process. Both muscles can also be found in the Eugnatha (Fechter, 1961; Manton, 1964, 1977; Naumann et al., 2020; Snodgrass, 1952; Wilson, 2002).

The pharynx in *Dolistenus* spp. and *B. lecontii* is equipped with several pharyngeal dilator muscles, which insert dorsally (p1–p3), laterally (p4, p7), and ventrally (p5, p6) on the pharynx. The pharyngeal muscles p1–p3, p4, and p7 were also depicted by Manton (1961: figure 28a) in *D. savii*. The same pharyngeal muscles found in *Dolistenus* spp., *B. lecontii*, and *D. savii* are also present in the Juliformia (e.g., Naumann et al., 2020; Wilson, 2002). These muscles correspond to each other in their insertion and origin. Naumann et al. (2020) furthermore described an eighth pair of pharyngeal muscles (lateral postoccipital flange pharyngeal dilator m.) in the Julida, which is absent in the Platydesmida and the other Juliformia.

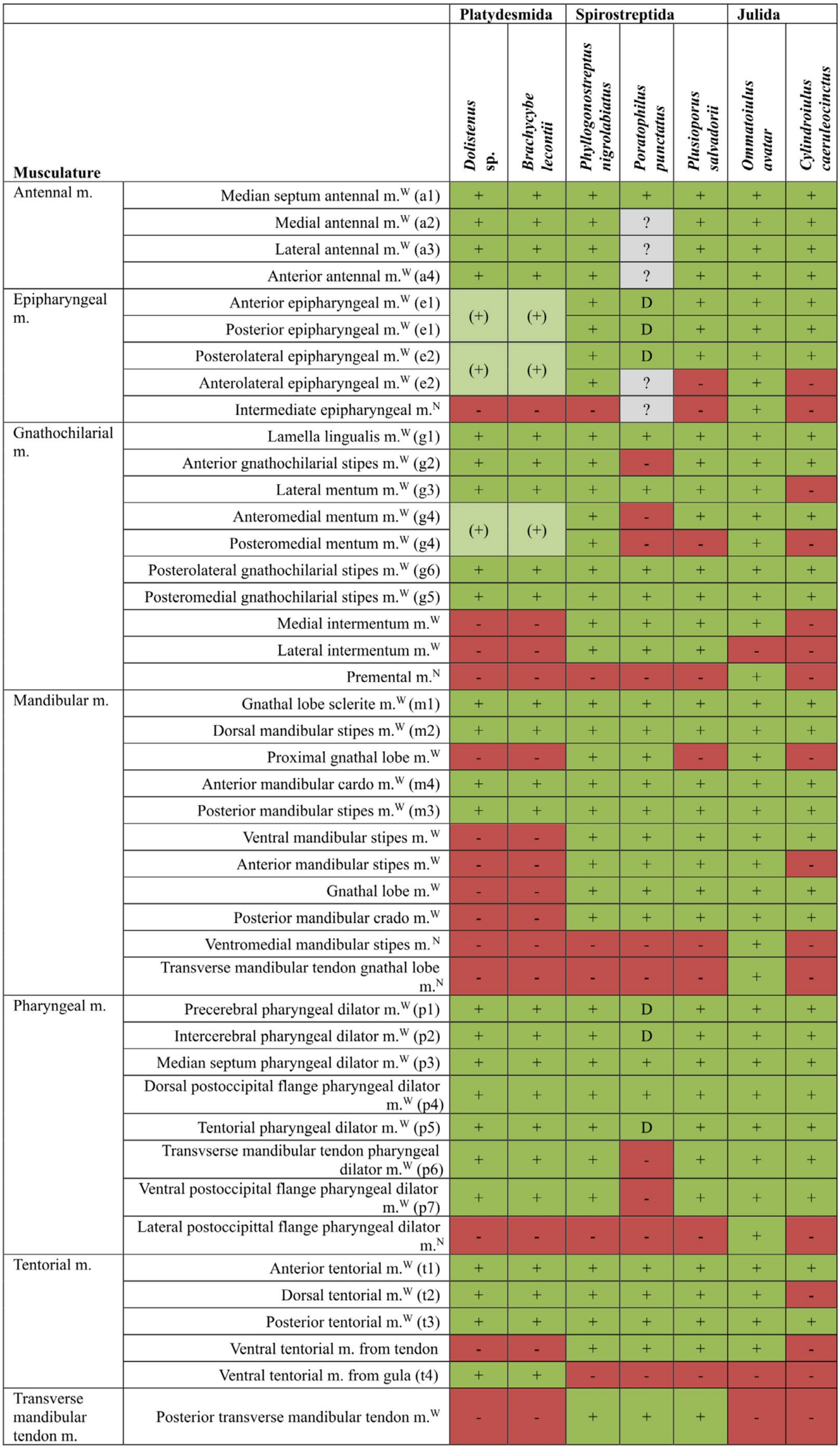
The tentorial muscles in *Dolistenus* spp. and *B. lecontii* are consistent with those described for the Juliformia (Naumann et al., 2020; Wilson, 2002), except for the ventral tentorial muscle from the transverse mandibular tendon, which is absent in the Platydesmida. The anterior tentorial m. (t1) and the dorsal tentorial m. (t2) in the Platydesmida insert on the short projection arising from the transverse bar. Those muscles insert in the Eugnatha directly on the transverse bar (*sensu* Koch, 2003; transverse process *sensu* Manton, 1977; lateral bar *sensu* Wilson, 2002) and the epipharyngeal bar (*sensu* Koch, 2003; anterior process *sensu* Wilson, 2002). Therefore, this anterior projection might correspond to the distal part of the epipharyngeal bar in the Eugnatha or the frontal process of the Polyxenida, described by Koch (2003). In her study of *D. savii*, Manton (1961: figure 28a) showed the protractor tentorii, which corresponds to our t2. The Platydesmida lack the robust anterior extension of the epipharyngeal bar, which arches over the gnathal lobe and is present in other millipedes (compare anterior process in Wilson, 2002; epipharyngeal process in Naumann et al., 2020). In Juliformia the ventral tentorial muscle arises from the transverse mandibular tendon (Manton, 1964; Naumann et al., 2020; Wilson, 2002), and its reduction in the Platydesmida might be the result of the reduction of the tendon and its shift between the tentorial posterior processes. A ventral tentorial muscle is also absent in the Sphaerotheriida (Moritz et al., 2018). An additional muscle associated with the tentorial complex of the Platydesmida, which is absent in Juliformia (Fechter, 1961; Manton, 1964; Naumann et al., 2020; Silvestri, 1903; Wilson, 2002), is the ventral tentorial m. from the gula (t4), which extends from the gula (sternite I *sensu* Manton, 1961) to the tentorial posterior process and corresponds to the retractor ventralis to tentorium of Manton (1961: figure 28d) in *D. savii*. Because of the shape of the tentorium and its intrinsic musculature, it can be assumed that the tentorial complex remains movable to a certain degree in the Platydesmida and might perform the tentorial swing. The tentorial swing is assumed to be typical for the Myriapoda (e.g., Koch, 2003) and might serve the indirect opening of the mandibular gnathal lobes, which lack abductor muscles, as hypothesized by Fechter (1961) and Manton (1964) for the Juliformia.

4.5 | Head modifications and potential feeding mode of the Platydesmida

The Platydesmida show several unique head characters compared to other millipedes (Diplopoda), which can be observed in all studied species of the group. The Platydesmida studied so far are fungivorous (Gardner, 1975; Macias et al., 2019; Wong et al., 2020) and are thought to exhibit a scraping–sucking mode of feeding (Koch, 2015; Verhoeff, 1911–1914; Wong et al., 2020), although Verhoeff (1911–1914) called it slurping ("Schlürfen" *sensu* Verhoeff) rather than a real sucking. This special mode of feeding is most likely possible because of modifications of the head, including the mandibles, the muscle attachment sites, and the shape of the head.

4.5.1 | Modifications to mandibles

The internalized mandible of the studied Platydesmida consists of a gnathal lobe and a base (Backen *sensu* Attems, 1926; Kopfpleurite



		Platydesmida		Spirostreptida			Julida	
Musculature		*Dolistenus* sp.	*Brachycybe lecontii*	*Phyllogonostreptus nigrolabiatus*	*Poratophilus punctatus*	*Plusioporus salvadorii*	*Ommatoiulus avatar*	*Cylindroiulus caeruleocinctus*
Antennal m.	Median septum antennal m.^{W} (a1)	+	+	+	+	+	+	+
	Medial antennal m.^{W} (a2)	+	+	+	?	+	+	+
	Lateral antennal m.^{W} (a3)	+	+	+	?	+	+	+
	Anterior antennal m.^{W} (a4)	+	+	+	?	+	+	+
Epipharyngeal m.	Anterior epipharyngeal m.^{W} (e1)	(+)	(+)	+	D	+	+	+
	Posterior epipharyngeal m.^{W} (e1)			+	D	+	+	+
	Posterolateral epipharyngeal m.^{W} (e2)	(+)	(+)	+	D	+	+	+
	Anterolateral epipharyngeal m.^{W} (e2)			+	?	-	+	-
	Intermediate epipharyngeal m.^{N}	-	-	-	?	-	+	-
Gnathochilarial m.	Lamella lingualis m.^{W} (g1)	+	+	+	+	+	+	+
	Anterior gnathochilarial stipes m.^{W} (g2)	+	+	+	-	+	+	+
	Lateral mentum m.^{W} (g3)	+	+	+	+	+	+	-
	Anteromedial mentum m.^{W} (g4)	(+)	(+)	+	-	+	+	+
	Posteromedial mentum m.^{W} (g4)			+	-	-	+	-
	Posterolateral gnathochilarial stipes m.^{W} (g6)	+	+	+	+	+	+	+
	Posteromedial gnathochilarial stipes m.^{W} (g5)	+	+	+	+	+	+	+
	Medial intermentum m.^{W}	-	-	+	+	+	+	-
	Lateral intermentum m.^{W}	-	-	+	+	+	-	-
	Premental m.^{N}	-	-	-	-	-	+	-
Mandibular m.	Gnathal lobe sclerite m.^{W} (m1)	+	+	+	+	+	+	+
	Dorsal mandibular stipes m.^{W} (m2)	+	+	+	+	+	+	+
	Proximal gnathal lobe m.^{W}	-	-	+	+	-	+	-
	Anterior mandibular cardo m.^{W} (m4)	+	+	+	+	+	+	+
	Posterior mandibular stipes m.^{W} (m3)	+	+	+	+	+	+	+
	Ventral mandibular stipes m.^{W}	-	-	+	+	+	+	+
	Anterior mandibular stipes m.^{W}	-	-	+	+	+	+	-
	Gnathal lobe m.^{W}	-	-	+	+	+	+	+
	Posterior mandibular crado m.^{W}	-	-	+	+	+	+	+
	Ventromedial mandibular stipes m.^{N}	-	-	-	-	-	+	-
	Transverse mandibular tendon gnathal lobe m.^{N}	-	-	-	-	-	+	-
Pharyngeal m.	Precerebral pharyngeal dilator m.^{W} (p1)	+	+	+	D	+	+	+
	Intercerebral pharyngeal dilator m.^{W} (p2)	+	+	+	D	+	+	+
	Median septum pharyngeal dilator m.^{W} (p3)	+	+	+	+	+	+	+
	Dorsal postoccipital flange pharyngeal dilator m.^{W} (p4)	+	+	+	+	+	+	+
	Tentorial pharyngeal dilator m.^{W} (p5)	+	+	+	D	+	+	+
	Transvserse mandibular tendon pharyngeal dilator m.^{W} (p6)	+	+	+	-	+	+	+
	Ventral postoccipital flange pharyngeal dilator m.^{W} (p7)	+	+	+	-	+	+	+
	Lateral postoccipittal flange pharyngeal dilator m.^{N}	-	-	-	-	-	+	-
Tentorial m.	Anterior tentorial m.^{W} (t1)	+	+	+	+	+	+	+
	Dorsal tentorial m.^{W} (t2)	+	+	+	+	+	+	-
	Posterior tentorial m.^{W} (t3)	+	+	+	+	+	+	+
	Ventral tentorial m. from tendon	-	-	+	+	+	+	-
	Ventral tentorial m. from gula (t4)	+	+	-	-	-	-	-
Transverse mandibular tendon m.	Posterior transverse mandibular tendon m.^{W}	-	-	+	+	+	-	-

FIGURE 10 Homology of the musculature of the Platydesmida and Juliformia. Data for the Juliformia from the literature. The names of muscles (m.) follow the terminology introduced by Wilson (2002)^{W} or Naumann et al. (2020)^{N}. Abbreviations in parentheses are those used in this study. *Phyllogonostreptus nigrolabiatus* from Wilson (2002), *Poratophilus punctatus* from Manton (1964), *Plusioporus salvadorii* from Silvestri (1903), *Ommatoiulus avatar* from Naumann et al. (2020), *Cylindroiulus caeruleocinctus* from Fechter (1961). +, present; (+), present but homology unclear; -, absent; ?, unknown; D, depicted but not described

sensu Verhoeff, 1926–1928). The base is divided into a distinct cardo and stipes, which are articulated via a syndesis. This division of the mandibular base has already been described by Verhoeff (1911–1914, 1926–1928) and Attems (1926), although Silvestri (1903) stated that such a division is only present in Polyzoniida among the Colobognatha. In this aspect, the platydesmidan mandibular base resembles the state observed in most Eugnatha (Koch, 2015), in contrast to the Polyxenida and Pentazonia, which possess an entire mandibular base (Blanke & Wesener, 2014, their character 13). Therefore, we suggest that the division of the mandibular base is an autapomorphy of the Helminthomorpha (Colobognatha + Eugnatha) contrary to Blanke and Wesener (2014), who assume it to be an autapomorphy of the Eugnatha. In the Platydesmida, the surface of the mandibular basal segments, cardo and stipes, is narrower and bears fewer muscles than in non-colobognathan millipedes, which show a biting–chewing mode of feeding and often exhibit large lateral cheeks (Koch, 2015; Manton, 1977; Naumann et al., 2020; Wilson, 2002). The armature of the mandibular gnathal lobe is partially reduced with modifications of the internal and external teeth, the pectinate lamellae, and the molar plate (Ishii & Tamura, 1995). In some species of the Platydesmida, the molar plate is absent (Semenyuk et al., 2011 for *Pseudodesmus*; Wong et al., 2020 for *B. lecontii*), and in *B. lecontii* the pectinate lamella is also absent (Wong et al., 2020). These modifications of the gnathal lobe suggest a shift toward softer and/or liquid food sources.

4.5.2 | Modifications to muscle attachment sites

In contrast to eugnathan millipedes (Fechter, 1961; Koch, 2015; Manton, 1964; Naumann et al., 2020; Silvestri, 1903; Verhoeff, 1926–1928; Wilson, 2002), a median septum, which serves as attachment site for mandibular muscles, is absent in the Platydesmida, and the mandibular adductor muscle (m1) is not as strongly developed as in other Diplopoda which exhibit a biting–chewing mode of feeding (Fechter, 1961; Manton, 1964; Naumann et al., 2020; Snodgrass, 1952; Wilson, 2002). The median projection arising from the head capsule in *Pseudodesmus camptotrichus* might be the remnant of this median septum because the median antennal m. (a1) and the posterior pharyngeal dilator m. (p3), which arise in eugnathan millipedes from the median septum (Naumann et al., 2020; Wilson, 2002), arise from this projection. Furthermore, the collagenous transverse mandibular tendon, which is the main attachment site for mandibular muscles in other millipede taxa (Fechter, 1961; Manton, 1964, 1977; Naumann et al., 2020; Wilson, 2002), is reduced in size and limited to a small bridge between the tentoria, lacking the branches and mandibular musculature typical for other Diplopoda (Fechter, 1961; Manton, 1964, 1977; Naumann et al., 2020; Wilson, 2002).

4.5.3 | Modifications to shape of the head

To accommodate the largest part of the stipes and gnathal lobe of the mandibles within the head (entognathy), the head capsule underwent significant changes when compared to other millipedes. The genae are largely bulged laterally, covering the mandibular stipes and mandibular gnathal lobe in all Platydesmida. The genae are appressed to the gnathochilarium and only diverge from it posteriorly, leaving the mandibular cardo visible externally. In eugnathan millipedes, the gena is located between the antennal groove and the mandibular base, without covering the latter.

4.5.4 | Potential feeding mode

The small head, which is tightly packed with muscles and other organs, the close proximity of the mandibular gnathal lobes, the partial internalization of the mandibles (entognathy), and the reduction of mandibular muscles and their sites of origin restrict the movement of the mandibular gnathal lobes. Our analysis of the opening angles of the mandibles only gives the minimal possible opening angle because the movement of the mandibular base and gnathochilarium is not taken into account. The mandibular bases might enable a ventral rotation of the mandibles, resulting in ventral displacement of the gnathochilarium and a wider opening angle of the gnathal lobe. Furthermore, Wong et al. (2020) report the fibrous structure of the labrum, which might indicate flexibility of the labrum to widen the opening of the preoral chamber.

In summary, the movability of the mandibles is likely limited compared to other millipede groups. We therefore suggest that the mandibles are used to scrape off particles or to penetrate surfaces by scraping (see also Verhoeff, 1911–1914) and not for a pronounced biting or chewing movement. Verhoeff (1911–1914) stated that the mandibles of the Platydesmida are biting organs but can only perform weakly; our study goes a step further toward a quantification of biting ability. The weak biting abilities are further supported by the reduction of the armature of the gnathal lobe (Ishii & Tamura, 1995; Koch, 2015; Wong et al., 2020).

Wong et al. (2020) hypothesized that negative pressure is created by the thickened foregut and that the invaginations of the star-shaped foregut might be related to suctorial feeding. Indeed, these invaginations of the foregut, the plicae *sensu* Fontanetti et al. (2015) are present in all studied millipede taxa (Fontanetti et al., 2015), including mainly biting–chewing representatives. We suggest that negative pressure is mainly generated by the pharyngeal dilator muscles of the head, although a real sucking pump as present in suctorial insects like Hemiptera (Forbes, 1977; Krenn, 2019) and Lepidoptera (Eberhard & Krenn, 2005; Krenn, 2019) is absent in the Platydesmida. Therefore, Verhoeff's (1911–1914) description as slurping ("Schlürfen" *sensu* Verhoeff) might be a more suitable term than sucking for the feeding mode of the Platydesmida.

4.6 | Eyespots in the Platydesmida

Eyespots, also termed supra-antennal convexities, were reported for the Platydesmida by Koch (2015) for *Platydesmus* sp., and by Hoffman (1982) and Hoffman et al. (1996: figure 6). Hoffman (1982) stated that eyes are absent but their typical position is in many species occupied by a convexity of the cranium, which shows reduced pigmentation. Humbert and de Saussure (1869) noted the resemblance of this structure to visual organs, and Pocock (1887) even stated that eyes are present in *Platydesmus* but lacking in *Pseudodesmus*. Sinclair (1905) reported those supra-antennal convexities, and related them to the presence of a "peculiar sense organ" in *Platydesmus mexicanus* HUMBERT & DE SAUSSURE 1869. According to Sinclair (1905), a thick nerve runs from the frontal lobes of the brain to a thickened and transparent area of the head, and he concludes that these represent an organ of Tömösváry. In the studied taxa, no such depressions (except in *G. claremontus*), no nerve, and no indication of an organ of Tömösváry could be found. Depressions on the head capsule of *G. claremontus* (Figure 6C) correspond to the origin of pharyngeal dilator muscles.

4.7 | Characters of the Platydesmida

The Platydesmida show several characters separating them from the remaining Diplopoda. Some of these characters might be intermediate states toward the strong modification seen in the Polyzoniida, Siphonocryptida, and Siphonophorida, as suggested by Verhoeff (1912–1914, 1926–1928). To clarify whether these characters are unique for the Platydesmida or are general colobognathan characters, investigations of the remaining Colobognatha taxa and a phylogenetic analysis of the Diplopoda with a resolved relationship of the Colobognatha are needed. The phylogenetic position of the Platydesmida within the Colobognatha remains controversial and every possible relationship was proposed. In recent phylogenetic analyses, the Colobognatha are unresolved (Blanke & Wesener, 2014; Enghoff et al., 1993), or the Platydesmida are retrieved as sister group to either Siphonocryptida (Sierwald & Bond, 2007), Siphonophorida (Regier et al., 2005), or Polyzoniida (Rodriguez et al., 2018).

4.7.1 | Tergites with dorsal suture

A mid-dorsal suture running along the tergites is present in all Platydesmida and can otherwise be found in the Chordeumatida and Stemmiulida (Enghoff et al., 2015) as well as in the Siphonocryptida (Hoffman, 1982). In the phylogenetic analyses performed by Sierwald and Bond (2007), this character unites the Platydesmida and Siphonocryptida (as opposed to a sister-group relationship of Siphonocryptida and Polyzoniida). As argued by Enghoff and Golovatch (1995), a mid-dorsal suture is embryologically primitive (Enghoff, 1990) and a similar groove is known for some Polyzoniida (Hoffman, 1977). Therefore, we suggest that the presence of a mid-dorsal suture is not a synapomorphy of the Platydesmida and Siphonocryptida but either a plesiomorphic character or evolved independently in both taxa.

4.7.2 | Pleurites fused to tergites

The fusion of pleurites and tergites to pleurotergites is otherwise known from the Siphonocryptida, the Nematophora and the ring-forming Polydesmida, and the Juliformia (Blanke & Wesener, 2014; Koch, 2015), whereas the remaining Colobognatha, Pentazonia, and Polyxenida show separate tergites and pleurites. Sierwald and Bond (2007) retrieve this character as an autapomorphy of a monophyletic taxon comprising Platydesmida and Siphonocryptida. As fused pleurotergites probably evolved convergently in some Colobognatha and Eugnatha (Koch, 2015; Wilson & Anderson, 2004), we assume that the fusion in the Platydesmida is secondary and evolved independently from the Siphonocryptida.

4.7.3 | Epicranium bulged, overlapping collum

The unique condition of a bulge formed by the head capsule (Scheitelwulst *sensu* Verhoeff, 1911–1914), which overlaps the collum, has already been pointed out by Verhoeff (1911–1914). Such a condition is not known from any other millipede group. Therefore, we suggest that this character is an autapomorphy of the Platydesmida.

4.7.4 | Mandible partially internalized

The Colobognatha show a reduction and internalization of their mandible (Enghoff et al., 2015; Koch, 2015; Verhoeff, 1911–1914), and only the cardo is externally visible. The mandibles of the Platydesmida differ from those of the Polyzoniida, Siphonocryptida, and Siphonophorida by their broader bases and the fact that the cardo is externally visible in the Platydesmida, whereas it is hidden in the remaining Colobognatha. Therefore, we suggest that the Platydesmida might show an intermediate state toward completely internalized and more elongated mandibles.

4.7.5 | Transverse mandibular tendon

The transverse mandibular tendon acts as a small bridge spanning between the tentorial posterior processes. A large transverse mandibular tendon with several arms can be found in all non-colobognathan millipede taxa including Polyxenida (Latzel, 1884: plate III figure 23), Glomerida (Verhoeff, 1926–1928: figure 400), Sphaerotheriida (µCT data from Moritz et al., 2018), Polydesmida (Snodgrass, 1952), and Juliformia (Fechter, 1961; Manton, 1964, 1977; Wilson, 2002). A mandibular tendon has not yet been reported in Colobognatha. The small transverse mandibular tendon of the Platydesmida without mandibular muscles therefore could be an intermediate form compared to a complete loss of the tendon in the derived Polyzoniida, Siphonocryptida, and Siphonophorida.

4.7.6 | Absence of incisura lateralis

The studied Platydesmida lack an incisura lateralis, an observation also made by Verhoeff (1911–1914), despite the presence of a tentorial transverse bar, which is often connected to the incisura lateralis in other millipedes. An incisura lateralis is present in the Eugnatha (Koch, 2015; Moritz & Koch, 2020 for Polydesmida) as well as in the Pentazonia (e.g., Koch, 2015; Moritz et al., 2018). Therefore, its absence in Colobognatha is most likely secondary.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Appendix Chapter 3

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EVOLUTIONARY BIOLOGY

A previously unknown feeding mode in millipedes and the convergence of fluid feeding across arthropods

Leif Moritz[1,2]*, Elena Borisova[3], Jörg U. Hammel[4], Alexander Blanke[2]†, Thomas Wesener[1]†

We report fluid feeding with a sucking pump in the arthropod class Diplopoda, using a combination of synchrotron tomography, histology, electron microscopy, and three-dimensional reconstructions. Within the head of nine species of the enigmatic Colobognatha, we found a pumping chamber, which acts as positive displacement pump and is notably similar to that of insects, showing even fine structural convergences. The sucking pump of these millipedes works together with protractible mouthparts and externally secreted saliva for the acquisition of liquid food. Fluid feeding is one of the great evolutionary innovations of terrestrial arthropods, and our study suggests that it evolved with similar biomechanical solutions convergent across all major arthropod taxa. While fluid-feeding insects are megadiverse today, it remains unclear why other lineages, such as Colobognatha, are comparably species poor.

INTRODUCTION

Arthropods are the most diverse group of animals, and they evolved an immense variety of feeding mechanisms. Fluid feeding is widespread among several arthropod lineages such as tardigrades, onychophorans, arachnids, crustaceans, and insects, and it has been suspected for a small group of millipedes, the Colobognatha (*1*–*3*). Hitherto, the internal morphology of the feeding apparatus and the feeding mechanism of these millipedes have remained largely unknown. To better understand how colobognaths take up food and how fluid feeding evolved in arthropods, a comparison with other suctorial arthropods is essential. Within several arthropod lineages, different pumping mechanisms evolved for the transport of fluids from the exterior into the alimentary canal. All these pumping systems rely on creating negative pressure to draw in liquids. This can be achieved in various ways, like the triradiate sucking pharynx of tardigrades and velvet worms (*4*), by peristaltic contraction of the gut as in Pauropoda (*5*), or by one or several more complex pumping chambers as in arachnids (*6*), parasitic crustaceans (*7*), and many insects (*8*). Complex pumping organs for fluid feeding are most diverse and best studied in fluid-feeding insects, in which they evolved independently in several major lineages contributing to half the insect diversity (*9*, *10*). In most fluid-feeding insects, a proboscis, formed by the mouthparts, is combined with a pumping chamber, which has a similar architecture in several orders (*11*), and might have played a role in the diversification of insects (*12*). Liquids are drawn into the food canal and transported into the foregut by a combination of capillary forces and a pressure gradient created by a volumetric change of the pumping chamber. These pumping chambers are usually formed by modifications of the cibarium, a preoral chamber anterior of the actual mouth, and show a common morphological pattern: The chamber consists of a rigid sclerotized floor, a flexible roof, which is raised by strong dilator muscles to expand the lumen, and anterior and posterior valves or muscles, which direct the flow of fluids. So far, similar structures are unknown from millipedes, which mainly feed on dead plant material with biting-chewing mouthparts (*13*).

For the colobognathan millipedes (colobo, reduced; gnathos, jaw), a species-poor remnant group of the Diplopoda, fluid feeding was suspected because of their acuminate heads and the largely reduced or modified mandibles, compared to biting-chewing millipedes (*1*–*3*, *14*, *15*). Nevertheless, the exact mode of fluid uptake is unclear, and a structure similar to the pumping organs of other suctorial arthropods is unknown. Not only their feeding mechanism but also their food source remains enigmatic. Colobognatha often inhabit moist habitats and can be found near fungi (Fig. 1A), and some have been observed to probe rotting plant material (*3*). Therefore, Colobognatha probably feed on algal films, bacterially degraded substances, or fungal hyphae (*16*–*18*). On the basis of their gut content, the latter is the main food source of the Platydesmida (*16*), which might even show external digestion (*18*). All these food sources share a more or less liquid consistency, which requires special adaptations of the feeding apparatus. Compared to fluid-feeding insects and arachnids, the Colobognatha are rather species poor and appear to be a remnant group (*19*) with ~250 species, divided into four groups, Platydesmida, Polyzoniida (Fig. 1B), Siphonocryptida (Fig. 1C), and Siphonophorida (Fig. 1D), which constitute 2% of the extant millipede diversity (*20*). Except for Platydesmida, which show the classic transverse moving mouthpart configuration without a sucking pump and rather feed by "slurping" (*21*, *22*), the head morphology of the other lineages has not been studied. Here, we study the head morphology of representatives of all families within the remaining three colobognathan lineages (table S1). Our results not only show that liquid feeding is also present in millipedes but also compare it to other suctorial arthropods to present a hypothesis on the general feeding mechanism in this group.

RESULTS

Our histology, scanning electron microscopy (SEM), and micro–computed tomography (μCT) observations showed that the three lineages Polyzoniida, Siphonocryptida, and Siphonophorida share several features regarding their head morphology and their feeding apparatus, which cannot be found in any other non–colobognathan millipedes (*13*, *15*). The heads of the studied specimens are minute and range between 128 and 504 μm in diameter and 162 and 815 μm

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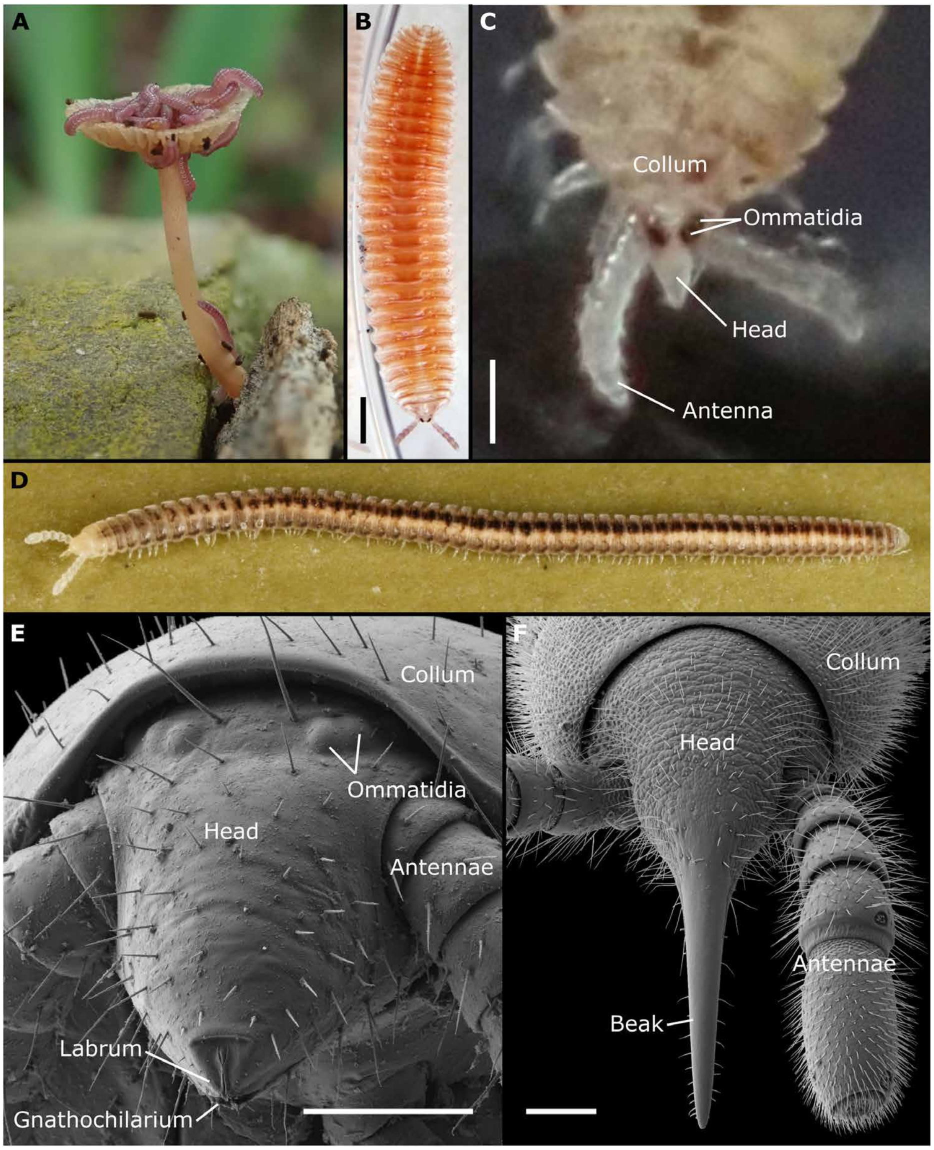

Fig. 1. The habitus and external head morphology of suctorial millipedes (Colobognatha). (**A**) *Rhinotus purpureus* (Polyzoniida, Siphonotidae) on a lamellate Basidiomycota in the greenhouse of the Botanical Garden Bonn. (**B**) *Hirudisoma roseum* (Polyzoniida, Hirudisomatidae) from Georgia, habitus. (**C**) *Hirudicryptus canariensis* (Siphonocryptida: Siphonocryptidae) from Tenerife, head. (**D**) *Siphonophora* cf. *zelandica* (Siphonophorida, Siphonophoridae) from New Zealand, habitus; photograph by H. Reip. (**E**) *Siphonetus* sp. (Polyzoniida, Siphonotidae) from New Zealand, fronto-lateral view, scanning electron microscopy (SEM). (**F**) *Siphonethus* sp. (Siphonophorida, Siphonophoridae) from New Zealand, head dorsal view, SEM. Scale bars, 100 μm (B, C, and F) and 20 μm (E).

in length (table S2). They are highly conical, taper anteriorly (Figs. 1, C and E, and 2A), and are even drawn out into a long "beak" in some species (Figs. 1F and 2C and fig. S1). Such a beak is formed by the head capsule and the plate-like lower lip called the gnathochilarium, a defining feature of all millipedes. The gnathochilarium is tightly appressed to the lower margin of the head capsule and the upper lip (labrum), leaving apically only a thin slit (Fig. 1E and fig. S2, A to J) or a circular pore (Fig. 2F), as the functional mouth opening to the preoral chamber. SEM data showed that the labrum and the gnathochilarium carry small pores (fig. S2), which are the external openings of the salivary glands, as evident from histological sections (Fig. 3, C to G). Such pores were absent in *Siphonophora*. The SEM data furthermore showed that the labrum (and the gnathochilarium of the Siphonophorida) carries a median incision, which is lined by teeth (Figs. 1E and 2F and fig. S2).

The paired, largely internalized mandibles consist of three parts: the cardo, the stipes, and the gnathal lobe (Fig. 2, A to C). High-resolution synchrotron (SR)–based μCT data showed that the short cardo, which lacks muscles, articulates with the head capsule and is inclined against an inward projecting protuberance. The long and slender stipes of the studied taxa articulates with the cardo and is equipped with a set of mandibular muscles, which arise mesally from a branched sclerotized endoskeleton, the so-called tentorial complex, and a single muscle arising posteriorly from the head capsule

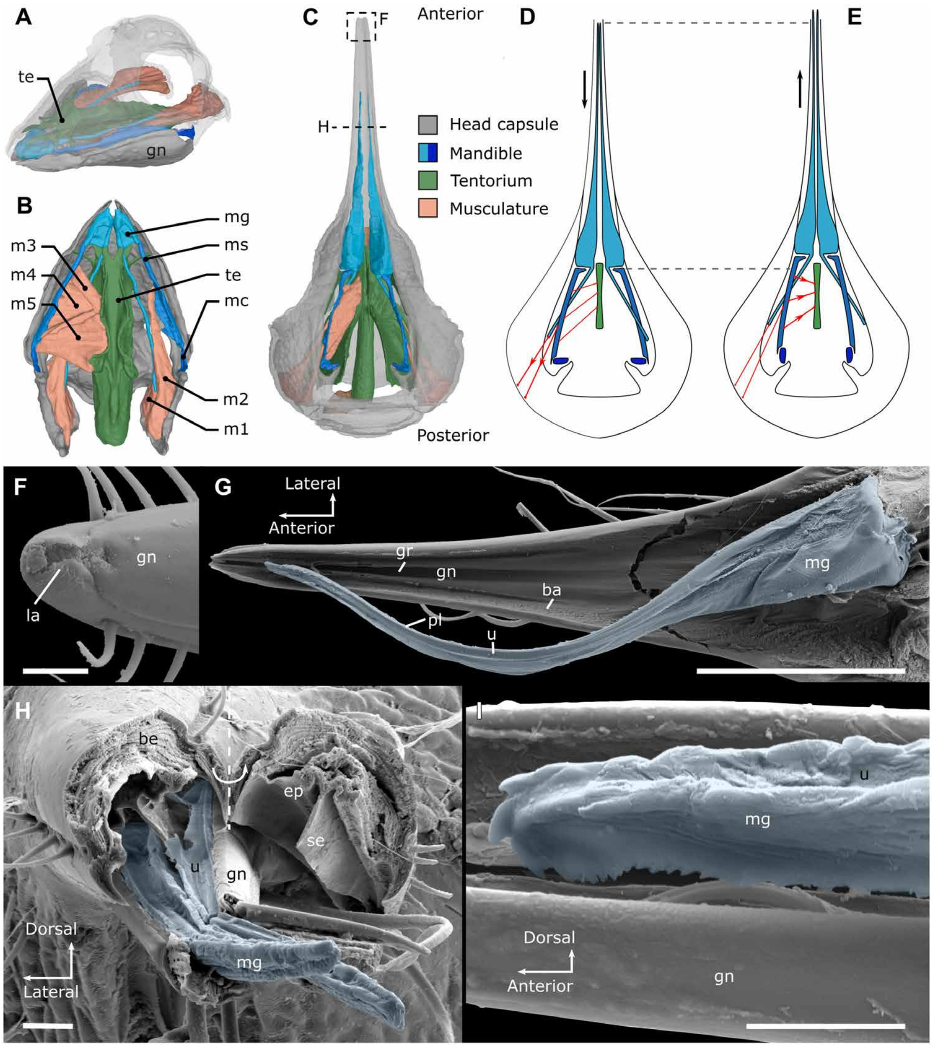

Fig. 2. The mandibles of fluid-feeding millipedes. (**A** to **C**) Musculoskeletal system of the mandibles, segmentation based on µCT data, not to scale. (A) *H. roseum* (Polyzoniida), fronto-lateral view. (B) *H. roseum* (Polyzoniida), ventral view with gnathochilarium removed. (C) *Siphonophora* sp. (Siphonophorida), ventral view with gnathochilarium removed, head capsule transparent; the apical tip of the gnathal lobe was not visible in the SR-µCT data. (**D** and **E**) *Siphonophora* sp. schematic representation of mandibular musculature when the mandibles are retracted (D) and protruded (E), arrows indicate movement of gnathal lobe. (**F** to **I**) *Siphonophora* sp., SEM, mandibular gnathal lobe in blue. (F) Tip of beak, ventral view, as indicated in (C). (G) Right mandibular gnathal lobe on top of gnathochilarium, dorsal view, second mandible removed. (H) Cross section through the rostrum, as indicated in (C) showing both mandibular gnathal lobes resting within the gnathochilarium, distal part of rostrum folded to the right. (I) Mandibular gnathal lobe, apical tip, lateral view. Scale bars, 10 µm (F, H, and I) and 100 µm (G). ba, band of cuticular fibers on the gnathochilarium; be, beak; ep, epipharynx; gn, gnathochilarium; gr, groove on inner surface of gnathochilarium; la, labrum; m1 to m5, mandibular muscles; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; pl, lamellae on the lateral surface of the gnathal lobe; se, median septum from epipharynx; u, dorsal u-shaped excavation of gnathal lobe.

(Fig. 2, B and C, figs. S3 to S11, and table S3). Anteriorly, the stipes articulates with the mandibular gnathal lobe, which is small and triangular in Polyzoniida and Siphonocryptida (figs. S3D and S4D) and extremely elongated and stylet-like in some Siphonophorida (Fig. 2, C and G, and fig. S10). Apically, the gnathal lobes carry larger and smaller teeth, which form a ventral saw-like structure in Siphonophoridae (Fig. 2, H and I, and fig. S12). At its base, the gnathal lobe of all studied taxa gives rise to an apodeme, the gnathal lobe sclerite, to which a single muscle (m1), arising posteriorly from the head capsule, inserts (Fig. 2, B and C, and figs. S3C, S4C, S8B, and S10B).

Behind the mandibular gnathal lobes, the preoral chamber opens into a pumping chamber in all studied taxa (Fig. 3, A and B, and fig. S14). The u-shaped floor of this chamber is thick and high in contrast, which might be due to sclerotization, and it is supported by the tentorial complex, a part of the head endoskeleton in arthropods (Fig. 3). The channel formed by the floor is closed dorsally by a thin roof to which large dilator muscles (p1, p2, and p3), arising from the head capsule, insert (Fig. 3 and fig. S14). In Polyzoniida and Siphonocryptida, additionally, a pair of large compressor muscles (c1) spans above the roof (Fig. 3, A and F, and figs. S3C and S4C). Posteriorly, the pumping chamber opens into the foregut and is surrounded by a well-developed circular muscle (s1), which appears distinct from the general muscular wall of the pharynx in most species (Fig. 3, A and C, and figs. S3C, S4C, and S8B).

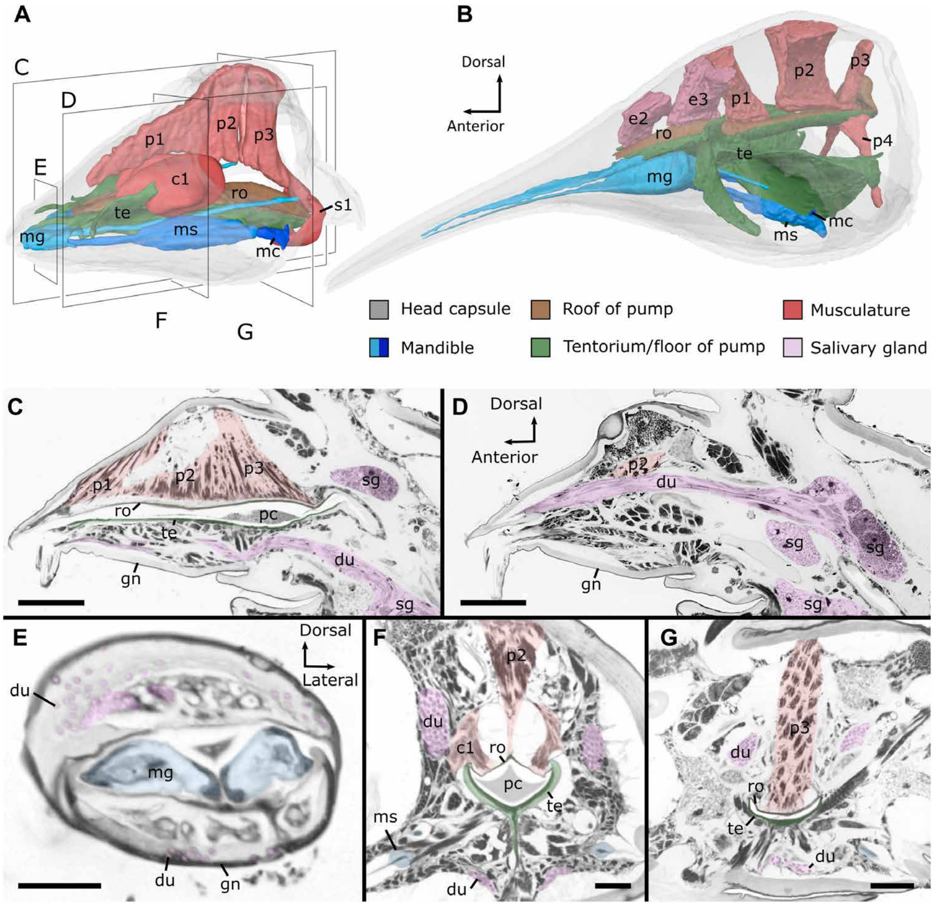

Fig. 3. The sucking pump and salivary glands of fluid-feeding millipedes. (**A** and **B**) Sucking pump and its associated musculature, three-dimensional segmentation based on SR-μCT data, fronto-lateral view. (A) *H. roseum* (Polyzoniida), cutting planes of (C) to (G) are indicated. (B) *Siphonophora* sp. (Siphonophorida). (**C** to **G**) *H. canariensis* (Siphonocryptida), histological sections of the head. (C and D) Adult male, sagittal sections: trough pumping chamber (C) and salivary gland (D). (E to G) Adult female, cross sections: trough mandibular gnathal lobe and pores of salivary glands (E), middle portion of pumping chamber (F), and posterior portion of pumping chamber (G). Scale bars, 50 μm (C and D), 20 μm (E), and 10 μm (F and G). c1, compressor muscles; du, ducts of salivary glands; e, epipharyngeal muscles; gn, gnathochilarium; mc, mandibular cardo; mg, mandibular gnathal lobe; ms, mandibular stipes; p, pharyngeal dilator muscles; pc, pumping chamber; ro, roof of pumping chamber; s1, sphincter muscle; sg, salivary glands; te, tentorial complex.

However, such a sphincter muscle is absent in Siphonophoridae (Fig. 3B and fig. S10).

Fluid feeding in other arthropod groups often relies on passive capillary forces in addition to active suctorial feeding. We assessed the potential for a capillary effect by calculating the equilibrium height for water for Siphonophoridae, which have the longest beak reported here. The lumen of their beak is mesally divided by a septum, resulting in two separated cylindrical food canals (Fig. 2H and fig. S15, A and B). The equilibrium height for water was calculated for two scenarios following Jurin's law (*23*) to account for morphologies with and without a septum. In the first scenario, the food canal is a single cylinder with a diameter of 15 μm, and in the second scenario, the food canal is subdivided into two cylinders, each with a diameter of 7 μm, reflecting the septum reported above. If the inner surface of the food canal is hydrophilic and the contact angle (θ) is 0° (*24*, *25*) (*10*), the equilibrium height is 1.982 m for the first scenario and 4.248 m for the second scenario. Assuming a less hydrophilic surface and therefore a contact angle of 45°, the equilibrium height is 1.402 and 3.004 m, respectively. Assuming a very weakly hydrophilic surface (θ = 89°), the equilibrium height is 0.035 and 0.074 m, respectively (see Supplementary Text). Given that even theoretical values at the upper and lower extremes of the theoretically possible fluid and surface characteristics result in capillary effects several times higher than the longest beak lengths, it can be assumed that the studied taxa can also access fluids with a higher viscosity such as particles (e.g., from fungi or algae) suspended in saliva or bacterially degraded substances.

DISCUSSION

The pumping chamber and feeding mechanism of colobognathan millipedes

An active pumping mechanism for the intake of liquid food evolved independently in several arthropod lineages (Fig. 4 and table S4), but all share a uniting functional principle: A positive displacement pump forces fluid in or out of a chamber by a change of its volume (*26*, *27*). Tardigrades, onychophorans (*4*), and sea spiders (*28*) have a triradiate sucking pharynx without constrictor muscles, while

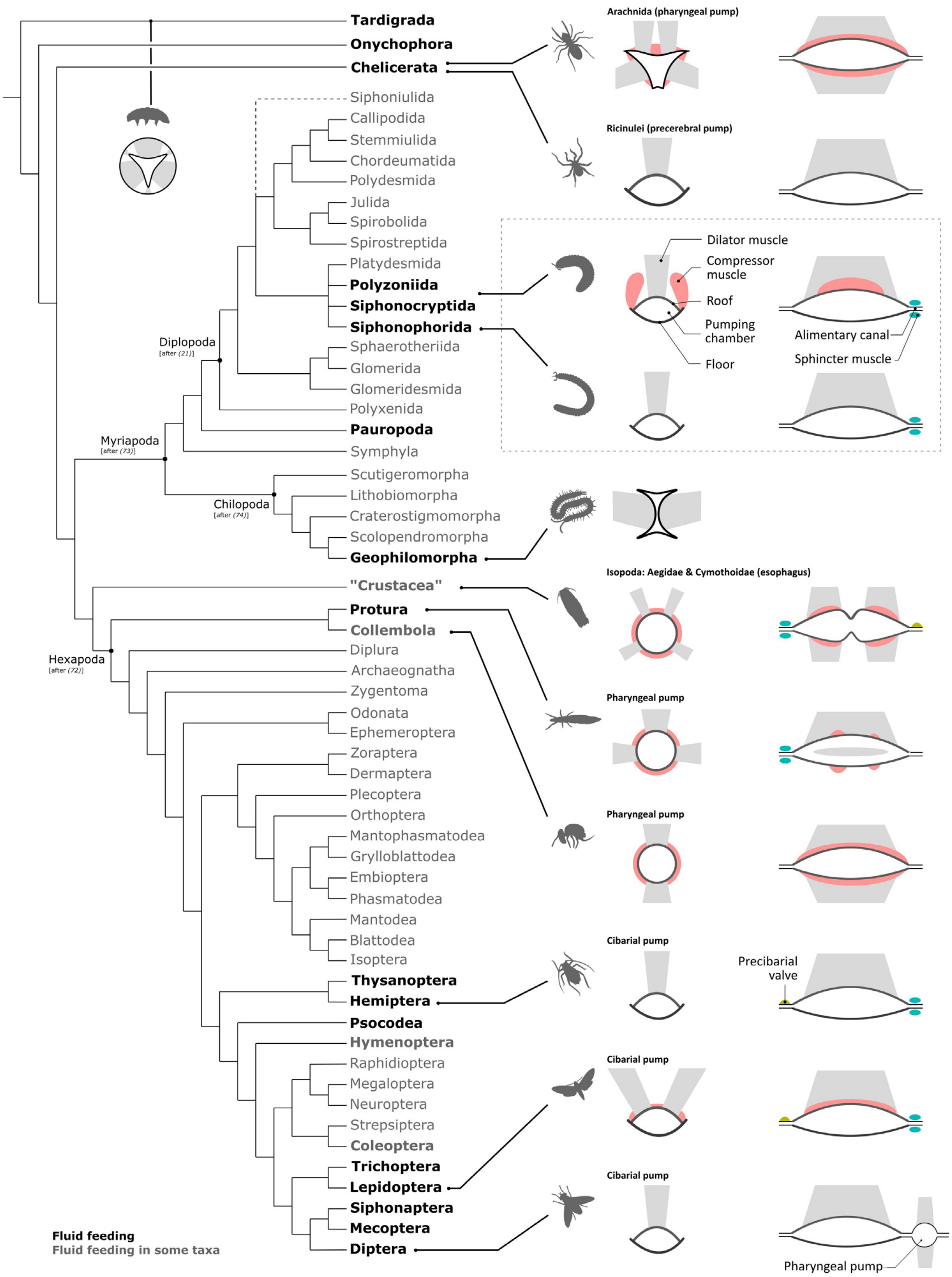

Fig. 4. Sucking pumps and their functional components in arthropods. The backbone phylogeny is based on (*71*), internal relationships within Hexapoda according to (*72*), within Myriapoda according to (*73*), within Chilopoda according to (*74*), and within Diplopoda according to (*21*). For references on the sucking pumps in the taxa, see table S4. Schematic and simplified representations of the pumping organs of various arthropods are shown on the right. Cross sections of the pump are shown on the left, and longitudinal sections are shown on the right; for Tardigrada and Geophilomorpha only the cross section is shown. Colors do not indicate homology but functional analogy.

many arachnids (*6*), springtails (*29*), and proturans (*30*) share a pumping chamber with dorsal and ventral or lateral dilator muscles and surrounding compressor muscles.

Within myriapods, the predatory geophilomorph centipedes might have a pharyngeal pump with a double-anchor cross section and lateral dilator muscles (*31*, *32*), while the minute Pauropoda suck out the contents of fungal hyphae by peristaltic movements of the midgut (*5*). For millipedes (Diplopoda), we demonstrate the presence of a sucking pump and active suction feeding, which resembles that of most fluid-feeding insect lineages even in fine structural details. All studied Colobognatha share a pumping chamber with a thick floor, which is formed by the tentorial complex, and a thin, supposedly flexible roof, to which large dilator muscles adhere. The pumping chamber of millipedes might have been formed by parts of the preoral chamber and the pharynx because epipharyngeal muscles and pharyngeal muscles insert on its roof (see Supplementary Text and table S3).

The basic pattern of the colobognathan pumping chamber resembles that of various suctorial insects like the Hemiptera (*33*, *34*), Lepidoptera (*8*, *35*), some Hymenoptera (*36*, *37*), Diptera (*38*), Siphonaptera (*8*), and Thysanoptera (*11*, *38*, *39*) (Fig. 4 and table S4). These insects all show a cibarium, modified into a pumping chamber with a sclerotized rigid floor and a thin flexible roof, to which large dilator muscles adhere. A similar pumping system with a sclerotized floor and a flexible roof with dilator muscles can also be observed in the precerebral sucking pump of Ricinulei within Chelicerata (*40*). Lepidoptera (*35*, *41*), some Hymenoptera (*36*), and some Coleoptera (*42*) have dorsal compressor muscles spanning above the pumping chamber's roof, as present in Polyzoniida and Siphonocryptida, while these are absent in other insect lineages and Siphonophorida. This common detailed structure of the pumping chamber in the suctorial millipedes, various suctorial insect lineages, and some Arachnida apparently evolved convergent.

In the studied millipedes, the volume of the sucking pump can increase by the contraction of the large dilator muscles, while the thick ventral wall prevents deformation due to negative pressure buildup. This is also the case in most suctorial insects with a similarly structured pumping chamber (Fig. 4) (*8*, *33*–*38*). During fluid intake, the posterior sphincter muscle closes the sucking pump posteriorly in Polyzoniida, Siphonocryptida, and Siphonorhinidae, similar to Lepidoptera (*35*, *43*). When the sphincter muscle relaxes, the content of the sucking pump is emptied into the foregut passively by the elastic retraction of the dorsal wall in Siphonophorida, as is the case in Hemiptera and Diptera (*34*, *44*, *45*), or actively by the action of muscles dorsally of the chamber, which are only present in Polyzoniida and Siphonocryptida and might function similarly to the compressor muscles spanning across the roof of the pumping chamber in Lepidoptera (*10*, *35*) and in some Hymenoptera (*37*, *46*, *47*) and Coleoptera (*42*, *48*, *49*). A mechanism closing the sucking pump anteriorly to prevent fluid flow out of the mouthparts was reported for butterflies, moths, and Hemiptera (*43*, *44*) but could not be identified in the studied millipedes. Available evidence suggests that, in suctorial millipedes, the filled pump is closed anteriorly by the labrum and gnathochilarium, which can be tightly appressed to each other [fig. S2; (*15*, *50*)]. Fluid intake might be further facilitated by capillary forces acting at the minute slit-like opening of the preoral chamber. The minute opening of the preoral chamber, with an incised labrum, results in capillary forces, which are sufficient to fill even the elongated beak of Siphonophoridae, as is the case in butterflies (*10*). The upper estimate of the height of water that rises within the proboscis of Siphonophorida is more than 4 m for a beak with a diameter of 7 μm, which surpasses the beak length by multiples and suggests that no suction pressure is needed to fill the proboscis. A similar phenomenon was observed in butterflies, where the height of water can range between 14.7 m for a 2-μm diameter and 14.7 cm for a 200-μm food canal (*10*). Considering that the hydrophilic properties of the beak are unknown and that the food might be more viscous, lower values can be expected for Siphonophorida. We suggest that a mixture of capillary forces and active pumping is used to transport liquids into the alimentary canal.

Protrusion-retraction mechanism of the mandibles

Although Colobognatha have been observed on fungi or probing rotting plant material (*3*), the actual food intake and their internal morphology have not been documented to date. On the basis of the arrangement of muscles and skeletal elements, compared to biting-chewing millipedes and Platydesmida, with mandibles that move in a transverse plane (*13*, *22*, *51*–*53*), the most likely movement of the mandibles in the studied millipedes is a protrusion-retraction through the minute functional mouth opening, similar to the protrusion movement of insect maxillae (*30*, *54*). Therefore, the mandibles can only be used to penetrate surfaces or to loosen particles by scraping or piercing instead of chewing. When the mandible is retracted, the cardo is inclined against a ventral protuberance of the head capsule (Fig. 2D). By contraction of the muscles spanning between mandibular stipes and tentorial complex (m3, m4, m5; Fig. 2), the mandibular base straightens and moves forward, resulting in the protrusion of the gnathal lobe (Fig. 2E). The mandible is retracted by contraction of the muscles spanning from the cranium to the mandibular stipes (m2) and to the gnathal lobe sclerite (m1; Fig. 2D). On the basis of the lengths of the gnathal lobe muscle, the mandibular cardo, and the mandibular stipes, the tips of the gnathal lobes can protrude through the opening of the preoral chamber (see Supplementary Text). In addition, the endoskeleton (tentorial complex) is mesally fused and supports the pharynx and is therefore probably immobile. This contrasts the swinging movement of the tentorium, which is essential for the mandibular abduction in biting-chewing millipedes (*13*, *15*, *51*).

Externally secreted saliva

In biting-chewing millipedes, the salivary glands open within the preoral chamber each via a single duct (*55*), and the released saliva is involved in the enzymatic digestion of polysaccharides, lipids, and proteins (*56*). In contrast, the salivary glands in fluid-feeding millipedes open via several cuticular tubes and small pores externally nearby the functional mouth opening. These pores and ducts (fig. S13) are a potentially apomorphic character for Colobognatha. For Siphonorhinidae, the release of secretion has been observed from these pores (*50*). Saliva released from the pores might aid in lubrication of the beak, in suspending detached particles for fluid intake, or in external digestion (*18*, *57*). The release of saliva via several small pores spread on the labrum and gnathochilarium, compared to larger amounts via a single opening, might also serve in creating a thin film of saliva instead of larger droplets, which would move away from the conical heads' tip toward the point of the lowest curvature (*58*, *59*).

Diversity and evolution of fluid-feeding millipedes

In insects, the evolution of a sucking pump paired with a proboscis might have played a role in their enormous diversification (*12*).

While fluid-feeding insects are extremely diverse and represent nearly half of all insect species (*9*, *10*), the fluid-feeding millipedes (Colobognatha) constitute only around 2% of the extant millipede diversity [ca. 250 of the 11,000 described species (*20*)]. In extant samples from tropical forests, less than 6% of millipede abundance is attributed to the Colobognatha [e.g., (*60*)], while they were the dominant millipede group ca. 100 million years ago based on the oldest known remains found in Burmese amber (*61*). The external morphology of these Cretaceous age Colobognatha is almost identical to that of extant representatives (*62*). The lower diversity of extant Colobognatha compared to the megadiverse suctorial insects might be related to the lower dispersal ability and dependence on moist habitats of colobognathan millipedes, which makes them more prone to extinction in changing environments.

Our discovery of a fluid-feeding mode in this group of millipedes shows that similar feeding strategies and biomechanical adaptations toward assessing liquid food evolved across all major arthropod taxa. In this context, the high degree of morphological analogy, even in fine structural details, is remarkable and underlines the strength of selection toward common functional solutions once a new type of food constituted an evolutionary advantage. However, our overview of fluid-feeding strategies across arthropods also suggests that, although suctorial feeding and specialization might have led to diversification in various insect lineages, this is not the case in other arthropod groups. Fluid feeding per se is not a universal driver of diversification.

MATERIALS AND METHODS

Taxon sampling and data deposition

The morphology of the head of nine species representing all six higher taxa (families) of the Polyzoniida, Siphonocryptida, and Siphonophorida was studied (table S1). For comparisons to the Platydesmida, already available µCT data (*21*, *22*) were used. All µCT data, segmentations, and digitalized histological data are deposited on Zenodo (https://doi.org/10.5281/zenodo.5215894). Voucher specimens were deposited in the collections of the Zoological Research Museum Alexander Koenig (ZFMK) (table S1). Specimens were examined and dissected with an Olympus Discovery.V12 stereo microscope.

SR-µCT and three-dimensional segmentation

For SR-µCT, specimens were fixed in Bouin solution (Morphisto, Art.Nr. 12588) or 95% ethanol (EtOH) (table S1) and critical point dried with a Leica EM CPD 300. SR-µCT data were obtained at the Imaging Beamline P05 (IBL) operated by Helmholtz-Zentrum Hereon (*63*–*65*) at PETRA III [Deutsches Elektronen-Synchrotron (DESY), Hamburg, Germany], at the PSI (Paul Scherrer Institut) SLS Beamline TOMCAT-X02DA (Villingen PSI, Switzerland) (*66*), and at the Super Photon ring-8 GeV (SPring-8, Hyogo, Japan) at Beamline BL47XU (table S1) (*67*). Cropping as well as brightness and contrast adjustments of image stacks were done in Fiji ImageJ version 1.50e (*68*). Segmentations were carried out in ITK-SNAP 3.8.0 (*69*) and further processed in MeshLab v2020.07 (*70*) and Blender 2.77 (www.blender.org) for final rendering. Measurements were taken in Blender 2.77 and Fiji ImageJ version 1.50e.

Histology

Histological sections were obtained for a male and a female *Hirudicryptus canariensis* (Siphonocryptida), which were fixed in Bouin solution and embedded in epoxy resin (Araldite CY212, Agar Scientific Ltd., R1030) following the manufacturer's protocol. Semithin cross and sagittal sections (1 µm) were obtained with a Leica HistoCore NANOCUT R microtome with a DiATOME histo Jumbo diamond blade and stained with 1% toluidine blue (PanReac AppliChem, A3842.0010) for 2 min. The obtained sections were photographed with an Olympus BX61VS light microscope equipped with a VS120-S6-W slide loader system and are deposited at the ZFMK [ZFMK-HIST000002 (*H. canariensis*, female, cross sections) and ZFMK-HIST000003 (*H. canariensis*, male, sagittal sections)].

Scanning electron microscopy

For SEM, specimens fixed in 70 or 95% EtOH were dehydrated and critical point dried using a Leica EM CPD 300. The specimens were mounted to SEM stubs using conductive tape and sputtered with gold using the Cressington Sputter Coater 108auto. SEM images were obtained with a Zeiss Sigma 300 VP scanning electron microscope at the ZFMK.

Capillary forces

To estimate the capillarity with Jurin's law (*23*), the equilibrium height was calculated without and with accounting for the contact angle of the fluid and the food canal (*10*, *24*, *25*), here calculated (see Supplementary Text) for a contact angle of 0° (high hydrophily), 45° (medium hydrophily), and 89° (low hydrophily).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abm0577

View/request a protocol for this paper from *Bio-protocol*.

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Appendix Chapter 4

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An apparently non-swinging tentorium in the Diplopoda (Myriapoda): comparative morphology of the tentorial complex in giant pill-millipedes (Sphaerotheriida)

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Abstract

The presence of a swinging tentorium is a key apomorphy of Myriapoda, but this character has been studied in detail in only few species. Here the tentorium, i.e., the peristomatic skeleton of the preoral chamber, is comparatively studied in three species of the millipede order Sphaerotheriida Brandt, 1833. Since dissections of the fragile tentorial components proved to be difficult, despite the large head size, they were analysed mainly in situ via micro-computed tomography. Our results confirm previous observations of large differences in the tentorial construction in the giant pill-millipedes compared to chilognathan diplopods. The tentorium of Sphaerotheriida consists of a curved, plate-like epipharyngeal bar with distal projections, an elongate and thin hypopharyngeal bar, and a plate-like triangular posterior process; a transverse bar is absent. Only seven muscles attach at the tentorium in giant pill-millipedes, including two antennal muscles and two muscles of the gnathochilarium. Within the order Sphaerotheriida, the composition of the tentorium and its muscular equipment seems to be conserved, except for some variability in the shape of the epipharyngeal bar. As the transverse bar has been considered essential for the mobility of the tentorium in myriapods, its absence in Sphaerotheriida may indicate that their tentorium is not capable of performing a swing. Loss of tentorial mobility may also pertain to the order Glomerida Brandt, 1833, inferred here from the absence of a posterior process. An apparently immobile tentorium in Glomerida and Sphaerotheriida can straightforwardly be correlated with transformations of the head related to their ability of volvation. The different transformations of the tentorium, here hypothesised to cause immobility, may support current assumptions that the ability of volvation evolved convergently in Glomerida and Sphaerotheriida. This conclusion, however, still requires more detailed studies of the head anatomy in Glomerida and Glomeridesmida Cook, 1895.

Keywords

Arthrosphaeridae, micro-CT, 3D-reconstruction, swinging tentorium, volvation

Introduction

Recent molecular studies (Gai et al. 2006, Regier et al. 2010, Miyazawa et al. 2014, Fernández et al. 2016) as well as studies combining molecular and morphological data (Lee et al. 2013, Giribet et al. 2001) consistently retrieve the Myriapoda as a monophylum. Unique synapomorphies shared by its taxa Chilopoda, Symphyla, Pauropoda, and Diplopoda, however, are sparse. The most striking character in favour of myriapod monophyly is the so-called swinging tentorium, i.e. an apparently mobile skeleton of the head supporting the preoral chamber and movements of the mandibles (Edgecombe and Giribet 2002, Koch 2003, Edgecombe 2004, Shear and Edgecombe 2010, Edgecombe 2011, Koch et al. 2015). The tentorial complex consists of sclerotised exoskeletal bars and endoskeletal processes (Koch 2003, Koch 2015). This complex provides stability to the largely membranous epi- and hypopharynx and serves as muscle attachment sites. The tentorium is considered essential for the movement of the mandibles and the gnathochilarium, although the mechanism is not yet understood (Manton 1964, Fechter 1961, Koch 2015). In general the tentorium of the Diplopoda is composed of four parts: the exoskeletal (1) hypopharyngeal bar, (2) the epipharyngeal bar, (3) the transverse bar, and (4) the endoskeletal posterior process (sensu Koch 2003). Associated with the tentorial complex is an additional sclerite, the nebententorium (sensu Attems 1926, Verhoeff 1928, Koch 2015) or hypopharyngeal lateral sclerite (sensu Wilson 2002), also serving as an attachment site for musculature (Verhoeff 1928). Details on the structure of the tentorium and its musculature have been described for only few representatives of the Diplopoda. Four descriptions for members of the order Sphaerotheriida date back more than 100 years, and three of them (vom Rath 1886, Silvestri 1903, Attems 1926) describe the state for *Sphaeropoeus* Brandt, 1833 (Zephroniidae Gray, 1843). There is only one description for the Arthrosphaeridae Jeekel 1974 by Verhoeff (1928) for *Arthrosphaera dentigera* Verhoeff, 1930. Recently, the tentorium of *Zoosphaerium bemanevika* Sagorny & Wesener, 2017 from Madagascar was visualised in 3D using volume renderings of a μCT scan (Sagorny and

Wesener 2017), but it was not described in detail. The Arthrosphaeridae are the second largest family with 119 species and 4 genera within the order Sphaerotheriida. The Arthrospaeridae have a very interesting biogeography with *Arthrosphaera* from the Indian sub-continent, and the three genera *Sphaeromimus*, *Zoosphaerium* and *Microsphaerotherium* Wesener & VandenSpiegel, 2007 only known from Madagascar (Wesener and VandenSpiegel 2009, Wesener et al. 2010). All descriptions indicate that the tentorium in the Sphaerotheriida deviates markedly from the pattern described for other myriapods. In order to reveal the deviating characteristics more comprehensively, the tentorium of three representatives of the Arthrosphaeridae genera *Arthrosphaera* Pocock, 1895, *Sphaeromimus* de Saussure & Zehntner, 1902 and *Zoosphaerium* Pocock, 1895 are described and compared.

Materials and methods

Vouchers are stored in natural history collections of the Zoological Research Museum A. Koenig (**ZFMK**) and the California Academy of Science (**CAS**). One head of *Zoosphaerium* sp. was used for light microscopy of the skeletal components with a Keyence VHX 700 digital stack imaging system. For this purpose the mandibles were removed with micro-scissors and the head bisectioned by slicing along the mouth with a razor blade. Micro-CT scans were taken from the heads of the three giant pill-millipede species *Arthrosphaera brandtii* (Humbert, 1865) (ZFMK MYR 06265), *Sphaeromimus kalambatritra* Moritz & Wesener, 2017 (CAS ENT 9058301) and *Zoosphaerium bemanevika* Sagorny & Wesener, 2017 (ZFMK MYR 6144), all belonging to the family Arthrosphaeridae Jeekel, 1974. The heads were dissected and critical point dried (CPD) after dehydration via an ascending ethanol series. X-ray micro-computed tomography (μCT) was performed with a SKYSCAN 1272 (Bruker microCT, Kontich, Belgium), using the following settings: source voltage = 60 kV, source current = 166 μA , exposure = 915 ms, rotation of 180° in rotational steps of 0.2°, frame averaging = 6, random movement = 15 px, filter = Al 0.25 mm. Isotropic voxel resolution varied in the following manner: *Arthrosphaera brandtii*: 5.99 μm; *Sphaeromimus kalambatritra*: 7.86 μm; *Zoosphaerium bemanevika*: 7.99 μm. Reconstruction and thermal drift correction was performed in NRecon 1.7.0.4 (Bruker microCT, Kontich, Belgium). Reduction of the data size by scaling to 50 % and conversion from 16- to 8-bit greyscale, and the adjustment of contrast and brightness was performed in IMAGE J 1.50e (Schneider et al. 2012). The resulting image stacks are deposited in MorphoBank as Project 2795 (http://morphobank.org/permalink/?P2795) Automated segmentation with subsequent manual corrections and 3D visualisation of the studied structures was performed in ITK-SNAP 3.6.0 (Yushkevich et al. 2006). Terminology follows Koch (2015) for components of the endoskeleton, and Wilson (2002) for the musculature. Illustrations and figure plates were prepared with Adobe Photoshop CS2 and Adobe Illustrator CS2.

Results

Skeletal elements of the tentorium in the Sphaerotheriida

A connection of the tentorium to the head capsule by a transverse bar (sensu Koch 2003) is missing in the three analysed species, despite the presence of an incisura lateralis (Fig. 1A). The paired tentorial complex consists of only four major parts: the epipharyngeal bar (Fig. 1C, eb), the hypopharyngeal bar (Fig. 1B, hb), the posterior process (Fig. 2, pp), which forms a single tripartite sclerite (the tentorium sensu stricto) along the mouth opening, and the separate nebententorium (nt; hypopharyngeal lateral sclerite sensu Wilson 2002) located on the hypopharynx (Fig. 1B, hy).

(1) The epipharyngeal bar:

The plate-like epipharyngeal bar (eb) is in connection with the wall of the epipharynx (Fig. 2A–J, ep). The distal part of the epipharyngeal bar is a triangular plate with one slightly curved lateral projection (e1) and a stout median projection (Fig. 1C, D, e2). The shape of the projections of the epipharyngeal bar is variable within the Arthrosphaeridae (Fig. 2D, F, H): the lateral projection (e1) is rather stout and short in *Sphaeromimus kalambatritra* (Fig. 2F), more elongate in *Arthrosphaera brandtii* (Fig. 2D) and long, slender and curved in *Zoosphaerium bemanevika* (Fig. 2H). The median projection (e2) is triangular in *A. brandtii* (Fig. 2D) and *S. kalambatritra* (Fig. 2F), and rectangular in *Z. bemanevika* (Fig. 2H). The distal part of the epipharyngeal bar (eb) is curved, following in shape the curvature of the mandible condyles (Fig. 2A, B, I, J, co) in all analysed specimens. The proximal part of the epipharyngeal bar (eb) is a rectangular plate, which is wider in *A. brandtii* (Fig. 2D) and *S. kalambatritra* (Fig. 2F), while it is more slender in *Z. bemanevika* (Fig. 2H).

(2) The hypopharyngeal bar:

In the three analysed species, the epipharyngeal bar (eb) of the tentorium (Fig. 2A–J) passes over into the hypopharyngeal bar (hb) posteriorly to the pharyngeal opening. The hypopharyngeal bar is elongate and rod-like (Fig. 2A–J, hb). The bar is strongly curved inward and extends ventrally on the hypopharynx towards the gnathochilarium where it is associated to the nebententorium (nt) via a membranous connection (Fig. 2C, E, G). A small cone-shaped medial projection (h1) close to the center of the hypopharyngeal bar is present (Fig. 2C–H), pointing to the hypopharyngeal suspensorial sclerites (Fig. 1B, ss) (Stützgerüst sensu Attems, 1926; Verhoeff, 1928). Although the hypopharyngeal bar of *A. brandtii* (Fig. 2C) is slightly shorter in relation to its width than in *S. kalambatritra* (Fig. 2E) and *Z. bemanevika* (Fig. 2G), its general rod-like appearance can be seen in all analysed species.

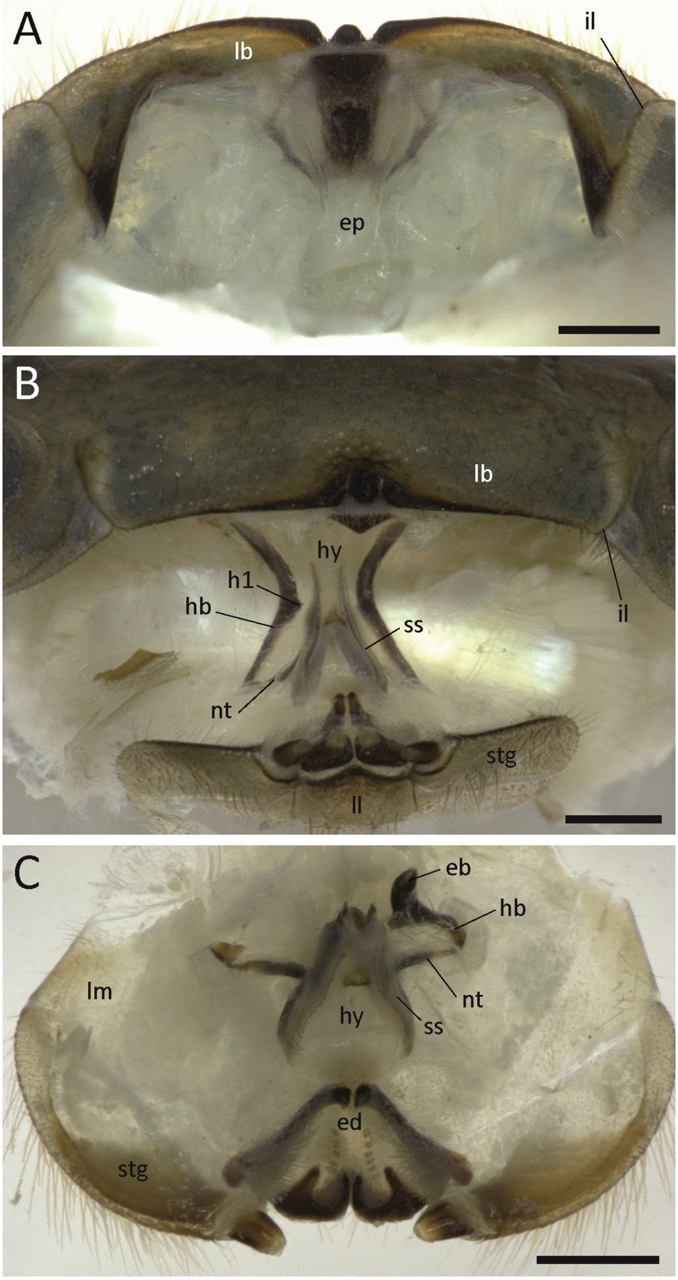

Figure 1. *Zoosphaerium* sp., light micrographs of peristomatic structures. **A** Epipharynx, showing absence of the tentorial transverse bar **B** Preoral chamber, frontal view on hypopharynx (mandibles removed) **C** Hypopharynx and endochilarium, dorsal view (hypo- and epipharyngeal bar of right tentorium broken off). Scale bars: 500 µm. **Abbreviations: eb** = epipharyngeal bar of left tentorium; **ed** = endochilarium; **ep** = epipharynx; **h1** = projection of hypopharyngeal bar; **hb** = hypopharyngeal bar of tentorium; **hy** = hypopharynx; **il** = incisura lateralis; **lb** = labrum; **ll** = lamella lingualis; **lm** = lamella-mentum; **nt** = nebententorium; **ss** = suspensorial sclerite; **stg** = stipes of gnathochilarium.

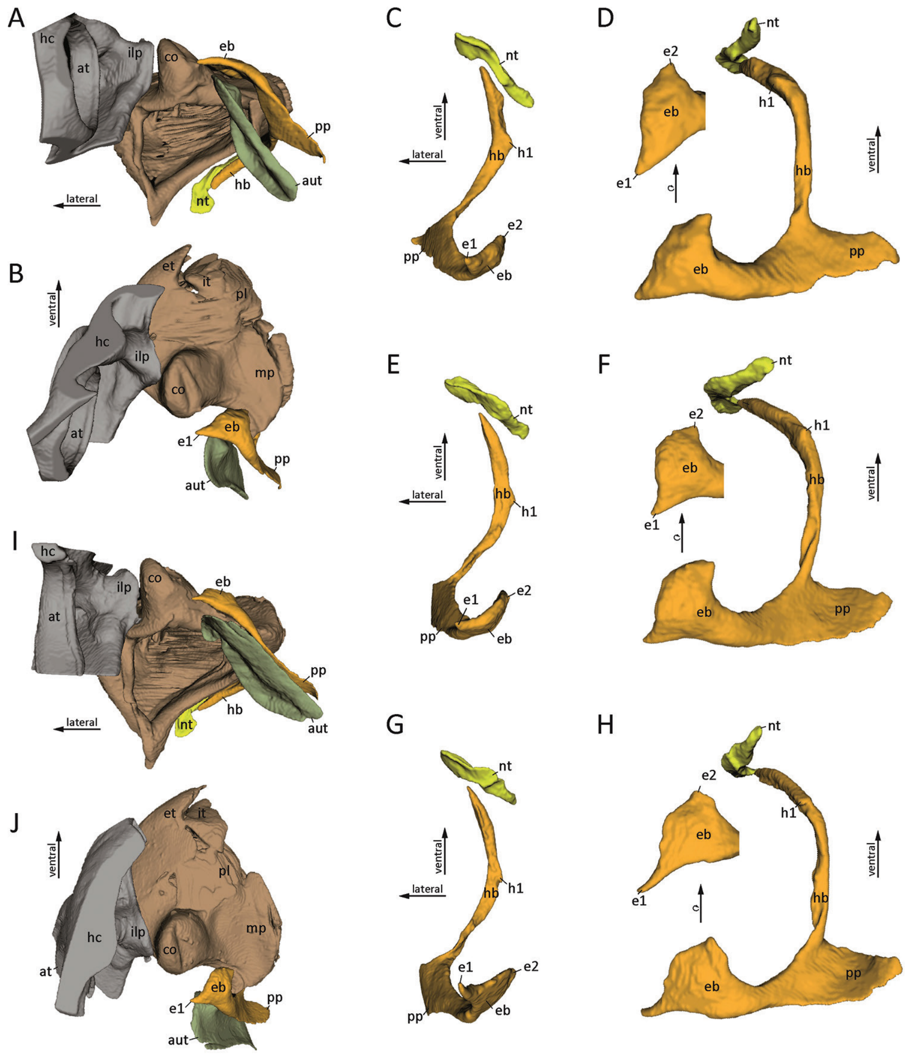

Figure 2. The tentorial complex of the Sphaerotheriida, 3D visualization. GREY = Head capsule; BROWN = mandible; ORANGE = tentorium; YELLOW = nebententorium; OLIVE = außententorium. **A–D** *Arthrosphaera brandtii* (Humbert, 1865), ZFMK MYR6265 **E, F, I, J** *Sphaeromimus kalambatritra*, CASENT 9058301 **G, H** *Zoosphaerium bemanevika* Sagorny & Wesener, 2017, ZFMK MYR6144. **A** tentorial complex and its association with the mandibular gnathal lobe and the head capsule of *A. brandtii*, dorsal view **B** same as A frontal view **C** tentorial complex of *A. brandtii,* frontal view **D** same as C medial view, with rotated epipharyngeal bar **E** tentorial complex of *S.* sp., frontal view **F** same as E medial view, with rotated epipharyngeal bar **G** tentorial complex of *Z. bemanevika*, frontal view **H** same as G medial view, with rotated epipharyngeal bar **I** tentorial complex and its association with the mandibular gnathal lobe and the headcapsule of *S.* sp., dorsal view **J** same as I, frontal view. **Abbreviations**: **at** = antennal socket; **aut** = mandibular gnathal lobe sclerite (außententorium); **co** = condylus of mandible; **e1** = lateral projection of epipharyngeal bar; **e2** = medial projection of epipharyngeal bar; **eb** = epipharyngeal bar; **et** = external tooth; **h1** = projection of hypopharyngeal bar; **hb** = hypophayrangeal bar; **hc** = head capsule; **ilp** = projection arising from incisura lateralis; **it** = internal tooth; **mp** = molar plate; **nt** = nebententorium; **pl** =pectinate lamellae; **pp** = posterior process.

(3) The posterior process:

The posterior process (pp) is a large triangular plate projecting posteriorly into the head capsule parallel to the mandibular gnathal lobe sclerite (sensu Wilson 2002; äußeres Tentorium sensu Voges 1916, Attems 1926; Außententorium sensu Seifert 1932; (German for "outer tentorium")) (Fig. 2A, B, I, J, aut). It arises from the transition point between the epi- (eb) and hypopharyngeal bars (hb). There is no variation in the shape of the posterior process (pp) within the studied Arthrosphaeridae (Fig. 2C–H).

(4) The nebententorium:

The nebententorium (nt) is a short, flat sclerite parallel to the distal portion of the hypopharyngeal bar (hb) of the tentorium (Fig. 2A, B, I, J, YELLOW). It bypasses the distal tip of the hypopharyngeal bar slightly and broadens, forming an articulation with the tentorium (Fig. 2C–H).

The connection of the mandible to the tentorium in Arthrosphaeridae

The strong condylus (co) of the mandibular gnathal lobe (Fig. 2A, B, I, J, BROWN) is not in direct contact with the tentorium, but medially faces the epipharyngeal bar (Fig. 2A, B, I, J, eb). Lateral of the condylus arises a sclerotised socket-shaped projection (Fig. 3A, ilp) from the incisura lateralis (il) of the head capsule (Fig. 3B, hc). The mandibular condylus hence appears to be encompassed by both the epipharyngeal bar and the sclerotised projection of the incisura lateralis.

Musculature of the tentorium in Arthrosphaeridae

The tentorial complex of the Arthrosphaeridae is associated with a set of seven muscles (Fig. 3C), which do not vary in the studied species. The proximal part of the epipharyngeal bar (eb) gives rise to the anterior tentorial muscle (t1), which inserts on the anterior part of the head, and to the dorsal tentorial muscle (t2), which inserts medial of the antennal socket (Fig. 3D). The posterior tentorial muscle (t3) inserts on the whole length of the posterior margin of the posterior process (pp) and originates from the postoccipital flange close to the transition to the collum (Fig. 3C, F). The lateral antennal muscle (a1) originates from the posterior margin of the transitional area between the epipharyngeal bar (eb) and the posterior process (pp), anteriorly of t3, and inserts on the posterior margin of the first antennomere (at1). The anterior antennal muscle (a2) inserts on the anterior margin of the first antennomere (at1) and originates from the epipharyngeal bar (eb) lateral of t3 (Fig. 3E). Median to t3, the pharyngeal dilator muscle (p1), which inserts on the lateral pharyngeal wall (ph),

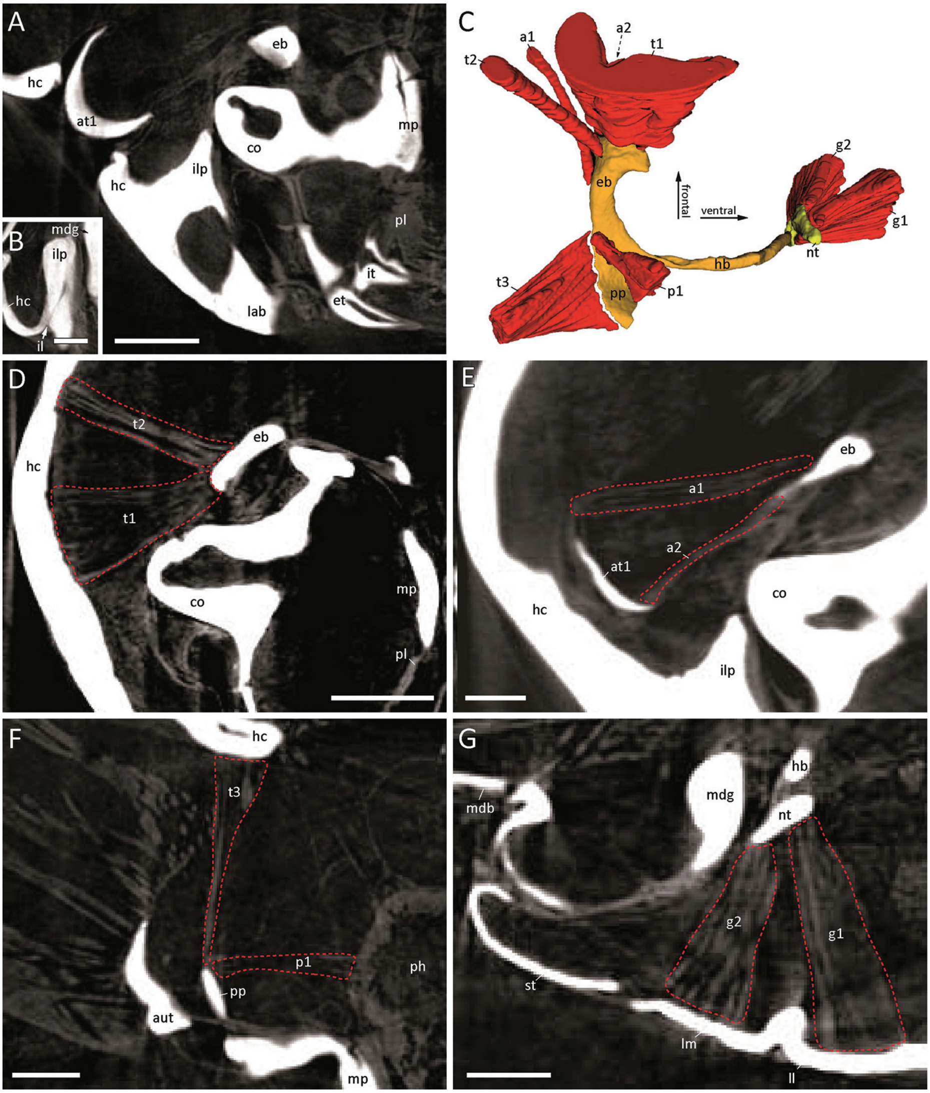

Figure 3. Head musculature of *Sphaeromimus kalambatritra*. **A, B, D–G** micro-CT images **C** 3D segmentation. **A** Arrangement of mandible, tentorium and head capsule, frontal section **B** Incisura lateralis in detail, frontal section **C** 3D segmentation of the isolated tentorium and its muscles, medial view **D, E** Muscles of the epipharyngeal bar, fronto-medial view **F** Muscles of the posterior process, frontal section **G** Muscles of the nebententorium, frontal section. Top is frontal, left is lateral. Scale bars: **A, D** 1000 µm **B, E–G** 500 µm **C** not to scale. **Abbreviations**: **a1** = lateral antennal muscle (m.); **a2** = anterior antennal m.; **at1** = first antennomere; **co** = condyle of mandibular gnathal lobe; **eb** = epipharyngeal bar; **et** = external tooth of mandible, **g1** = lamella lingualis m.; **g2** = lamello-mentum m.; **aut** = mandibular gnathal lobe sclerite (außententorium); **hb** = hypophayrangeal bar; **hc** = head capsule; **il** = incisura lateralis; **ilp** = projection arising from incisura lateralis; **it** = internal tooth of mandible; **lab** = labrum; **ll** = lamella lingualis of gnathochilarium; **lm** = lamellomentum; **mdb** = mandibular base; **mdg** = mandibular gnathal lobe; **mp** = molar plate; **nt** = nebententorium; **p1** = pharyngeal dilator m.; **ph** = pharynx; **pl** = pectinate lamellae of mandible; **pp** = posterior process; **st** = stipes of gnathochilarium; **t1** = anterior tentorial m.; **t2** = dorsal tentorial m.; **t3** = posterior tentorial m.

originates from the frontal anterior portion of the posterior process (Fig. 3F, pp). The nebententorium (nt) gives rise to a muscle (g1) inserting medially on the lamella lingualis (ll) of the gnathochilarium. Another muscle (g2) of the gnathochilarium passes from the lamello-mentum (lm) to the posterior surface of the nebententorium (nt) lateral to g1 (Fig. 3G).

Discussion

Structure of the tentorium in the Sphaerotheriida

The tentorium of the three studied representatives of Sphaerotheriida shows the same basic structure (Fig. 2C–H), as already described by vom Rath (1886), Silvestri (1903) and Attems (1926) for *Sphaeropoeus*, and by Verhoeff (1928) for *Arthrosphaera dentigera*. Vom Rath (1886) stated that the tentorium of the Sphaerotheriidae *Sphaerotherium* Brandt, 1833 resembles that of the Zephroniidae *Sphaeropoeus* Brandt, 1833, without a detailed description. Therefore, the general structure of the tentorium seems to be highly conserved within the Sphaerotheriida.

The most striking character of the giant pill-millipede tentorium is the absence of the transverse bar (Fig. 1C), which is present in all other millipede orders as far as known. Although the transverse bar differs among millipedes in its shape and articulation to the head capsule, it is known to be present in the Polyxenida (Koch 2003), Glomerida (vom Rath 1886, Silvestri 1903, Voges 1916), Julida (vom Rath 1886, Silvestri 1903, Voges 1916, Attems 1926, Verhoeff 1928, Fechter 1961), Spirobolida (vom Rath 1886, Snodgrass 1950), Spirostreptida (vom Rath 1886, Silvestri 1903, Manton 1964), Polydesmida (vom Rath 1886, Silvestri 1903, Verhoeff 1928, Seifert 1932, Snodgrass 1950), Chordeumatida (vom Rath 1886, Verhoeff 1928), Callipodida (Verhoeff 1928) and Platydesmida (Koch 2015). The state of the transverse bar (or the tentorium in general) for Glomeridesmida, Stemmiulida, Siphoniulida and most Colobognatha has not been documented yet. A transverse bar can nevertheless be assumed for the ground pattern of Diplopoda. Its reduction can be viewed as a derived state of the Sphaerotheriida.

Impact on tentorial mobility

The general function of the transverse bar is the connection of the tentorial complex to the head capsule at the incisura lateralis (= clypeal notch), around which the tentorium is deemed to perform its swinging movements (Manton 1964). Furthermore, the transverse bar is the insertion site for tentorial protractor muscles (Manton 1964, Wilson 2002). Along with the reduction of the transverse bar in Sphaerotheriida, the mobility of the tentorium must have undergone tremendous changes and must differ from the mechanism assumed by Fechter (1961) and Manton (1964) for Juliformia, as the muscular equipment of the tentorium in Sphaerotheriida (Fig. 3 C) varies greatly from that of Juliformia. While Wilson (2002) reported 13 muscles attached to the tentorium in Spirostreptida, Sphaerotheriida only maintain seven muscles (Fig. 3C). In Sphaerotheriida, the anterior tentorial muscle and the dorsal tentorial muscles originating on the epipharyngeal bar (Fig. 3D), as well as the posterior tentorial muscle and pharyngeal dilator muscles originating from the posterior process (Fig. 3F) correspond to the state in Juliformia (Wilson 2002). The tentorial protractor muscles, however, apparently shifted their position from the transverse bar (as described by Wilson 2002) to the distal part of the epipharyngeal bar. Further differences concern the antennal muscles that arise from the posterior process of the tentorium in Juliformia, but from the epipharyngeal bar in Sphaerotheriida (Fig. 3E). In Sphaerotheriida, contrarily to Juliformia (Silvestri 1903, Manton 1964, Wilson 2002), no mandibular muscles arise from the tentorium. In the Sphaerotheriida all muscles of the mandibular base instead originate from the transverse mandibular tendon and cranial wall, respectively. Among the three gnathochilarial muscles arising from the nebententorium in Juliformia, only two are present in Sphaerotheriida, i.e., the one (g1) inserting on the lamella-mentum, and the one (g2) inserting on the lamellae linguales (Fig. 3G). The muscle extending from the gnathochilarial stipes to the nebententorium is apparently reduced in giant pill-millipedes. This reduction of gnathochilarial muscles is likely due to strong modifications of the gnathochilarium in Sphaerotheriida, which are considered as autapomorphies of this taxon (Wesener 2016).

Despite these differences, the main muscles considered essential for movements of the tentorial complex are present. Manton (1964) states that the movement of the tentorium in Spirostreptida results from the tension of the protractor tentorii (anterior tentorial muscle sensu Wilson 2002, t1) and the depressor tentorii, which correspond to the lamella lingualis muscle (g1) and the lamello-mentum muscle (g2) of Sphaerotheriida.

The differences in the composition of the tentorium and in its muscular equipment might not only be correlated with the absence of the tentorial transverse bar, but also with the presence of a strong condylus on the mandibular gnathal lobes of Sphaerotheriida that unquestionably impacts on the mandibular mechanism. In Juliformia, the connection of the transverse bar to the incisura lateralis is deemed to fix a swing of the tentorium, causing the mandibular gnathal lobe to abduct (Fechter 1961, Manton 1964). In Sphaerotheriida, we propose that abduction of the mandibular gnathal lobes instead is caused by forces exerted from the epipharyngeal bar on the condylus of the gnathal lobes. The shape of the epipharyngeal bar indicates that it interlinks to the notch present on the condylus (Fig. 2A, J) to cause abduction of the gnathal lobe by pushing its condylus into the projection of the incisura lateralis. The theory about this movement is further corroborated by the shape correlation between the shape of the notch and the curvature of the epipharyngeal bar. The curvature of the epipharyngeal bar is more shallow in *A. brandtii*, where the notch forms a larger plateau (Fig. 2A) than in *S. kalambatritra*, in which the notch of the condylus is more strongly curved (Fig. 2I). The projections of the incisura lateralis and the epipharyngeal bar appear to form an anchor around which the mandibular gnathal lobe rotates during its abduction. According to this interpretation, the tentorial protractor and retractor muscles likely do no longer cause the tentorium to swing. The modified muscles instead likely serve to stabilise the tentorium in a position enabling the condylus of the gnathal lobe to rotate between the incisura lateralis and the epipharyngeal bar.

Correlations of the shape of the tentorium with volvation: a comparison with Glomerida

The tentorium of Sphaerotheriida contributes more characters to the list of head modifications that likely correlate with adaptations to volvation (see, e.g., Golovatch 2003, Blanke and Wesener 2014, Tuf et al. 2016). These adaptations include a reduction of the head lumen and coincident size reduction or entire loss of endoskeletal formations (surveyed by Koch 2015). Among Pentazonia, both Sphaerotheriida and Glomerida are able to roll themselves up into a ball, but their tentorium displays different transformations: as inferred from *Glomeris marginata*, the transverse bar is primarily maintained (e.g., Voges 1916, Dohle 1964), albeit with a remarkably loose connection to the head capsule. However, unlike in Sphaerotheriida, in the Glomerida the posterior process of the tentorium seems to be absent (Koch 2015). Since the posterior process provides the origin of the tentorial retractor muscle (t3), the loss of the posterior process may indicate that the tentorium in Glomerida is, as in Sphaerotheriida, no longer capable of performing swinging movements. The different modifications of the tentorium, the reduction of the transverse bar in Sphaerotheriida and of the posterior process in Glomerida, corroborate the view that anatomical adaptations to volvation are non-homologous (Sierwald and Bond 2007, their Supplemental Appendix 1), i.e., that volvation evolved convergently in Glomerida and Sphaerotheriida. This view is particularly supported by recent molecular analyses (Regier et al. 2005, Fernández et al. 2016), as well as by characters of the gnathochilarium (Wesener and Van den Spiegel 2009) favouring a sister group relationship between Glomerida and Glomeridesmida over the traditional classification of Glomerida and Sphaerotheriida in the taxon Oniscomorpha. Our ongoing studies focus on a detailed comparison of the cephalic musculature in Glomerida and Glomeridesmida to test the hypothesis of convergent loss of a swinging tentorium in correlation with convergent gain of volvation in Glomerida and Sphaerotheriida.

The tentorium as a taxonomic character inside Sphaerotheriida

Although the general appearance of the tentorium is conserved within Arthrosphaeridae there are some differences in details. These mainly concern the epipharyngeal bar, with its projections varying in their shape and length (Fig. 2D, F, H). Slight variations in shape are also displayed by the hypopharyngeal bar and the nebententorium (Fig. 2C, E, G). The tentorium of *Sphaeromimus* (Fig. 2E, F) is more similar to the state in *Arthrosphaera* (Fig. 2C, D) than in *Zoosphaerium* (Fig. 2G, H). This corresponds to the interrelationship within Arthrosphaeridae retrieved by Wesener et al.

(2010) from molecular analyses, according to which the Malagasy genus *Sphaeromimus* is more closely related to the Indian genus *Arthrosphaera* than to the other Malagasy genera *Zoosphaerium* and *Microsphaerotherium*. The structure and shape of the tentorium accordingly might also serve as an informative character not only for phylogenetic reconstructions, but also for taxonomic studies, which can be assessed quite rapidly with high-throughput techniques like μCT and automated 3D-segmentation. However, not investigated yet were the changes in the structure of the tentorium in different life stages of millipedes and intraspecific variations. We recommend that internal characters should more often be considered in taxonomic descriptions.

Conclusions

The reduction of the transverse bar of the tentorial complex as well as the presence of the mandible condyles in Sphaerotheriida must have an enormous impact on the mandibular abduction, resulting in a probably non-swinging tentorium. The reduction of the transverse bar in Sphaerotheriida is probably correlated to the volvation and suggests a convergent evolution of volvation in the pentazonian orders Sphaerotheriida and Glomerida. In Glomerida the posterior process of the tentorial complex is reduced as an adaptation to volvation. This could furthermore support a previously suggested (Regier et al. 2005, Fernández et al. 2016, Wesener and van den Spiegel 2009) closer relationship between Glomerida and Glomeridesmida. Furthermore the tentorium offers taxonomic characters to distinguish at least genera. Therefore we recommend considering internal characters more often in taxonomic descriptions. Despite its importance as apomorphy, supporting the monophyly of Myriapoda, and its functional role, the tentorial complex is largely understudied and the knowledge on it throughout the Myriapoda is only fragmentary. This study can be seen as first step towards a broader assessment of the tentorial complex in the Diplopoda.

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Appendix Chapter 5

Leif Moritz & Thomas Wesener (2021) A tarsal spinning organ in glomeridesmid millipedes (Diplopoda: Pentazonia: Glomeridesmida). *Arthropod Systematics & Phylogeny* 79: 555–567.

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A tarsal spinning organ in glomeridesmid millipedes (Diplopoda: Pentazonia: Glomeridesmida)

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Abstract

The production of sticky threads from spinnerets is known from various myriapod groups including some representatives of the millipedes (Diplopoda). In Diplopoda the thread-producing glands are mostly seta-like and positioned terminally on the telson, and the secretion product is typically used to build molting chambers or egg sacs. So far, no such secretions or organs have been documented for the subgroup Pentazonia. Here we describe thread-producing glands from the species-poor Glomeridesmida. These putative spinning organs are single circular fields of small pores (spinning fields) positioned on the outer side of the tarsi of all walking legs of mature and juvenile individuals of both sexes. These pores are the openings of cuticular tubuli (conducting canals), which extend from the tarsus to an aggregation of cells, a putative gland, within the femur. In several specimens thin threads were observed to be extruded from the pores. The tarsal spinning fields are present in all 21 investigated Glomeridesmida morphospecies, including Termitodesmidae and Glomeridesmidae from South East Asia, the Indian subcontinent, Oceania, and South and Central America. These organs might constitute an apomorphic character of the Glomeridesmida, as similar organs are absent in other Myriapoda. The function of the extruded threads in Glomeridesmida remains speculative, because observations of living specimens of the group are almost non-existing. We suggest that the secretion might be used for defense, to build molting chambers or to secure tunnels burrowed in the substrate.

Keywords

exocrine gland, *Glomeridesmus*, leg, Limacomorpha, scanning electron microscopy, silk, spinnerets, *Termitodesmus*

1. Introduction

Exocrine glands, which produce superficially silk-like threads and are often referred to as spinning organs or spinnerets, can be found in several Myriapoda taxa, including millipedes (Diplopoda), and differ in their structure and position on the body. Spinning glands are not known for Pauropoda but are present in Symphyla, where they open on appendages of the preanal segment (spinnerets) (Verhoeff 1934; Szucsich and Scheller

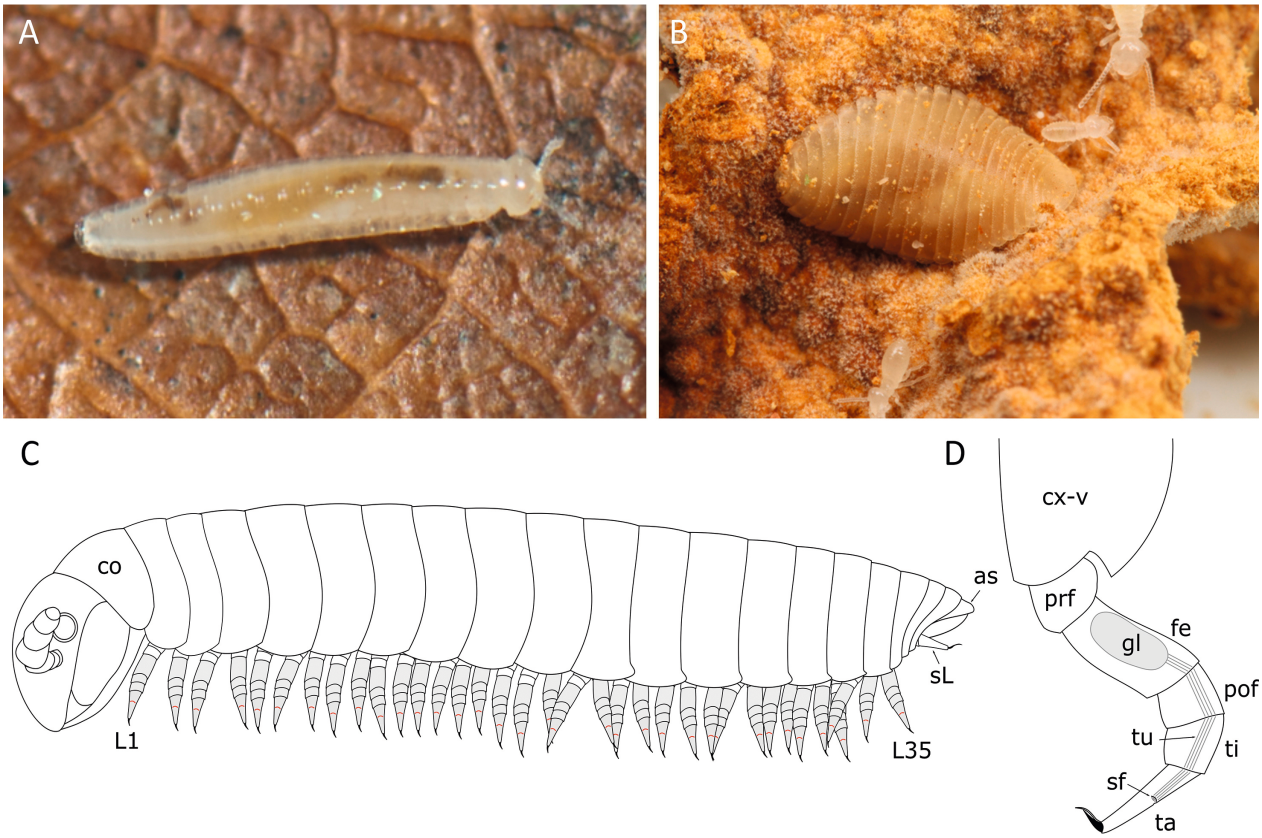

Figure 1. Glomeridesmida and the distribution of the tarsal spinning organ. **A**: Glomeridesmidae (*Glomeridesmus* cf. *javanicus*), photograph of living specimen in Indonesia by Jan-Philip Oeyen. **B**: Termitodesmidae (*Termitodesmus* sp.) in a termite nest in Malaysia, photograph of living specimen by Munetoshi Maruyama and Taisuke Kanao. **C**: Habitus of adult female with 35+1 legs, schematic drawing, each walking leg is equipped with a spinning organ. **D**: Schematic representation of the spinning organ in a walking leg. **Abbreviations**: as = anal shield, co = collum, cx = coxa, fe = femur, gl = glandular cells, L1 = walking leg 1, L35 = walking leg 35, pof = postfemur, prf = prefemur, sf = spinning field, sL = sensory leg, ta = tarsus, ti = tibia, tu = tubuli, v = stigmatic plate.

2011). In Symphyla the secreted threads can be attached to the ground and used for abseiling, and as defense by blocking the passage for predators in narrow crevices (Verhoeff 1934; Dunger 1983; Betz and Kölsch 2004). Lithobiomorph centipedes (Chilopoda, Lithobiomorpha) produce long sticky fibers, which are used for defense and predation, from telopodal pores on the four distal podomeres of the posterior leg pairs 12–15 (Blower 1952; Rosenberg and Müller 2009; Kenning et al. 2019). When disturbed, many Lithobiomorpha raise their ultimate legs and release a milky secretion, which hardens immediately and can entangle predators as well as prey (Verhoeff 1925; Attems 1926; von Byern et al. 2017; Kenning et al. 2019). Within Diplopoda, exocrine glands which release threads can be found in the Polyxenida, Polydesmida, Nematophora, and potentially in the Siphoniulida and Siphonophorida. Some male pincushion millipedes (Polyxenida, Polyxenidae) possess coxal spinning glands on their 2nd leg pair associated with their gonopores (penes), which secrete threads for the placement of sperm (Schömann 1956; Huynh and Veenstra 2016). Furthermore, male Polyxenidae produce guide threads from glands on leg-pairs 8 and 9 to help females find fresh spermatophores (Schömann and Schaller 1954; Schömann 1956). Polydesmida and Nematophora (Callipodida, Chordeumatida, Stemmiulida) share terminal fiber-producing glands or spinnerets, which are located on the epiproct of the telson, and largely resemble setae (Verhoeff 1928; Adis et al. 2000; Shear 2008; Enghoff and Akkari 2011; Enghoff and Reboleira 2013; Blanke and Wesener 2014; Iniesta and Ferreira 2015; Fiemapong et al. 2017). A detailed overview of these spinnerets in Polydesmida and Nematophora was provided by Shear (2008). In the Polydesmida and Nematophora the (silk-like) threads produced by the spinnerets are used to build molting chambers or in some cases egg-sacs (Adis et al. 2000; Shear 2008; Enghoff and Akkari 2011). Similar telsonian spinnerets can also be found in the enigmatic Siphoniulida (Sierwald et al. 2003; Liu et al. 2017), although the homology of these terminal spinning organs among the orders remains unknown (Sierwald et al. 2003; Shear 2008). Furthermore, tergal setae, which extrude a silk-like substance, might be present in the Siphonoporida (Marek and Bond 2006; Read and Enghoff 2009; Marek et al. 2012, 2016). The internal morphology of these glands and the chemical composition of the secreted threads in millipedes is largely unknown, but staining experiments show, that it is not true silk (i.e. made of fibrous protein) but consists of mucopolysaccharides in the Polydesmida (Adis et al. 2000).

For the Pentazonia, which include large and conspicuous animals like the Holarctic pill millipedes (Glomerida) and the giant pill-millipedes (Sphaerotheriida), as well as the small and poorly known glomeridesmid millipedes (Glomeridesmida) (Enghoff et al. 2015), no spinning organs or spinning activity have been reported so far. Silvestri (1902, 1903) probably misinterpreted the tips of the sensory legs in Glomeridesmida as cylindrical spinning organs opening on the preanal tergite. The taxon Glomeridesmida is among the least-diverse and least-studied millipede orders, and its members are thought to be particularly similar to the chilognathan ground-plan (Enghoff 1990). Glomeridesmida comprises 36 described species from South-East Asia, India, and South and Central America (undescribed species are also known from Oceania: Shelley 2011), classified in two families, Glomeridesmidae (Fig. 1A) and Termitodesmidae (Fig. 1B) (Jeekel 2003; Enghoff et al. 2015; Wesener et al. 2021). Most of the few described species are only known from their type series, the majority of them collected more than 100 years ago (Jeekel 2003). Unlike Helminthomorpha and Glomerida, which possess defense secretions (Shear et al. 2011; Shear 2015), or the strongly sclerotized Glomerida and Sphaerotheriida, which can roll into a ball (Blanke and Wesener 2014; Enghoff et al. 2015), the weakly sclerotized Glomeridesmida appear to be relatively defenseless against predation. Observations of living specimens and therefore information on the behavior and ecology of this group are rare and mostly anecdotal (Escherich 1911; Hirst 1913; Wesener et al. 2021). Observations show that *Glomeridesmus spelaeus* Iniesta & Wesener, 2012 is able to dig tunnels into compact substrate (Iniesta et al. 2012), and that *Termitodesmus* Silvestri, 1911 (Escherich 1911; Hirst 1913) and *Glomeridesmus* Gervais, 1844 (Wesener et al. 2021) are capable of (relatively) fast and swift movements, showing great flexibility of the body.

Here we present first evidence for the presence of tarsal thread-producing organs (putative spinning organs) in the Glomeridesmida combining scanning electron microscopy, histology, and light microscopy.

2. Methods

2.1. Abbreviations

FMNH – Field Museum of Natural History, Chicago, USA; **ISLA** – Subterranean Invertebrate Collection of Lavras, Center for Studies on Subterranean Biology, Federal University of Lavras, Brazil; **LM** – Light microscopy; **MHNG** – Muséum d'histoire naturelle de la ville de Genève, Geneva, Switzerland; **NHMD** – Natural History Museum of Denmark, University of Copenhagen, Denmark; **SEM** – Scanning electron microscopy; **SMNG** – Senckenberg Museum für Naturkunde Görlitz, Germany; **VMNH** – Virginia Museum of Natural History, Martinsville, USA; **ZCSWU** – Zoological Collection of Srinakharinwirot University, Bangkok, Thailand; **ZFMK** – Zoological Research Museum A. Koenig, Leibniz Institute for Animal Biodiversity, Bonn, Germany.

2.2. Material examined

19 morphospecies of the family Glomeridesmidae from South East Asia, the Indian subcontinent, Oceania, and South and Central America, spanning the known geographical distribution of the family, and two morphospecies of the family Termitodesmidae from Vietnam and Malaysia were studied. Investigated specimens included mature females (20 tergites (T) + anal shield (AS)) and males (19 tergites + anal shield) and immatures/juveniles (9–19 tergites + anal shield) (tergite number including collum). All material, including mostly undescribed species, is deposited in the collections of different museums (Table 1) and had initially been fixed and stored in ethanol (70% or 96%). Additional images are provided as supplementary files (Figs S1–S5; https://doi.org/10.3897/asp.79.e70002.suppl1).

2.3. Scanning electron microscopy (SEM)

To study the external morphology and structure of the putative spinning organs SEM was used. SEM data was obtained for three *Glomeridesmus* and one *Termitodesmus* morphospecies (Table 1). Specimens were dehydrated in an ascending ethanol series and dried overnight. The dried samples were sputtered with gold (ca 35 nm) using a Cressington 108 auto sputter coater (TESCAN GmbH, Dortmund, Germany). Images were obtained using a Zeiss Sigma 300 VP SEM (Carl Zeiss AG, Oberkochen, Germany) at the ZFMK. Furthermore, SEM images previously obtained for *Glomeridesmus* spp. (Philippines, Ecuador) and *Termitodesmus* sp. (Malaysia) by TW and for *Glomeridesmus* sp. (Ecuador) by William A. Shear, were checked for the absence or presence of the described structure.

2.4. Light microscopy (LM) and histology

The legs were examined microscopically with transmitted light to check for the presence of the putative spinning organs and to investigate their internal morphology. Legs, unstained or stained (Table 1) for 3–4 minutes with 1% Toluidine blue (PanReac AppliChem, ITW Reagents (Chicago, USA), A3842.0010) to enhance contrast, and subsequently washed with ethanol, were temporarily mounted onto microscopic slides, and investigated with an Olympus BX51 light microscope (Olympus, Shinjuku, Tokio, Japan).

Histological sections were obtained for mid-body legs of a female *Glomeridesmus* cf. *sumatranus* Pocock, 1894 (SUM06/08 01). Legs were embedded in epoxy resin (Araldite CY212, Agar Scientific Ltd (Stansted, UK), R1030) and semi-thin sections with a thickness of 0.5 µm were obtained with a Leica HistoCore NANOCUT R microtome (Leica Biosystems, Wetzlar, Germany) with a DiATOME histo Jumbo diamond blade (Diatome Ltd,

Table 1. Material examined. **Abbreviations**: H = histology, LM = light microscopy without Toluidine, LMT = light microscopy with Toluidine, SEM = scanning electron microscopy, $^{\mathrm{TW}}$ = SEM images obtained previously by TW, $^{\mathrm{WS}}$ = SEM images obtained previously by William A. Shear.

Species	Locality	Method
Glomeridesmus siamensis Wesener, Wongthamwanich & Moritz, 2021	THAILAND • 1 ♂, 1 ♀, 2 juv (19T+AS); Krabi Province, N. of Krabi Town, western aspect of Tiger Cave temple (Wat Tham Suea); 08°07′23.8″N, 098°55′18.9″E; 27.VII.2018; Wesener, Wongthamwanich, Nawanetiwong, Moritz leg.; overgrown rocks next to rubber plantation; ZCSWU-MyrD000011, ZFMK-MYR10302, ZFMK-MYR10301; ZFMK-MYR10304	SEM + LM
Glomeridesmus spelaeus Iniesta & Wesener, 2012	BRAZIL • 1 ♀; Pará, Curionópolis, iron cave SL 31; E 0650189m, N 9339714m; R.A. Zampaulo, leg.; in bat guana pile far from entrance; ISLA MII GEM 176150	LMT
Glomeridesmus cf. *sumatranus* Pocock, 1894	INDONESIA • 1 ♂, 1 ♀, 2 juv (19+AS; 15+AS); Sumatra, West Sumatra Province, Mt. Merapi, ca. 15 km SE of Bukittinggi; 0°23′32″S, 100°26′54″E; 1650–1700 m a.s.l.; 4.VI.2006; A. Schulz leg.; hill forest; MHNG SUM06/08 01	SEM + LMT + H
Glomeridesmus sp.	INDONESIA • 1 ♂, 1 ♀, 1 juv (19T+AS); Sumatra, West Sumatra Province, Mt. Merapi, ca. 15 km SE of Bukittinggi; 0°23′32″S, 100°26′54″E; 1650–1700 m a.s.l.; 4.VI.2006; A. Schulz leg.; hill forest; MHNG SUM06/08 02	SEM + LMT
Glomeridesmus cf. *javanicus* Attems, 1907	INDONESIA • 1 ♀; Java, Jawa Barat, Cikaniki Research Station, Erstes Bachtal von der Station aus (HAL92); 6°44′54″S, 106°32′21″E; 1082 m a.s.l.; 19.IV.2016; Myriapoda Team leg.; Winklerextraktion; ZFMK-MYR07870	LMT
Glomeridesmus sp.	MALAYSIA • 1 juv (19T+AS); Pahang, Cameron Higlands, “Orang Asli vill.“ env. Gunung Perdah [Mt.]; 4°29.2N, 101°22.1E; 1575 m a.s.l.; 2–14.V.2009; Petr Baňař leg.; Sifting leaf litter in shallow ravine; NHMD	LMT
Glomeridesmus sp.	THAILAND • 1 juv (9T+AS, 8 leg-pairs); Doi Sutep; 1150 m a.s.l.; 29.IX.1958; B. Degerbøl leg.; Lok 3a; Zool. Mus. Kbh. 1/7 59; NHMD	LM
Glomeridesmus sp.	PAPUA NEW GUINEA • 1 ♀; New Britain, Valoka; 12.V.1962; Noona Dan Exp. 61–62 leg.; NHMD	LM
Glomeridesmus sp.	FIJI • 1 juv (19T+AS); Colo-i-Surva Forest Park; 29.III.–6.IV.199?; van Harten A. leg.; NHMD Fiji 580	LM
Glomeridesmus sp.	FIJI • 1 juv (15T+AS); Colo-i-Surva Forest Park;9.II.1997; van Harten A. leg.; NHMD glomeridesmids553	LM
Glomeridesmus sp	PHILLIPINES • 1 ♀; Panay, Sibaliw; 11°49′37″N, 121°56′21″E; 220 m a. s. l.; 2007; leg. Prof. Curio leg.; SMNG	SEM$^{\mathrm{TW}}$
Glomeridesmus sp.	INDIA • 1 ♀, 1 juv (10T+AS, 13 leg-pairs); Chennai (Madras), Anamalai Hills, au-dessud d’Aliyar Dam; 1150 m a.s.l.; 18.XI.1972; C. Besuchet & I. Löbl leg.; tamisages en foret, au pied d′un groupe d′arbras envahis par les lianes; MHNG Inida34	LMT
Glomeridesmus sp.	INDIA • 1 ♀; Meghalaya, Khasi Hills: en-dessous de Cherapunjee;1200 m a.s.l.; 26.X.1978; C. Besuchet & I. Löbl leg.; MHNG India28	LMT
Glomeridesmus sp.	SRI LANKA • 1 ♂; Sinharaja; 400 m a.s.l.; 2.XII.1979; V. Mahler leg.; NHMD	LM
Glomeridesmus sp.	PANAMA • 1 juv (19T+AS); Gamboa; 01.XI.1983; W. Netwig leg.; NHMD; PR4 B	
Glomeridesmus sp.	ECUADOR • VMNH	SEM$^{\mathrm{WS}}$
Glomeridesmus sp.	ECUADOR • 1 ♀; Pichincha, Río Palenque Station, 47 km S Santo Domingo; 700 ft; 18.V.1975; S. B. Peck & J. Kukalova-Peck leg.; FMNH INS 0000 011 916	SEM$^{\mathrm{TW}}$
Glomeridesmus sp.	COLOMBIA • 1 ♂; Nevada del Ruiz; 3700–3800 m a.s.l; 10.X.1978; H. Sturm leg. (78/94); under Calamagrostis; NHMD Colombia78/94	LM
Glomeridesmus sp.	BRAZIL • 1 juv (18T+AS); Taperinka, Santarém; 3./11.70, Pr. 9–10 Myriapoda, S.I. Tuxen & O. Densen; NHMD	LM
Termitodesmus calvus Attems, 1938	VIETNAM • 1 ♀; Cat Tien; 16.I.2012; Semenyuk leg.; termite nest; ZFMK	SEM + LM
Termitodesmus sp.	MALAYSIA • 1 ♀; 06.VI.2012; M. Maruyama leg.; *Odontotermes* termite nest; ZFMK MYR596	SEM$^{\mathrm{TW}}$

Nidau, Switzerland). Semi-thin sections were stained with 1% Toluidine blue for 2 minutes. The mounted legs and histological sections were photographed with a Zeiss AxioCam HRc camera mounted to a Zeiss Imager.Z2m light microscope (Carl Zeiss AG, Oberkochen, Germany). The histological sections are deposited at the ZFMK (ZFMK-HIST00001).

2.5. Terminology

The terminology for the podomeres of the walking legs follows Iniesta et al. (2012) and Wesener et al. (2014) with a basal coxa fused to a ventral sclerite (stigmatic plate) followed by prefemur, femur, postfemur, tibia and tarsus. Terminology for the musculature follows Manton (1961).

3. Results

3.1. External morphology

The investigated mature males, mature females, and juveniles of the 19 Glomeridesmidae morphospecies and two Termitodesmidae morphospecies (Table 1, Figs S1–S5) carried a single circular field of pores, hereafter referred to as a spinning field, on the tarsi of all walking legs (Figs 1C, 2, 3). A spinning field was absent on the modified posterior legs of mature individuals (telopods in males; sensory legs in males and females). Already in the earliest stadium studied, a juvenile from Thailand (NHMD 1/7 59) with 9 tergites + anal shield, 8 leg pairs and ca. 1 mm in length, the tarsal spinning fields were present on all walking legs (Fig. S1). Each spinning field was located on the dorsal/outer side of the tarsus. In the studied Glomeridesmidae it was located ca at mid-length of the tarsus distally of a faint suture and the most apical setae (Fig. 2B). In Termitodesmidae, where the podomeres were generally shorter and thicker (Fig. 3A), the spinning field was located in the distal third of the tarsus, distally of the most apical setae (Fig. 3B).

The tarsal spinning fields were 3–5 µm in diameter, slightly recessed into the tarsal cuticle, and comprised of 20–30 pores, which faced distally. Each pore had a diameter of ca 0.35 µm in the studied specimens (Figs 2C, 3C). In *Glomeridesmus* sp. (SUM06/08 02) from Sumatra the field of pores appeared clean on the SEM images and only in few pores small buds (the tips of threads) could be seen (Figs 2C, S2A, B). In *Glomeridesmus* cf. *sumatranus* (SUM06/08 01), from the same locality as the previous species, thin threads with a regular diameter of ca 0.35 µm were extruded from the pores of some legs. The threads coiled up separately (Figs 2D, S2C, D) or unified distally to a larger mass (Fig. 2E). In some cases small particles stuck to the threads (Fig. 2D). In *Glomeridesmus siamensis* Wesener, Wongthamwanich & Moritz, 2021 (Fig. S3) and *Glomeridesmus* spp. from Ecuador (Fig. S4A–C) and the Philippines (Fig. S4D) the separate pores could not be identified on the SEM images, as these were obscured, but a depression was present at the corresponding position.

3.2. Internal morphology

In the 16 Glomeridesmidae and the single Termitodesmidae species studied with light microscopy (Table 1), thin cuticular tubuli (conducting canals), which arose internally from the pores of the spinning field, were visible through the translucent cuticle (Figs 3D, 4A, B, S5). These tubuli ran in a bundle proximally within the tarsus (Figs 3D, 4B). In the stained mounts of the whole legs, the femur appeared to be filled by a granular mass between well-defined muscular strands in Glomeridesmidae (Fig. 4A) and Termitodesmidae. The histological sections show suboptimal preservation of soft tissue and a clear or reliable identification of glandular tissue is not possible. Nevertheless, the cuticular tubuli arising from the sieve plate can be discerned. The cuticular tubuli run as a bundle from the tarsal sieve plate (Fig. 4C, D) through the tibia (Fig. 4E) and postfemur into the femur, where they lead to an accumulation of tissue (Figs 1D, 4F, G), probably the secretory cells of the gland.

4. Discussion

4.1. A tarsal spinning gland in Glomeridesmida

We suggest that Glomeridesmida possess tarsal spinning organs in the form of exocrine glands, which release threads through a field of pores on their walking legs' tarsi. Such threads are thin filaments of unknown composition and were observed to be extruded from the pores on some legs (Fig. 2D, E). The buds observed in some of the pores (Fig. 2C) are the tips of these filaments. Furthermore, in the figures of Iniesta et al. (2012; fig. 2A, C), the first publication of photos of a living Glomeridesmida specimen, threads can be observed on the substrate close to *Glomeridesmus spelaeus* Iniesta & Wesener, 2012, although it is not clear if these threads have been produced by the glomeridesmid. The putative tarsal spinning organs could be observed in all 21 Glomeridesmida morphospecies studied here, which span the whole biogeographic distribution of the group, and include Glomeridesmidae as well as Termitodesmidae (Table 1). Therefore, we suggest that Glomeridesmida in general possess tarsal thread-producing organs, although the recently described genus *Glomeridesmoides* Mauriès, 2020 needs to be studied in this respect. Although the legs of some Glomeridesmidae have been studied and depicted before (e.g. Silvestri 1903; Carl 1942; Iniesta et al. 2012; Wesener et al. 2014; Mauriès 2020) and Carl (1942) described glandular tissue within the syncoxite of the first leg-pair of a female, this spinning organ has not been reported. This is mainly due to the small size of the external structure (3–5 µm in diameter) and its position in a depression on the outer side of the tarsus, as it is not clearly visible in anterior or posterior views, which are typically depicted in taxonomic description. We initially discovered the sieve plate only by means of SEM and it is only visible at certain angles (Fig. 2). Only then we were able to also locate the organ using transmitted light microscopy of whole legs (Figs 3D, 4A, B, S5). Furthermore, the sieve plates are often obscured by dirt, the secretion, or setae.

The spinning organ of the Glomeridesmida is an aggregated gland with several secretory units; cells are clustered within the femur, but open via separate tubuli (conducting canals), which run through the podomeres to the tarsus (Figs 1D, 4C–G). To understand the detailed structure of these glandular units, ultrastructural investigations are needed. Aggregated glands are known from various myriapods, like the defense glands of millipedes

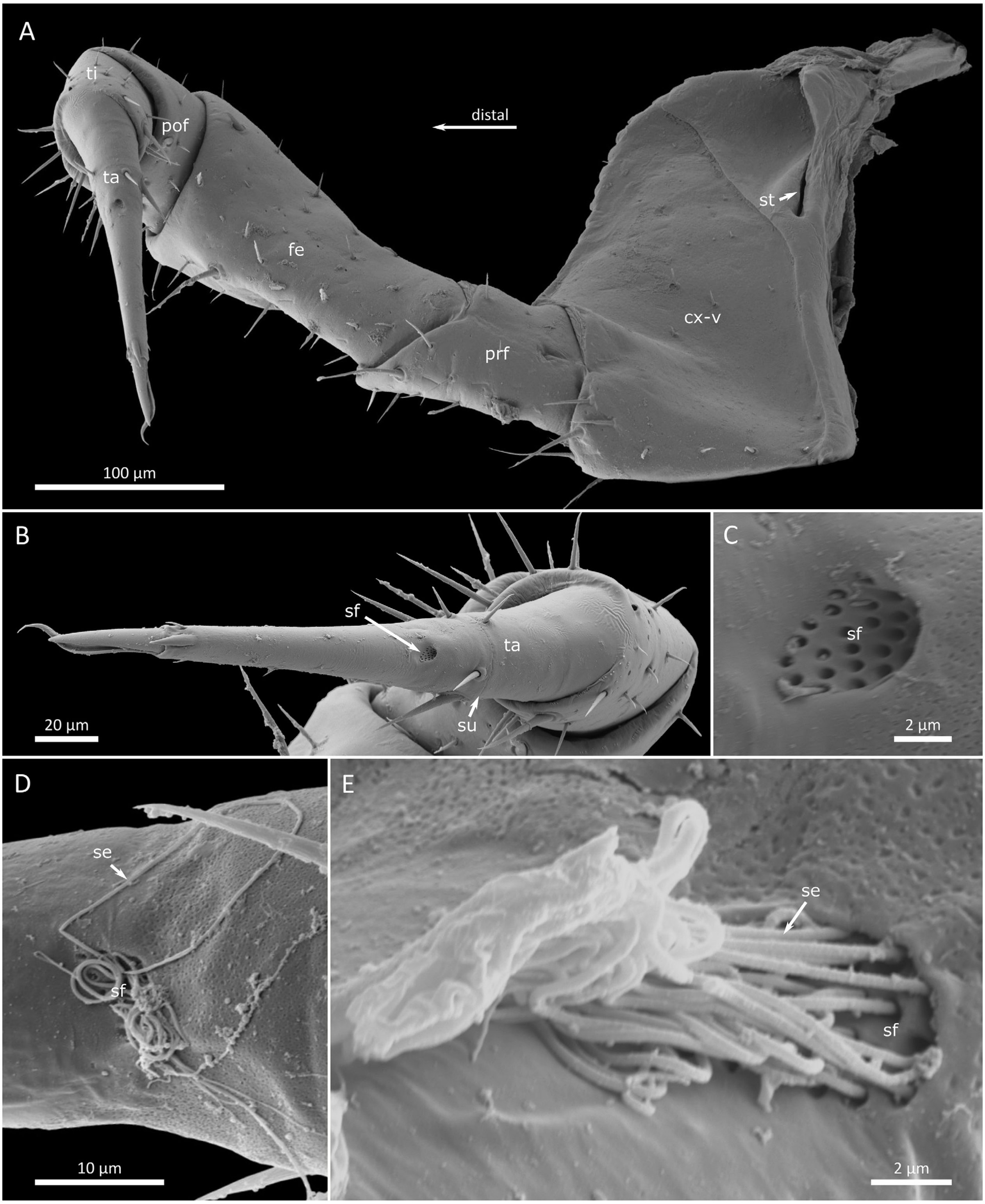

Figure 2. The putative spinning organ in the Glomeridesmidae, external morphology, SEM. **A–C**: *Glomeridesmus* sp. (SUM06/08 02), female, mid-body leg. **A**: Overview of leg. **B**: Detail of tarsus. **C**: Detail of spinning field. **D, E**: *Glomeridesmus* cf. *sumatranus* (SUM06/08 01), female, spinning fields on tarsi of leg pair 2 with extruded threads/secretion. **Abbreviations**: cx = coxa, fe = femur, pof = postfemur, prf = prefemur, se = secretion, sf = spinning field, st = stigmata, su = suture, ta = tarsus, ti = tibia, v = stigmatic plate.

(Weatherston and Percy 1969). The various aggregated glands of centipedes have been summarized by Rosenberg et al. (2011), and include the vesicular glands (Hilken and Rosenberg 2009), the maxillary organ gland (Hilken et al. 2003), the epidermal maxilla II-gland (Hilken et al. 2005) and the poison gland (Rosenberg and Hilken 2006), as well as the sternal glands of Geophilomorpha (Turcato and Minelli 1990) and the telopodal glands of Lithobiomorpha (Keil 1975). In the latter two the conducting canals open directly to the outside and not into a common duct or lumen, as is also the case in the spinning organs of the Glomeridesmida.

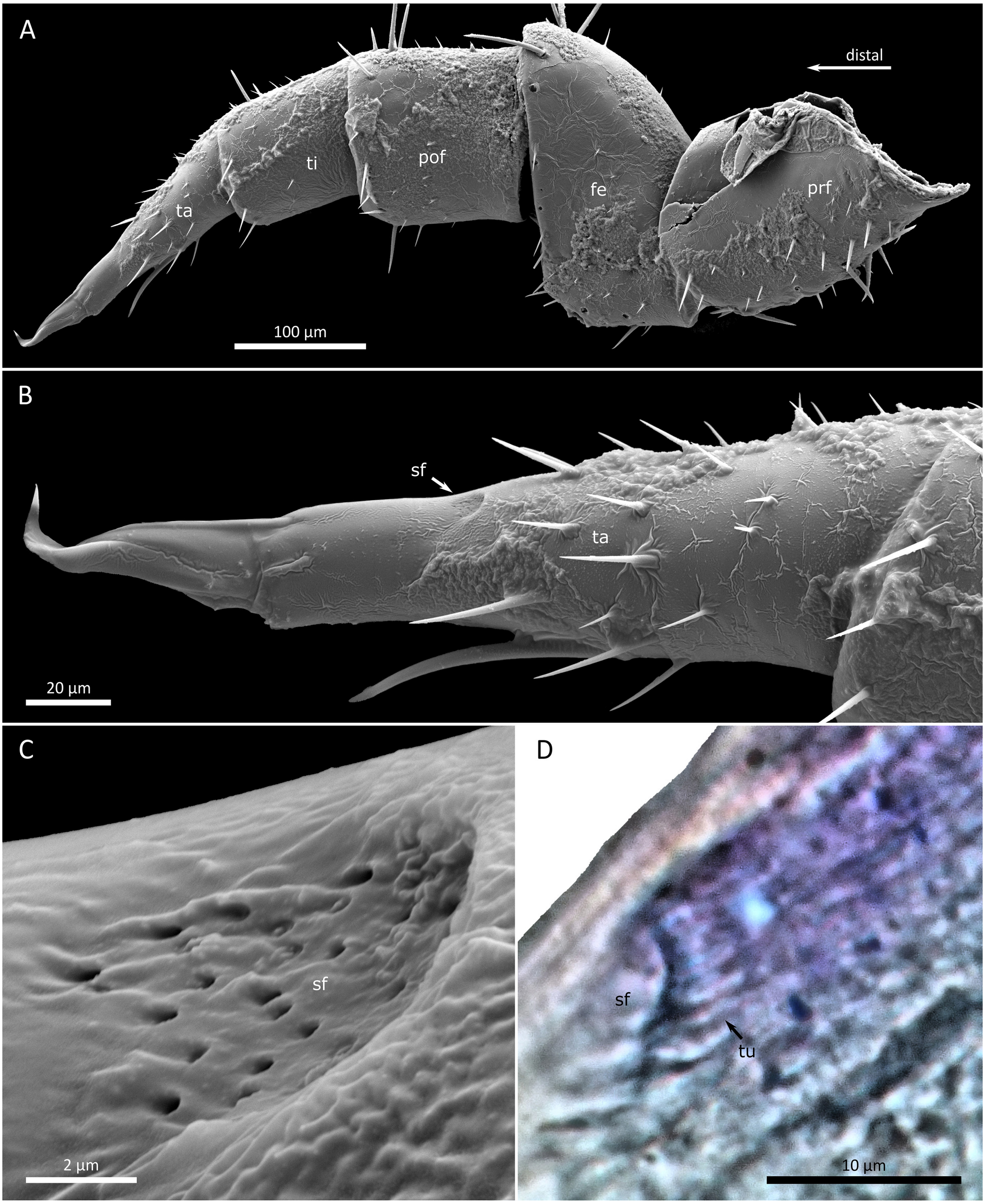

Figure 3. The putative spinning organ in the Termitodesmidae, mid-body leg of *Termitodesmus calvus* Attems, 1938, female. **A–C**: External morphology, SEM. **A**: Overview of leg. **B**: Detail of tarsus. **C**: Detail of spinning field. **D**: Detail of the spinning field with the underlying tubuli, mid-body leg stained with Toluidine blue, light microscopy. **Abbreviations**: fe = femur, pof = postfemur, prf = prefemur, sf = spinning field, ta = tarsus, ti = tibia, tu = tubuli.

Exocrine glands positioned on the legs are known from several millipede taxa but are typically restricted to males and differ in their position (i.e. on which legs and/or podomeres) from those in Glomeridesmida, in which pores are present in both sexes and in juveniles on the tarsi of all walking legs (Fig. 1C). Thus, pores can be found on the coxae of leg-pairs 7 and 9 in male Chordeumatida (Verhoeff 1928), leg-pairs 8 and 9 in male Polyxenida (Schömann and Schaller 1954; Schömann 1956) and on the posterior gonopods in male Julida. In the latter, these glands release long and viscose threads (zähe Sekretfäden sensu Verhoeff 1928). Furthermore, exo-

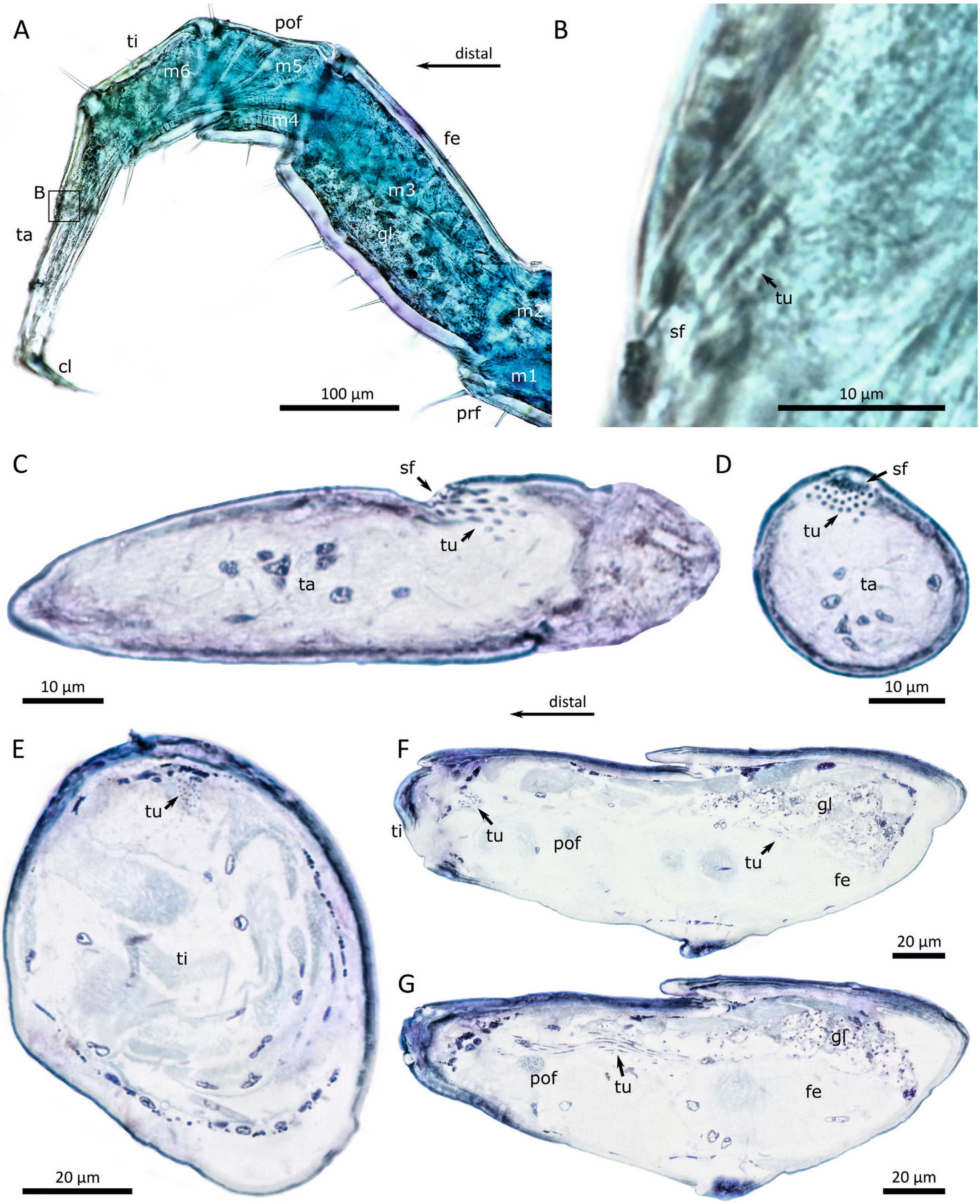

Figure 4. The putative spinning organ of the Glomeridesmidae, internal morphology of a mid-body leg of *Glomeridesmus* cf. *sumatranus* (SUM06/08 01), female, light microscopy. **A, B**: Mid-body leg stained with Toluidine blue. **A**: Overview, terminology for the musculature follows Manton (1961). **B**: Detail of the spinning field with the underlying tubuli, as indicated in A. **C–G**: Histological sections. **C**: Sagittal section through tarsus. **D**: Cross section through tarsus. **E**: Cross section through tibia. **F, G**: Sagittal sections through postfemur and femur. **Abbreviations**: cl = claw, fe = femur, gl = putative glandular tissue, m1 = depressor femoris, m2 = flexor femoris, m3 = flexor postfemoris, m4 = flexor tibiae, m5 = flexor tarsi, m6 = flexor unguiculi, pof = postfemur, prf = prefemur, sf = spinning field, ta = tarsus, ti = tibia, tu = tubuli.

crine glands open on the inner side of the femur of single or several walking legs of some male Polydesmida and Julida and might be related to mating (Verhoeff 1928).

Although spinning organs are reported from various millipede (Diplopoda) taxa, these differ largely in their location and structure from the putative spinning organs found in Glomeridesmida. In Glomeridesmida these are porous fields on the tarsi (Fig. 2B, C), while Polydesmida (Adis et al. 2000; Shear 2008), Nematophora (Shear 2008; Enghoff and Akkari 2011; Enghoff and Reboleira 2013; Blanke and Wesener 2014; Iniesta and Ferreira 2015; Fiemapong et al. 2017) and possibly Siphoniulida (Sierwald et al. 2003) carry terminal seta-like spinnerets on their epiproct, Siphonophorida carry potential seta-like spinning organs on their tergites (Marek and Bond 2006; Read and Enghoff 2009; Marek et al. 2012, 2016), and polyxenid millipedes have 'silk-producing coxal glands' (Schömann and Schaller 1954; Schömann 1956; Huynh and Veenstra 2016). The internal structure of these spinning glands in millipedes remains largely unknown.

In addition to the structure of the putative spinning organ, the structure of the threads observed in Glomeridesmida differs from that found in other millipede taxa with spinning abilities. Thus, the threads released from telsonian spinnerets are rather flattened in Chordeumatida (Enghoff and Reboleira 2013) or can have regular swellings in some Chordeumatida (Verhoeff 1928: p. 1061, figs 601–603), Callipodida (Enghoff and Akkari 2011) and Polydesmida (Adis et al. 2000; Shear 2008), while the threads in Glomeridesmida are of a uniform circular diameter (Fig. 2D, E).

From the other pentazonian taxa Glomerida and Sphaerotheriida, which are comparatively well-studied, including SEM images of the legs (e.g. Wesener and VandenSpiegel 2009; Wesener 2012; Oeyen and Wesener 2015; Wesener 2016; Wesener 2018), similar structures are unknown. Therefore, we suggest that the putative spinning organ of the Glomeridesmida constitutes an apomorphy of the group and supports the monophyly of Glomeridesmida (Glomeridesmidae + Termitodesmidae). Other characters supporting the monophyly of Glomeridesmida are a strongly elongated membranous fringe on the mandible molar plate, the presence of 20 tergites, and modifications of the penultimate and ultimate leg pairs in females (Oeyen and Wesener 2018). Apart from these characters, the Glomeridesmida are mainly characterized by reductive or supposedly plesiomorphic traits (Enghoff 1990; Iniesta et al. 2012).

4.2. Function of the putative tarsal spinning organ

The actual function of the extruded threads in the Glomeridesmida remains speculative because the biochemical composition of the threads (as for most millipedes) is unknown, and no spinning activity or spinning product has been observed so far in living specimens. For Polydesmida, it has been shown by staining experiments that the threads are not true silk (i.e. made of fibrous protein) but consist of mucopolysaccharides (Adis et al. 2000), while the threads in lithobiomorph centipedes are apparently a lipoid-protein complex or mixture (Blower 1952). Because small particles adhere to some threads in the studied Glomeridesmida (Fig. 2D), we suggest that the threads are adhesive. As tarsal spinning organs were not only found in mature male and female Glomeridesmida, but also in the smallest juveniles studied here (Fig. S1), we suggest that the threats are not primarily used for mating or brood care (although they might additionally play a role in it), but are rather used to build molting chambers, as is the case in Polydesmida and Nematophora (Adis et al. 2000; Shear 2008; Enghoff and Akkarin 2011), or as defense against predation as in Symphyla (Dunger 1983) and Chilopoda-Lithobiomorpha (Rosenberg and Müller 2009; Kenning et al. 2019). Glomeridesmida neither possess defensive glands as present in Glomerida and Helminthomorpha (Blanke and Wesener 2014; Enghoff et al. 2015; Shear 2015), nor can they roll up into a tight ball like Glomerida and Sphaerotheriida (Blanke and Wesener 2014; Enghoff et al. 2015) to ward off predators.

In insects thread-producing or spinning organs can be found on the apical podomeres of several taxa, like webspinners (Embioptera), dance flies (Diptera: Empididae), and some ants (Hymenoptera: Formicidae). In these insects the secretions serve to build tunnels as in Embioptera (Mukerji 1927; Büsse et al. 2015) and potentially in some ants (Billen and Peeters 2020), or to wrap nuptial gifts, as in dance flies (Young and Merrit 2003; Sutherland et al. 2007). Furthermore, thread-producing organs opening on apical podomeres are also known from some marine crustaceans, like the corophioid amphipod *Crassicorophium bonellii* (Kronenberger et al. 2012) and the order Tanaidacea (Kakui and Hiruta 2014; Kaji et al. 2016), in which the secreted threads (i.e. silk) are involved in tunneling and building tubes. Similar to the Glomeridesmida, the glands of these crustaceans are located in proximal podomeres or within the body, and ducts lead to the distal podomere.

To clarify the function of the spinning product in Glomeridesmida, behavioral observations of living specimens are needed. Until now such observations of living specimens are rare and mostly anecdotal (Escherich 1911; Hirst 1913; Wesener et al. 2021) and spinning activity has not been reported. *Glomeridesmus spelaeus* Iniesta & Wesener, 2012 specimens have been observed to dig small tunnels into compact guano substrate, despite their delicate appearance, and to shelter themselves in these small passages (Iniesta et al. 2012). Possibly the spinning product is used to secure the walls of these tunnels, as might be the case in some tunneling ants, which have similar exocrine glands and pores (the so called Delage-Darchen gland in *Melissotarsus* Emery, 1877) on the basitarsi of their walking legs (Delage-Darchen 1972; Billen 2009; Hölldobler et al. 2014; Billen and Peeters 2020). In these ants, solid 'toothpaste like' threads are excreted through the pores (Billen 2009; Hölldobler et al. 2014; Billen and Peeters 2020) and rubbed along the walls and roof of the ant's tunnels, which are made of silk (from a cephalic gland) and wood fragments (Delage-Darchen 1972; Billen and Peeters 2020). As the thread-producing organs found on the basitarsi of ants and on the tarsi of the Glomeridesmida are superficially similar in their position and structure, they might be used in a similar manner in Glomeridesmidae; i.e. the threads secreted serve in stabilizing the walls of their molting chambers or even tunnels. However, the latter function would not explain their presence in the termitophilous Termitodesmidae, which exclusively inhabit termite mounds (Silvestri 1911; Hirst 1911, 1913; Enghoff et al. 2015), in which tunnels are maintained by the termites, thus making tunneling and (if they are involved in this) the putative spinning glands obsolete. Therefore, a defensive function either during molting or against predation seems to be most plausible at the moment, although it might have multiple (e.g. tunneling and brood care) rather than a single function.

4.3. No terminal spinnerets in Glomeridesmida

For Glomeridesmida Silvestri (1902, 1903) reported paired cylindrical glands that open on the preanal tergite, and interpreted these as mucus glands (ghiandole mucipare sensu Silvestri 1902), which are possibly homologous to the spinnerets of Callipodida and Chordeumatida. We could not identify any such organs in the studied specimens using SEM or light microscopy. We suggest that Silvestri (1902, 1903) interpreted the apical podomeres of the ultimate legs, which are modified into sensory legs (Fig. 1C), as gland openings. The apical podomeres of the sensory legs are cylindrical, equipped with a terminal spine, and protrude (visible in dorsal view) from underneath the preanal tergite (tergite 19/20) beyond the anal shield, while its basal podomeres remain hidden (Iniesta et al. 2012; Enghoff et al. 2015; Mauriès 2020; Wesener et al. 2021), thus appearing superficially similar to telsonian spinnerets of other millipedes. Therefore, we conclude that such preanal glands are absent in Glomeridesmida.

4.4. Outlook

For this study we only had access to material from museum collections, which has been initially fixed and subsequently stored over a long period in ethanol (70% or 96% EtOH), resulting in the suboptimal preservation of soft tissue and in artefacts, as visible in the histological sections (Fig. 4). Therefore, the histological data presented here have to be taken with caution. To better understand the structure and function of the putative spinning glands in Glomeridesmida, future studies should focus on ultrastructure using transmission electron microscopy (TEM), as has been done for the cephalic glands and nephridia of several millipede taxa (e.g. El-Hifnawi and Seifert 1971, 1972; El-Hifnawi 1973, 1974) and epidermal glands in centipedes (e.g. Sombke and Müller 2021). A more complete series of postembryonic stadia might shed light on the first appearance and changes within the organ during development. In this study the earliest stadium has 9 tergites + anal shield and 8 leg pairs. Furthermore, the biochemical composition of the secretion should be analyzed, and, most importantly, the behavior of living specimens should be observed to better understand the function of the organ and the extruded threads.

5. Conclusion

Glomeridesmida possess exocrine glands in their walking legs, which open through a field of pores (spinning field) on their tarsi and extrude threads. These putative spinning glands, are present in both major groups of the Glomeridesmida, the Glomeridesmidae and Termitodesmidae, and are probably an apomorphic character of the group. The function of these threads remains speculative, but we suggest that the threads serve for a defensive function against predation and during molting, or that they are involved in tunneling. To clarify the structure and function of the glands and their excretion ultrastructural examination and behavioral observations of living specimens are needed.

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Supplementary material 1

Figures S1–S5

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Appendix Chapter 6

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No Tömösváry organ in flat backed millipedes (Diplopoda, Polydesmida)

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Abstract

The Tömösváry organ is a sensory structure of the head in myriapods and some other terrestrial arthropods. Due to its variable shape, size, and position in millipedes (Diplopoda) the Tömösváry organ is commonly used as diagnostic character in taxonomic descriptions and often included in phylogenetic analyses. For the Polydesmida, the largest millipede order, the Tömösváry organ is inconsistently stated as being either absent or present as a pear-shaped pit covered by a membrane or cuticular disc. In order to resolve this inconsistency, we investigated the morphology of the presumable Tömösváry organ in four polydesmidan species based on paraffin-histology, semi-thin sections and micro-computed tomography. Our results unambiguously favor the view that the articulation of the cephalic tentorium with the head capsule was misidentified as the Tömösváry organ in previous studies, and thus that the Tömösváry organ indeed is absent in the Polydesmida. The pear-shaped pit proved to represent the distal roundish expansion of the incisura lateralis, to which – similarly as in julidan millipedes – the tentorial transverse bar is articulated. The absence of the Tömösváry organ in the Polydesmida does not affect the topology of the interrelationships among the millipede orders retrieved in previous cladistic analyses based on morphology. As a character shared by Colobognatha and Juliformia, however, absence of a Tömösváry organ in Polydesmida favors the optimization of its presence in nematophoran millipedes as a reversal. Further studies are needed to clarify whether among chilognathan millipedes a Tömösváry organ really exists in taxa such as Stemmiulida, and whether the Tömösváry organs are homologous across millipedes.

Keywords
Anatomy, Histology, Micro-Computed Tomography, Morphology, Tentorium

Introduction

The Tömösváry organ is a paired sensory organ, situated on the head of millipedes (Diplopoda) and other Myriapoda posterior of the antennal base. It is also referred to as postantennal organ (e.g., Altner and Thies 1976), temporal organ (e.g., Bedini and Mirolli 1967; Yamana et al. 1986) or in German as either "Schläfenorgan" (e.g., Tömösváry 1883; Latzel 1884; Verhoeff 1926–1928; Seifert 1932) or "Schläfengrube" (e.g., Vom Rath 1886) or as "foveae lateralis capitis" (e.g., Vom Rath 1886). The function of the Tömösváry organ is still unclear and several competing hypotheses exist, as discussed in detail by Müller and Sombke (2015), such as reception of vibration (e.g., Pflugfelder 1933; Meske 1961), olfaction (e.g., Zograf 1899; Bedini and Mirolli 1967), gravitation (e.g., Krishnan 1968), or humidity (e.g., Bedini and Mirolli 1967).

Tömösváry organs can be present in various shapes, forming a groove, pit or tube (Hennings 1906; Müller and Sombke 2015), and possess a sensory cavity with a thin and porous cuticle formed by a peg-like or hemispherical epidermis (Müller and Sombke 2015). In addition to studies on the physiology and morphology of the Tömösváry organ it is also used as an important taxonomic and phylogenetic character due to the variation in its shape, position or size (e.g., Hennings 1906; Attems 1926; Sierwald and Bond 2007; Blanke and Wesener 2014; Müller and Sombke 2015; Bouzan et al. 2017a, b). This is also true for the Polydesmida, where it has been coded in phylogenetic analyses as present and small (Blanke and Wesener 2014, characters 6 and 7) or as small pit (Sierwald and Bond 2007, character 18).

The order Polydesmida is the most diverse order of the millipedes (Diplopoda), with more than 5000 described species (Brewer et al. 2012) in 30 families (Shelley 2002), which contribute to over a third of the more than 12,000 known millipede species. The flat body of the blind Polydesmida consists of usually 17 or 18 fully fused body-rings (plus one apodous ring and telson) with large paranota (Enghoff et al. 2015), a habitus known as litter-splitter (Golovatch and Kime 2009).

There is uncertainty about the absence or presence of the Tömösváry organ in the Polydesmida. Its presence in Polydesmida was first stated by Attems (1899) and subsequently reported by him repeatedly (e.g., Attems 1926, 1937). Since the first detailed description of the presumable Tömösváry organ in the Polydesmida by Hennings (1906), this peculiar organ has not been re-examined for the order. Attems (1926) questioned its presence in some genera of the Polydesmida (Attems 1926, p. 55), while Verhoeff (1926–1928) depicted the Tömösváry organ for *Coromus thomsonii* (Verhoeff 1926–1928, p. 771, fig. 364), and Snodgrass (1952) described a Tömösváry organ for *Apheloria coriacea*. Seifert (1932), in contrast, stated its absence in the Polydesmida

(Seifert 1932, p. 436), without referring to previous records. Apparently Richard L. Hoffman likewise affirmed absence of the Tömösváry organ in the Polydesmida in an unpublished manuscript (P. E. Marek, pers. comm. December 2019). Although it seems to be mostly accepted among some taxonomic experts that the Tömösváry organ is absent in the Polydesmida (W. A. Shear and P. E. Marek, pers. comm. 12th May 2017) it is still stated as being present in recent cladistic analyses and taxonomic descriptions (e.g., Sierwald and Bond 2007; Blanke and Wesener 2014; Enghoff et al. 2015; Müller and Sombke 2015; Bouzan et al. 2017a, b), usually with reference to Hennings (1906) or Attems (1926).

In this study we aim to clarify whether the Tömösváry organ is present or absent in the Polydesmida, and which structure was originally described by Hennings (1906).

Material and methods

Specimens and data deposition

Four species representing four families (Polydesmidae, Paradoxosomatidae, Oxydesmidae, Gomphodesmidae) and three suborders (Polydesmidea, Strongylosomatidea, Leptodesmidea) were investigated. Specimens of *Polydesmus angustus* (Latzel, 1884) were collected in April 2015 in the Kottenforst (50°41'05.3"N, 07°05'19.4"E, Bonn, Germany) and fixed in Bouin-solution for paraffin-histology and micro-CT scanning, or in Karnovsky fixative (2.5% glutaraldehyde, 3.2% paraformaldehyde in 0.1M salted phosphate buffer, pH 7.2) for semi-thin sections. Furthermore, for investigations with micro-CT only, specimens of *Oxidus gracilis* (Koch, C. L., 1847) (collected in the Botanical Garden of the University of Bonn, Germany in April 2018), *Coromus vittatus* (Cook, 1896) (obtained via the pet trade from Nigeria) and *Tymbodesmus* sp. (obtained via the pet trade from Cameroon) were fixed in Bouin-solution. Micro-CT data and histological images of *Polydesmus angustus* are deposited on Morphobank (O'Leary and Kaufman 2011, 2012) under project number 3582 (http://morphobank.org/permalink/?P3582). The specimens studied by micro-CT are stored in the Zoological Research Museum Alexander Koenig (ZFMK) as vouchers (see Table 1).

Table 1. Taxon sampling and scanning parameters for micro-computed tomography.

	ID	Location	Voltage	Current	Pixel size	Exposure	Rotation steps	Rotation	Averaging
Polydesmus angustus	ZFMK-MYR08922	Kottenforst, Bonn, Germany	40 kV	200 µA	2.6 µm	1659 ms	0.1°	180°	7
Oxidus gracilis	ZFMK-MYR08923	Botanical garden, Bonn, Germany	50 kV	200 µA	1.2 µm	500 ms	0.1°	180°	7
Coromus vittatus	ZFMK-MYR08924	Pet trade, Nigeria	60 kV	166 µA	3.6 µm	500 ms	0.1°	180°	7
***Tymbodesmus* sp.**	ZFMK-MYR08925	Pet trade, Cameroon	43 kV	200 µA	1.8 µm	1800 ms	0.15°	360°	10

Histology

Following Hennings (1906) histological sections were acquired of specimens embedded in paraffin wax. Specimens fixed with Bouin-solution were decalcified in 5% nitric acid for 6 hours before embedding in paraffin (Paraplast, Sigma-Aldrich). Sections with a thickness of 7 µm were obtained with a Leica RM2165 microtome and stained with a trichrome Azan-staining. To obtain semi-thin sections, specimens fixed in Karnovsky fixative for one hour and decalcified in 5% nitric acid were embedded into an Epon-Araldite epoxy resin (Electron Microscopy Science). Semi-thin sections of the specimen's head with a thickness of 1 µm were made with a Diatome Histojumbo Hj4237 diamond knife at a Reichert Ultracut S ultramicrotome (Leica). The semi-thin sections were stained with Toluidine blue. Sections were photographed with a dotSlide Olympus BX51 light microscope and the software dotSlide 2.5 (Olympus Soft Imaging Solutions GmbH). The digital images were aligned in an image stack with the software Imodalign (B. Quast, https://www.q-terra.de/biowelt/3drekon/tools/imodalign/imodalign.htm).

Micro-computed tomography (micro-CT) and visualization

For micro-CT scanning one specimen each fixed in Bouin-solution of *Polydesmus angustus* (Latzel, 1884), *Oxidus gracilis* (Koch, C. L., 1847), *Coromus vittatus* (Cook, 1896) and *Tymbodesmus* sp. were transferred to 96% ethanol via an ascending ethanol series and stained with 3% Iodine solution for 24 hours. The specimens were washed in 100% ethanol and critical point dried using a Leica EM CPD 300. Micro-CT scanning was performed at the ZFMK using a SKYSCAN 1272 (Bruker micro-CT) with random movement = 15 and flat-filed correction and geometric correction switched on. For varying scanning parameters see Table 1. Post-alignment, ring-artefact reduction, beam-hardening correction and reconstruction were performed in NRecon 1.7.1.6 (Bruker microCT). The image stacks were modified using Fiji ImageJ 1.50e (Schindelin et al. 2012). Volume rendering was performed in Drishti Version 2.6.3 (Limaye 2012). Segmentation was done in ITK-SNAP 3.6.0 (Yushkevich et al. 2006). Images were edited in GIMP version 2.10.6 (https://www.gimp.org) and Inkscape 0.92 (www.inkscape.org).

Results

In all studied species the structure described as Tömösváry organ (Fig. 1A, *) in previous studies demarcates the distal roundish expansion of the incisura lateralis (Fig. 1B, C). At this point of the incisura lateralis the transverse bar of the tentorium projects through the head capsule. From the outside the tip of the transverse bar appears oval in shape and is surrounded by a rim (Figs 1C; 2A–C) formed by the cephalic cuticle

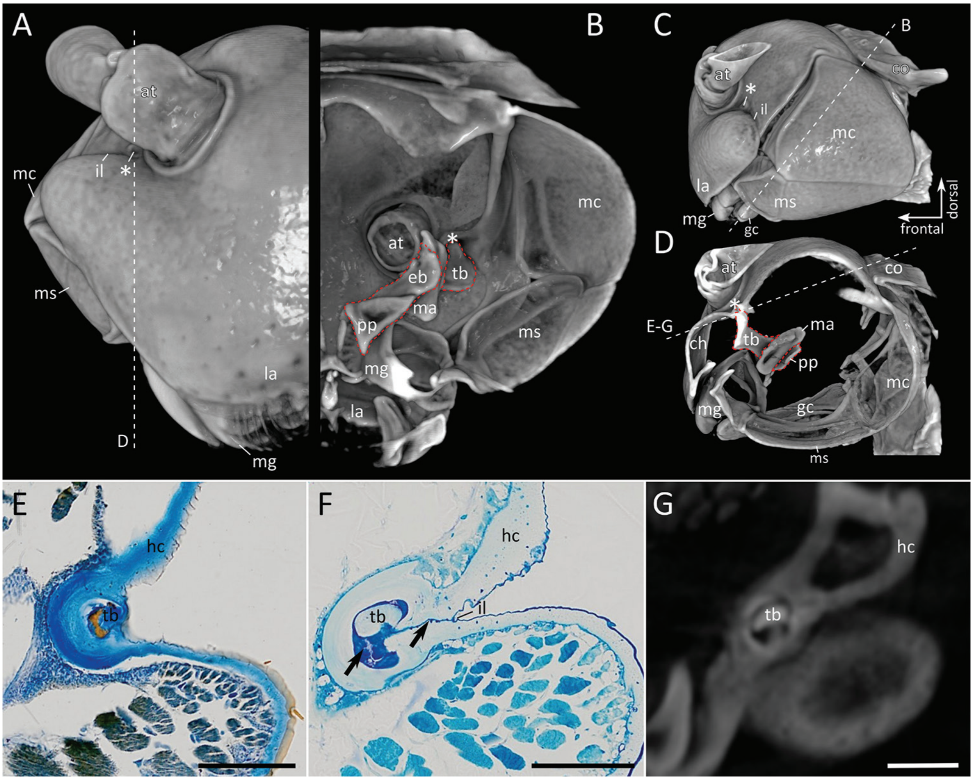

Figure 1. *Polydesmus angustus*, head **A–D** volume rendering based on micro-CT data: **A** Frontal view **B** cross-section, posterior view, plane indicated in C **C** lateral view **D** sagittal view, cutting plane indicated in A **E–G** details of connection of tentorial transverse bar to head capsule at incisura lateralis, plane as indicated in D: **E** histological section (Paraffin, Azan-staining) **F** histological section (Araldite, Toluidine blue) **G** optical section of micro-CT scan. Abbreviations: at = antenna, co = collum, eb = epipharyngeal bar of tentorium, gc = gnathochilarium, gls = gnathal lobe sclerite, hc = head capsule, il = incisura lateralis, la = labrum, mc = mandibular cardo, mg = mandibular gnathal lobe, ms = mandibular stipes, pp = posterior process of tentorium, tb = transverse bar of tentorium. Asterisk (*) indicates structure previously interpreted by Hennings (1906) as the Tömösváry organ in the Polydesmida. In the volume renderings the tentorium is marked with a red dotted line. Scale bar: 100 µm (**E–G**).

(Figs 1D; 2D–F). The cuticle of the head capsule is soft in this region and surrounds the tip of the transverse bar completely (Figs 1E, F; 2G–J). While in *Polydesmus angustus* and *Oxidus gracilis* the transverse bar does not project over the level of the surrounding cuticle (Figs 1A, D; 2A, D), it is more exposed in *Coromus vittatus* and *Tymbodesmus* sp. (Fig. 2B, C, E, F). No structure similar to a Tömösváry organ in other millipedes is associated with the flexible connection of the transverse bar to the head capsule.

The general structure of the tentorium of the studied species is the same as described by Seifert (1932) for *Strongylosoma pallipes*. The transverse bar (tb) extends from the incisura lateralis (Fig. 3A) posteriorly and bends mesially off about 90°, where

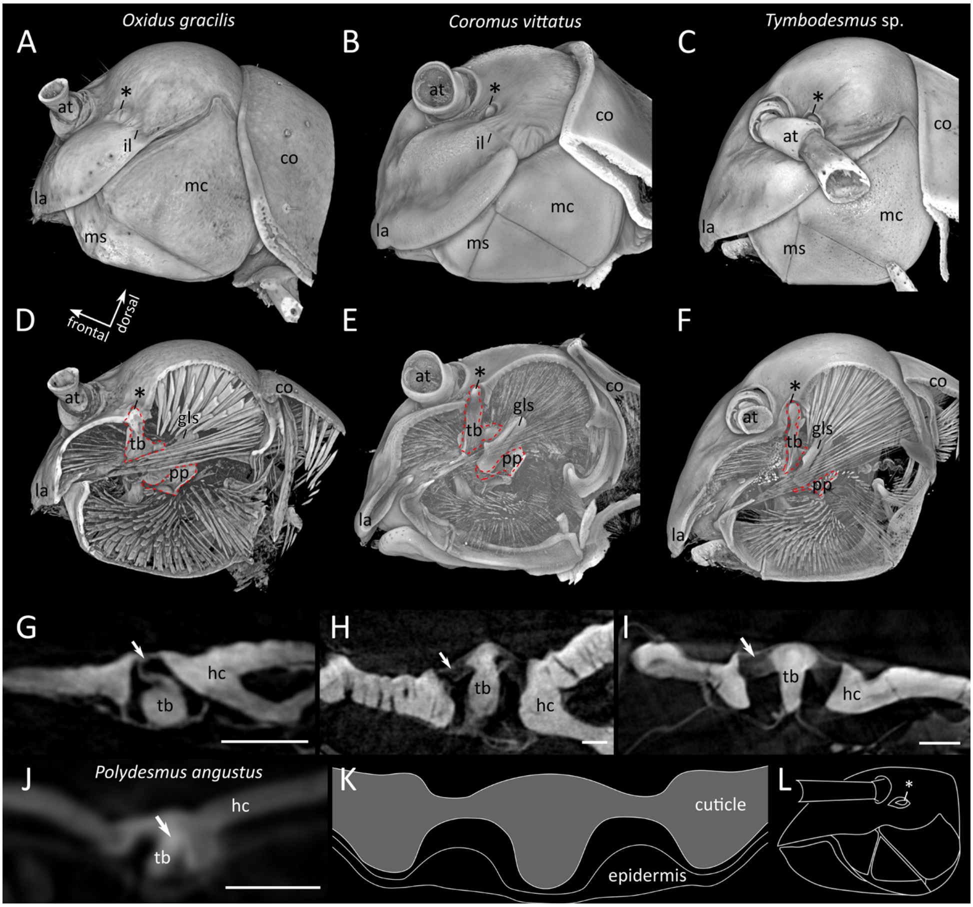

Figure 2. Articulation of the tentorial transverse bar to the head capsule in the Polydesmida **A–C** head in lateral view (anterior is left), volume rendering **D–F** head, sagittal section through tentorial transverse bar, volume rendering **A, D, G** *Oxidus gracilis* **B, E, H** *Coromus vittatus* **C, F, I** *Tymbodesmus* sp. **J** *Polydesmus angustus* **G–J** optical cross sections **K, L** the Tömösváry organ in *Eurydesmus laxus* after Hennings (1906): **K** cross-section, modified from Hennings (1906, plate XXXI, fig. 11) **L** head in lateral view, modified from Hennings (1906, plate XXXI, fig. 9). Abbreviations: at = antenna, co = collum, gls = gnathal lobe sclerite, hc = head capsule, la = labrum, mc = mandibular cardo, ms = mandibular stipes, tb = transverse bar of tentorium, pp = posterior process of tentorium. Asterisk (*) indicates the structure previously interpreted by Hennings (1906) as Tömösváry organ. In the volume renderings the tentorium is marked with a red dotted line. Arrows indicate the flexible connection of transverse bar and head capsule. Scale bars: 100 µm (**G–J**).

it becomes plate-like. Along its mesal extension towards the preoral chamber the tb serves as insertion for the anterior tentorial muscle (t1) which originates from the head capsule. Antero-laterally the transverse bar passes over into the epipharyngeal bar (eb) (Fig. 3B). On its distal tip the dorsal tentorial muscle (t2) inserts, which originates from the head capsule mesal of t1. Posteriorly the epipharyngeal bar passes

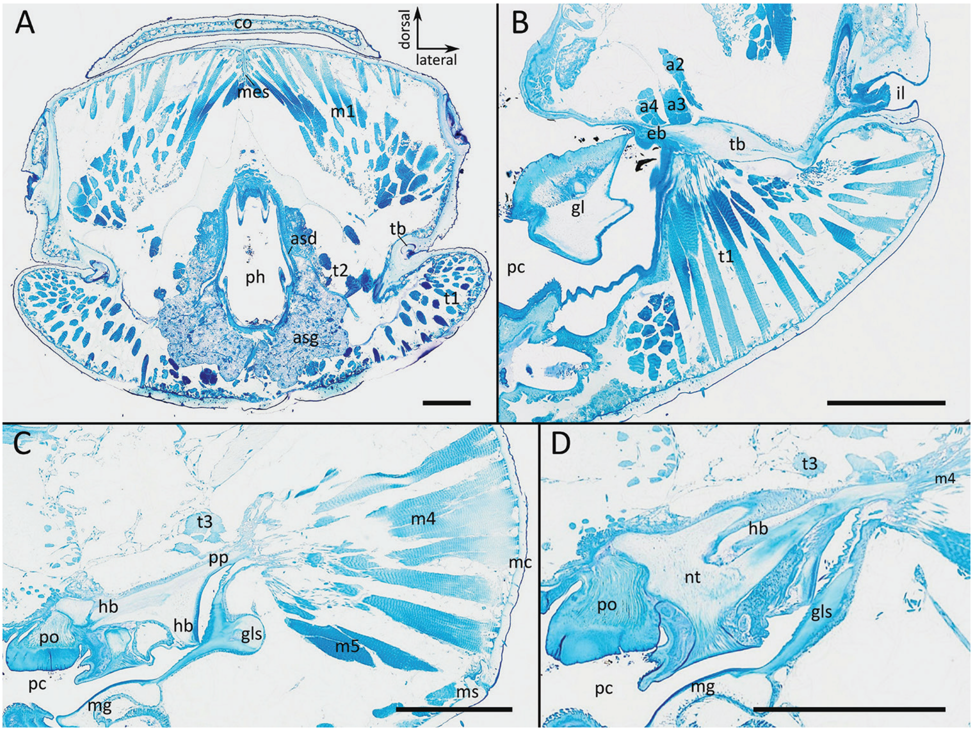

Figure 3. *Polydesmus angustus*, histological sections from anterior (**A**) to posterior (**D**) **A** articulation of tentorial transverse bar to head capsule **B** tentorial transverse bar and epipharyngeal bar **C** tentorial hypopharyngeal bar and posterior process **D** articulation of tentorium to "Nebententorium". Abbreviations: a2 = medial antennal muscle, a3 = lateral antennal muscle, a4 = anterior antennal muscle, asd = duct of anterior salivary gland, asg = anterior salivary gland, co = collum, eb = epipharyngeal bar of tentorium, gl = mandibular gnathal lobe, gls = gnathal lobe sclerite, hb = hypopharyngeal bar of tentorium, m1 = gnathal lobe sclerite muscle, m4 = anterior mandibular cardo muscle, m5 = posterior mandibular cardo muscle, mc = mandibular cardo, mes = median septum, mg = mandibular gnathal lobe, ms = mandibular stipes, nt = 'Nebententorium', pc = preoral chamber, ph = pharynx, po = 'Presshöcker', t1 = anterior tentorial muscle, t2 = dorsal tentorial muscle, tb = transverse bar of tentorium. Scale bars: 200 µm.

over into the hypopharyngeal bar (hb), which is located within the hypopharyngeal wall and distally articulated to the 'Nebententorium' (Fig. 3C, D). At the point where epipharyngeal bar and hypopharyngeal bar meet, the plate-like posterior process (pp) projects posteriorly into the head capsule. The posterior process serves as origin of three (medial, lateral and anterior) antennal muscles (a2, a3, a4) inserting on the antennal base, and of the tentorial pharyngeal dilator muscle (p5), which inserts laterally on the pharyngeal wall. Furthermore, the mandibular muscles m4/m5 originate from the posterior margin of the tentorial posterior process and insert at the mandibular base.

Attached on the distal margin of the posterior process is the posterior tentorial muscle (t3), which originates from the postoccipital flange, and the ventral tentorial muscle (t4), which originates from the transverse mandibular tendon (see Suppl. material 1: file S1).

Discussion

No Tömösváry organ in Polydesmida

Hennings (1906) described the alleged Tömösváry organ of the polydesmid *Eurydesmus laxus* Gerstaecker, 1873 as a pear-shaped pit covered by a membrane with a median hard swelling (Fig. 2K, L; compare also to Hennings 1906, p. 593). Snodgrass (1952) also described the Tömösváry organ of the Polydesmida as oval groove with a thickened central disc, beneath which sensory cells lie. Such a structure cannot be observed in the studied species. A structure resembling the general appearance of the Tömösváry organ in millipedes, with a sensory cavity lined by a thin porous cuticle and a peg-like epithelium is not present in the Polydesmida. All previous descriptions as well as the accompanying graphical depictions provided by Attems (1899, 1937), Verhoeff (1926–1928) and Snodgrass (1952) for the Polydesmida unambiguously refer to a structure we identified as the projection of the tentorial transverse bar through the head capsule. Snodgrass (1951) stated that the tentorial transverse bar (fulturae sensu Snodgrass 1951) attaches to the central disc of a horseshoe-shaped Tömösváry organ in the Polydesmida, but in the studied species no separate disc-like structure was found. Instead a knob-like distal tip of the tentorial transverse bar is present. Based on our data it is now obvious that the connection of the tentorial transverse bar to the head capsule was misidentified as the Tömösváry organ in Polydesmida. Hennings (1906) stated that the alleged Tömösváry organ only varies in size in the seven species of Polydesmida he studied (*Oranmorpha guerini*, *Orthomorpha coarctata*, *Orthomorpha tenuipes*, *Polydesmus complanatus*, *Spanobrachium collaris*, *Fontaria* sp., *Aphelidesmus uncinatus*), among which is one congener of *Polydesmus angustus* (*P. complanatus*), while being absent in species capable of volvation (i.e., *Lignydesmus rubriceps*, *Oniscodesmus aurantiacus* and *Aporodesmus gabonicus*).We accordingly hypothesize that the Tömösváry organ is generally absent in the Polydesmida.

This conclusion (absence of the Tömösváry organ in the Polydesmida) is further supported by previous doubts on the presence of a nervus tömösváryi in the Polydesmida (Sombke and Rosenberg 2015). The nerve innervating the Tömösváry organ in other myriapods could not be identified either in our histological studies, in contrast to Hennings (1906) who stated its presence, but absence of the nervus opticus. The absence of the alleged Tömösváry organ in *Lignydesmus rubriceps*, *Oniscodesmus aurantiacus* (Hennings 1906) and in *Cyclodesmus* (Attems 1899) can straightforwardly be related to their ability to volvate (Golovatch 2003). In the same context of volvation, the lateral connection of the tentorium to the head capsule via the transverse bar is also lost in Sphaerotheriida (Moritz and Wesener 2017; Moritz et al. 2018).

Phylogenetic significance of the Tömösváry organ

The absence of the Tömösváry organ in the Polydesmida is a character shared with the Colobognatha, Stemmiulida, Juliformia and Siphoniulida among the chilognathan millipedes (Sierwald and Bond 2007; Blanke and Wesener 2014). The phylogenetic analyses based on morphological data by Sierwald et al. (2003) and Blanke and Wesener (2014) suggest a sister-group relationship of Polydesmida and Nematophora. Other phylogenetic analyses resolve the Polydesmida as more closely related to either Juliformia (Enghoff et al. 1993; Cong et al. 2009), Stemmiulida (Rodriguez et al. 2018), or Colobognatha (Sierwald and Bond 2007), all of which do not possess a Tömösváry organ. Although Silvestri (1903) depicts the Tömösváry organ for the stemmiulid *Stemmiulus ortonedae*, its apparent presence likewise requires re-consideration according to Müller and Sombke (2015). The correction of the character coding related to the Tömösváry organ for Polydesmida in the character matrix compiled by Blanke and Wesener (2014) does not alter its topology, in which the Polydesmida is the sister group of the Nematophora. The resolution of the chilognathan orders in this analysis, however, now questions the homology of the Tömösváry organ across millipedes, since its presence in (some) Nematophora optimizes most parsimoniously as a reversal. Based on the available data, the question of whether the Tömösváry organ among chilognathans is exclusively maintained or instead regained in Nematophora remains an issue of debate. Therefore, detailed investigations of the head morphology for all millipede orders are needed combining various techniques including developmental studies.

Conclusion

Contrary to several old and recent publications (e.g., Hennings 1906; Verhoeff 1926–1928; Snodgrass 1951, 1952; Blanke and Wesener 2014; Müller and Sombke 2015; Bouzan et al. 2017a, b) the Polydesmida do not seem to possess a Tömösváry organ. Indeed, the connection of the tentorial transverse bar laterally to the head capsule has been misinterpreted as the Tömösváry organ, as we show here. The absence of the Tömösváry organ in the Polydesmida, Juliformia and Colobognatha may be due to multiple losses, but parsimony favors its sole presence in Nematophora among Chilognatha as a reversal. To further clarify the distribution, homology and evolution of the Tömösváry organ in the Diplopoda more detailed studies are needed.

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Supplementary material 1

***Polydesmus angustus*, head capsule and tentorium with associated musculature**

Authors: Leif Moritz, Markus Koch

Data type: multimedia

Link: https://doi.org/10.3897/zookeys.930.48438.suppl1

Appendix Chapter 7

Thomas Wesener & Leif Moritz (2018) Checklist of the Myriapoda in Cretaceous Burmese amber and a correction of the Myriapoda identified by Zhang (2017). *Check List* 14(6): 1131–1140.

https://doi.org/10.15560/14.6.1131

Check List the journal of biodiversity data

ANNOTATED LIST OF SPECIES

Check List 14 (6): 1131–1140
https://doi.org/10.15560/14.6.1131

PENSOFT

Checklist of the Myriapoda in Cretaceous Burmese amber and a correction of the Myriapoda identified by Zhang (2017)

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Abstract

An inventory of the Myriapoda (Diplopoda, Chilopoda, Symphyla) from Cretaceous Burmese amber, Myanmar, is presented, including the oldest and/or first fossil record for numerous orders. For millipedes (Diplopoda) 527 records, including 460 new specimens determined by us, belonging to 13 of 16 recent orders are listed: Polyxenida, Glomeridesmida, Glomerida, Siphonophorida, Polyzoniida, Platydesmida, Siphoniulida, Chordeumatida, Polydesmida, Stemmiulida, Callipodida, Spirostreptida and Spirobolida. For centipedes, 33 records for 4 of the 5 recent orders are listed: Scutigeromorpha, Lithobiomorpha, Scolopendromorpha and Geophilomorpha. For Symphyla, three records for both families, Scutigerellidae and Scolopendrellidae, are listed. The majority of Diplopoda records (30.5%) are Polydesmida. The record of the Polyzoniida includes first instar octopod juveniles. The checklist includes the first fossil representatives known of the Platydesmida, as well as the oldest known fossils of the Polyxenida, Glomeridesmida, Glomerida, Siphonophorida, Polyzoniida, Siphoniulida, Spirostreptida, as well as both Symphyla families. Misidentifications by Zhang (2017) are corrected; while most Chilopoda in that list are correct, almost all Diplopoda are misidentified.

Key words
Cretaceous; Myanmar; Fossil; Diplopoda; Chilopoda; Symphyla.

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Introduction

Burmese amber from the Hukawng Valley in northern Myanmar dates back to the Albian-Cenomanian boundary (Cretaceous), ca 99 mya (Shi et al. 2012). For a review on the history and geology of Burmese amber see Zherikhin and Ross (2000), Grimaldi et al. (2002), and Cruickshank and Ko (2003). Since the 1990s Burmese amber has yielded many important fossils. Thus many spectacular arthropod fossils were described, such as the extinct insect order Alienoptera (Bai et al. 2016). A checklist of arthropod taxa recorded from Burmese amber was compiled by Ross et al. (2010) and is regularly updated online (Ross 2018), currently comprising 1013 species, of which 941 species belong to the Arthropoda. Myriapoda are mainly soil animals (David 2015) and contain 4 classes: Diplopoda (millipedes), Chilopoda (centipedes), Pauropoda and Symphyla, altogether containing ca 18,000 described extant species (Bonato and Zapparoli 2011, Scheller 2011, Szucsich and Scheller 2011, Enghoff et al. 2015). The Myriapoda have an old (> 400 my) but also very fragmentary fossil record (see Shear and Edgecombe 2010, Edgecombe 2015), including the oldest known terrestrial animal (Wilson and Anderson 2004). From

Burmese amber Ross (2018) listed 21 records of Myriapoda, of which 5 species were described: 3 Diplopoda (Cockerell 1917, Liu et al. 2017a), 1 Chilopoda (Bonato et al. 2014), and 1 Symphyla (Moritz and Wesener 2018). Burmese amber yielded the oldest fossil of the class Symphyla, the first known fossil representative of the family Scolopendrellidae (Moritz and Wesener 2018), and the first known fossils of the millipede order Siphoniulida (Liu et al. 2017a).

The opportunity to study several hundreds of Myriapoda inclusion from 4 private collections in Germany resulted in the discovery of numerous new records, which are presented here. These new records include the oldest known fossils of the millipede orders Glomeridesmida, Glomerida, both Siphonophorida families, Polyzoniida, Platydesmida, Stemmiulida and Spirostreptida. Unfortunately, the vast majority of specimens are in the hands of private collectors, and it can only be hoped that they will be made accessible to the general scientific community in the near future.

Methods

Abbreviations:

NHML	Natural History Museum of London, UK
NMS	National Museums Scotland, Edinburgh, UK
In.	Prefixed registration number at the NHML (Rasnitsyn and Ross 2000)
AMNH	American Museum of Natural History, New York, USA
BuB	Private collection of Patrick Müller, Käshofen, Germany
Wu	Private collection of Jörg Wunderlich, Hirschberg, Germany
CG	Private collection of Carsten Gröhn, Glinde, Germany
RO	Private collection of Rainer Ohlhoff, Saarbrücken, Germany
ZFMK	Zoological Research Museum Alexander Koenig, Bonn, Germany

Data from two Museum collections (NHML, AMNH) are compiled from the literature, while specimens deposited in the ZFMK were observed. Literature references without any picture or source of the amber or specimens are not included in this list (e.g. specimens mentioned in conference abstracts, or online lists without any voucher information). In addition specimens, often fragmented, that cannot be securely determined to order level, are not included. Specimens belonging to 4 private collections, containing the majority of the records, were examined and determined to order, in some cases also family level. These examined collections include a total of 480 specimens.

All examined specimens are from the Noije Bum amber mine in the Hukawng Valley, Kachin State, Northern Myanmar (26°15′ N, 096°34′ E). All necessary permits are present and available upon request.

Determinations and classifications follow the characters given in the recent literature (Bonato and Zapparoli 2011, Szucsich and Scheller 2011, Blanke and Wesener 2014, Enghoff et al. 2015). In our list numbers in parentheses refer to the total number of specimens per taxonomic group; M = male, F = female, ? = sex unknown.

As many Diplopoda families can only be securely determined by studying the male copulatory legs (gonopods or telopods), which is beyond the scope of this list, such determinations were not undertaken in the Glomerida, Polydesmida, Callipodida, Stemmiulida, Spirostreptida and Spirobolida, even if the outer appearance of the fossils might be similar to extant species, because convergent evolution cannot be ruled out, and extinct representatives could resemble morphotypes of extant but different families.

Microphotographs were obtained using a Canon EOS 7D camera equipped with a magnifier lens. Image stacking was performed in Zerene Systems Stacker (Version 1.04).

Results

Class DIPLOPODA deBlainville in Gervais, 1844

Order Polyxenida Verhoeff, 1934

Family undetermined (5)

New records. 1 ?, BuB2658; 1 ?, BuB2659; 1 ?, BuB2984; 1 ?, BuB3028; 1 ?, RO my295.

Identification. Members of the order Polyxenida are among the easiest Diplopoda to identify. They are the only soft-bodied millipedes whose body is covered by tufts of hair and which carry caudal bundles of trichomes (Enghoff et al. 2015).

Family Polyxenidae Lucas, 1840 (8)

New records. 1 ?, BuB634; 1 ?, BuB2612; 1 ?, BuB2961; 1 ?, BuB2966; 1 ?, Wu F3358/Bu/CJW; 1 ?, Wu F3384/Bu/CJW; 1 ?, Wu F3389/Bu/CJW; 1 ?, Wu F3394/Bu/CJW.

Identification. These 8 specimens were thankfully determined to the family level by the taxonomic expert of the group, Megan Short, based on photographs (see Acknowledgements). The diagnostic characters include: presence of 10 tergites, 13 pairs of legs, as well as a special arrangement of hairs.

Family Synxenidae Silvestri, 1923 (68)

Literature records. 3 ?, AMNH (Grimaldi et al. 2002); NHML: 4 ?, In.19102-3; 6 ?, In.19104-6; 2 ? In.19177-22; 24 ?, In.19123; 5 ?, In.20149; 18 In.20150; 1 ?, In.20169 (Rasnitsyn and Ross 2000); 1 ?, (Zhang 2017: 146); 1 ?, NMS G.2010.41.41 (Ross and Sheridan 2013).

Phryssonotus burmiticus (Cockerell, 1917)

Cockerell 1917, Rasnitsyn and Golovatch 2004, Zhang 2017.

New records. 1 ?, Wu F3388/Bu/CJW; 1 ?, RO my107; 1 ?, RO my191.

Order Glomeridesmida Latzel, 1884

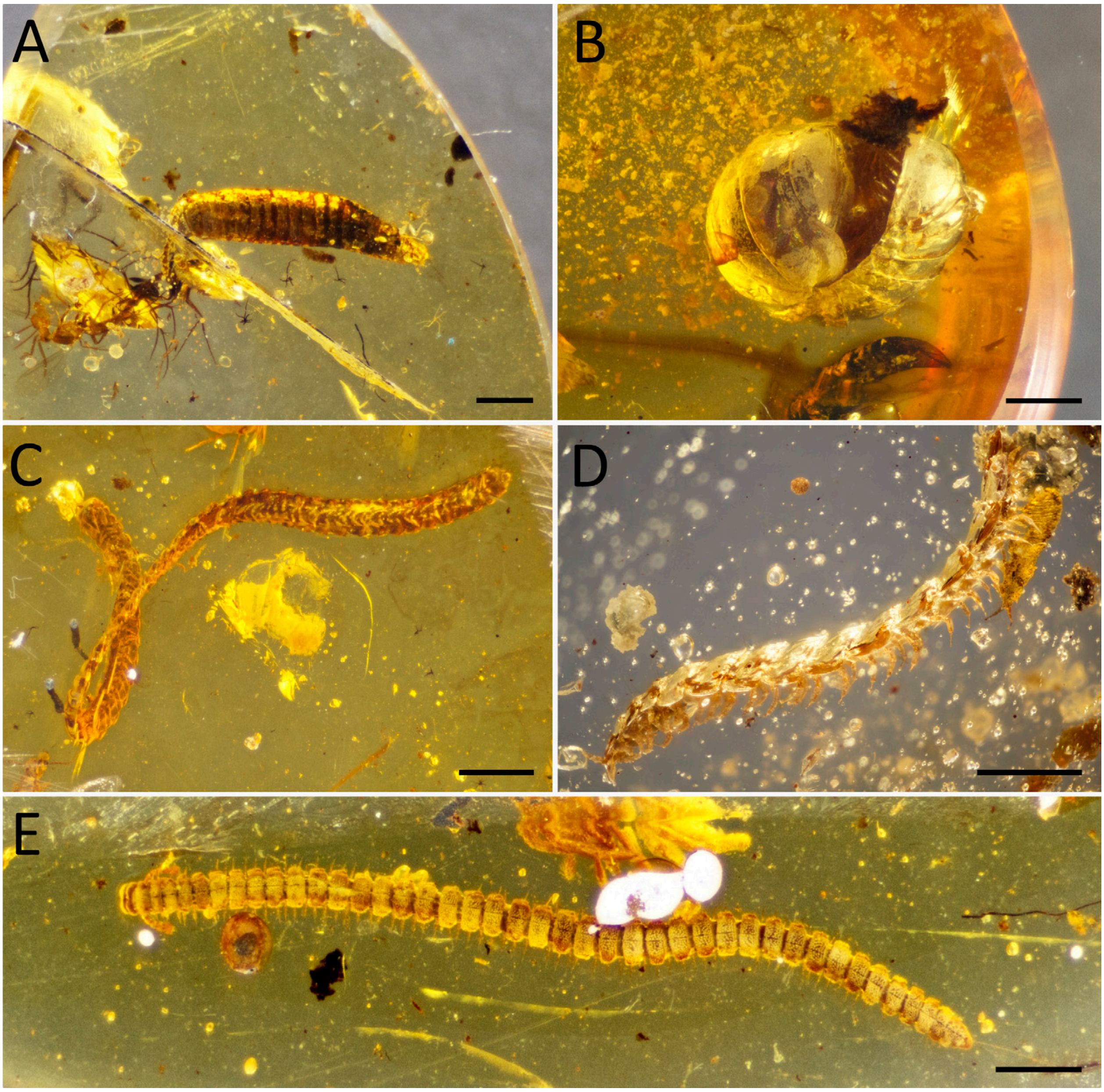

Figure 1. Pentazonia and Colobognatha in Burmese amber, microphotographs. **A.** Glomeridesmida (BuB2413), dorsal view. **B.** Glomerida (BuB2603) lateral view. **C.** Siphonophorida (BuB823), ventral view. **D.** Polyzoniida (BuB979) lateral view. **E.** Platydesmida (BuB2670), dorsal view. Scale bars = 1 mm.

Family Glomeridesmidae Latzel, 1884 (3)

New records. 1 M, ZFMK MYR06117; 1 M, BuB2423; 1 ?, BuB3285.

Figure 1A

Identification. Members of the Glomeridesmida, family Glomeridesmidae can be easily identified by the following head characteristics: the absence of ommatidia, a large Tömösváry organ which is circular. Furthermore, their body consists of 19 tergites plus anal shield (Enghoff et al. 2015). The determination can be further confirmed by the presence of well-developed and visible telopods as 2 of the specimens are males. The specimens also fit in all characters to the recent members of the family Glomeridesmidae; the only other family of the Glomeridesmida, Termitodesmidae, is morphologically strongly derived (Enghoff et al. 2015).

Order Glomerida Leach, 1814

Family undetermined (37)

New records. 1 M, CG-My7276; 1 ?, CG-BURMA11119; 1 ?, CG-BURMA11127; 3 ?, BuB992; 3 ?, BuB1821; 1 ?, BuB2438; 1 ?, BuB2603; 1 ?, BuB2604; 1 ?, BuB2703; 3 ?, BuB2704; 1 ?, BuB2705; 1 ?, BuB2706; 1 ?, BuB2707; 1 ?, BuB2718; 1 ?, BuB2957; 1 ?, BuB2990; 1 ?, BuB2995; 1 ?, BuB2996; 1 ?, BuB3013; 1 ?, BuB 3014; 1 ?, BuB3015; 1 ?, BuB3016; 1 ?, BuB3053; 1 ?, BuB3058; 1 F, BuB3257; 1 ?, BuB3259; 1 ?, ZFMK MYR06116; 1 ?, ZFMK MYR07365; 1 ?, ZFMK MYR07371; 1 ?, ZFMK MYR07372; 1 ?, ZFMK MYR07376.

Figure 1B

Identification. All these pill millipede specimens were determined as members of the Glomerida based on the characteristic head characters of the order. Glomerida can be distinguished from the Sphaerotheriida by the shape of the Tömösváry organ, the insertion point of the antenna and many other characteristics (Oeyen and Wesener 2018). The family classification of the Glomerida is based on the telopods, which currently prevents any more detailed determinations.

Order Siphonophorida Newport, 1844

Family undetermined (25)

New records. 1 F, BuB823; 1 M, BuB977; 1 ?, BuB982; 1 M, BuB905; 1 F, BuB1835; 1 F, BuB1951; 1 M, BuB1959; 1 ?; Bub1966b; 1 ?, BuB1970; 1 F, BuB1971; 1 F, BuB1977; 1 F, BuB1978; 1 F, BuB1980, 1 M, BuB1981; 1 M, BuB1991; 1 ?, BuB2605; 1 ?, BuB2973; 1 F, BuB3019; 1 M, BuB3035; 1 M, BuB3036; 1 ?, BuB3037; 1 ?, BuB343; 1 F, BuB3045; 1 F, BuB3054; 1 ?, BuB3057.

Figure 1C

Identification. Members of the order Siphonophorida can be easily identified based on the beak-like projection of the head, the absence of eyes, as well as the absence of a dorsal suture (Enghoff et al. 2015). The trunk is usually very slender with a length/width ratio of 1/10 or more.

Family Siphonophoridae Newport, 1844 (35)

Literature record. 4 ?, AMNH (Grimaldi et al. 2002).

New records. 1 F, RO my130; 1 ?, RO my330; 1 F, BuB1030; 1 F, BuB2243; 1 F (broken), BuB644; 1 ? (broken), BuB73; 1 ?, BuB828; 1 F, BuB978; 1 F, BuB981; 1 ?, BuB984; 1 ?, BuB986; 1 F, BuB1143; 1 F, BuB1159; 1 F, BuB2963; 1 F, BuB2973; 1 ?, BuB2986;1 M, BuB2989; 1 ?, BuB2997; 1 M, BuB3006; 1 M, BuB3007; 1 ?, BuB3010; 1 M, BuB3034; 1 ?, BuB3047; 1 F, BuB3052;1 F, BuB3239; 1 ?, BuB3245; 2 F, BuB3261; 1 F, BuB3262; 1 ?, Wu F3149/Bu/CJW; 1 ?, Wu F3393/Bu/CJW.

Identification. Members of the family Siphonophoridae can be easily identified based on the head characteristics (Enghoff et al. 2015): the antennae is straight, with large sensory pits on the antennomeres 5 and 6. The antennomere 2 is as long as the others.

Family Siphonorhinidae Cook, 1895 (12)

New records. 1 M, BuB1086; 1 M, BuB997; 1 F, BuB1123; 1 F, BuB1150; 1 F, BuB1822; 1 F, BuB1838; 1 F, BuB1842; 1 F, BuB1845; 1 ?, BuB1851; 1 ?, BuB2979; 1 F, BuB3243; 1 ?, BuB3283.

Identification. Members of the order Siphonorhinidae can be easily identified based on the head characteristics (Enghoff et al. 2015): the antennae is elbowed, the antennomere 2 is twice as long as antennomere 3, lacking any large sensory pits.

Order Polyzoniida Gervais, 1844

Family undetermined (35)

New records. 1 F, BuB112; 1 F, BuB219; 1 F, BuB913; 8 F, BuB914; 1 F, BuB919; 1 ?, BuB1031-B; 1 ?, BuB1994; 1 ?, BuB1996; 1 F, BuB2964; 1 ?, BuB2965; 1 F, BuB2980; 1 ?, BuB3017; 1 ?, BuB3033; 1 ?, BuB3044; 3 ?, Wu F3167/BU/CJW; 1 ?, Wu F3172/BU/CJW; 1 ?, Wu F3201/Bu/CJW; 1 ?, Wu F3202/Bu/CJW; 1 ?, Wu F3107/Bu/CJW; 1 F, Wu F3390/Bu/CJW; 1 ?, Wu F3395/Bu/CJW; 1 ?, Wu F3400/Bu/CJW; 1 F, Wu F3401/Bu/CJW; 1 F, RO my153; 1 F, RO my199.

Identification. Members of the order Polyzoniida are flat, wide, with a width/length ratio of 1/10 to 1/3. The head is characteristically elongated into a well-rounded snout, with 2+2 to 4+4 ommatidia. The tergites lack a dorsal suture (Enghoff et al. 2015).

Family Siphonotidae Cook, 1895 (67)

New records. 1 ?, BuB612; 1 F, BuB825; 1 F, BuB837; 1 F, BuB817; 1 F, BuB824; 1 F, BuB831; 1 F, BuB826;; 1 F, BuB836; 1 F, BuB840; 1 F, BuB925; 1 F, BuB979; 1 F, BuB1130; 1 F, BuB1034; 1 F, BuB1087;1 F, BuB1161; 3 ?, BuB1162; 1 F, BuB1163; 1 F, BuB1164; 1 F, BuB1166; 1 F, BuB1167; 1 F, BuB1834; 2 F, BuB1837; 1 F, BuB1853; 1 F, BuB1854; 1 F, BuB1855; 2 F, BuB1856; 1 F, BuB1956; 1 F, BuB1966; 1 F, BuB1972; 1 F, BuB1976; 1 F, BuB1983; 2 ?, BuB1984; 1 F, BuB1993; 1 F, BuB2607; 1 F, BuB2608; 1 F, BuB2609; 1 F, BuB2610; 1 F, BuB2611; 1 ?, BuB2615; 1 ?, BuB2656; 1 ?, BuB2657; 1 F, BuB3266; 1 F, BuB3268; 1 F, BUB3270; 2 F, BuB3272; 1 F; BuB3273; 1 juv., BuB3280; 5 F, BuB3281; 5 F, BuB3284; 1 F, ZFMK MYR06122; 1 F, ZFMK MYR06124; 1 F, ZFMK MYR07374; 1 F, ZFMK MYR07381.

Figure 1D

Identification. Members of the Siphonotidae have the following characters (Enghoff et al. 2015): the posterior tergal margins is "normal", not upturned. The telson is surrounding the anal valves in ventral view. The head is free, not covered by the first tergite. The leg claws carry a paronychium.

Order Platydesmida de Saussure, 1860

Family Andrognathidae Cope, 1869 (8)

New records. 1 M, Wu F3391/Bu/CJW; 1 F, BuB1413; 1 M, BuB2670; 1 M, BuB2991; 1 M, BuB3237; 1 F, BuB3291; 1 M, BuB3307; 1 F, BuB3308.

Figure 1E

Identification. Members of the order Platydesmida are morphologically variable, the head is only weakly elongated, lacking eyes. The tergites and pleurites are fused, but have dorsally a clearly evident suture (Blanke and Wesener 2014).

Order Siphoniulida Pocock, 1894

Family Siphoniulidae Pocock, 1894 (2)

Siphoniulus muelleri Liu, Rühr & Wesener, 2017

Literature record. 1 F, ZFMK MYR6098 (Liu et al. 2017a).

Siphoniulus preciosus Liu, Rühr & Wesener, 2017

Literature record. 1 F, ZFMK MYR5543 (Liu et al. 2017 a).

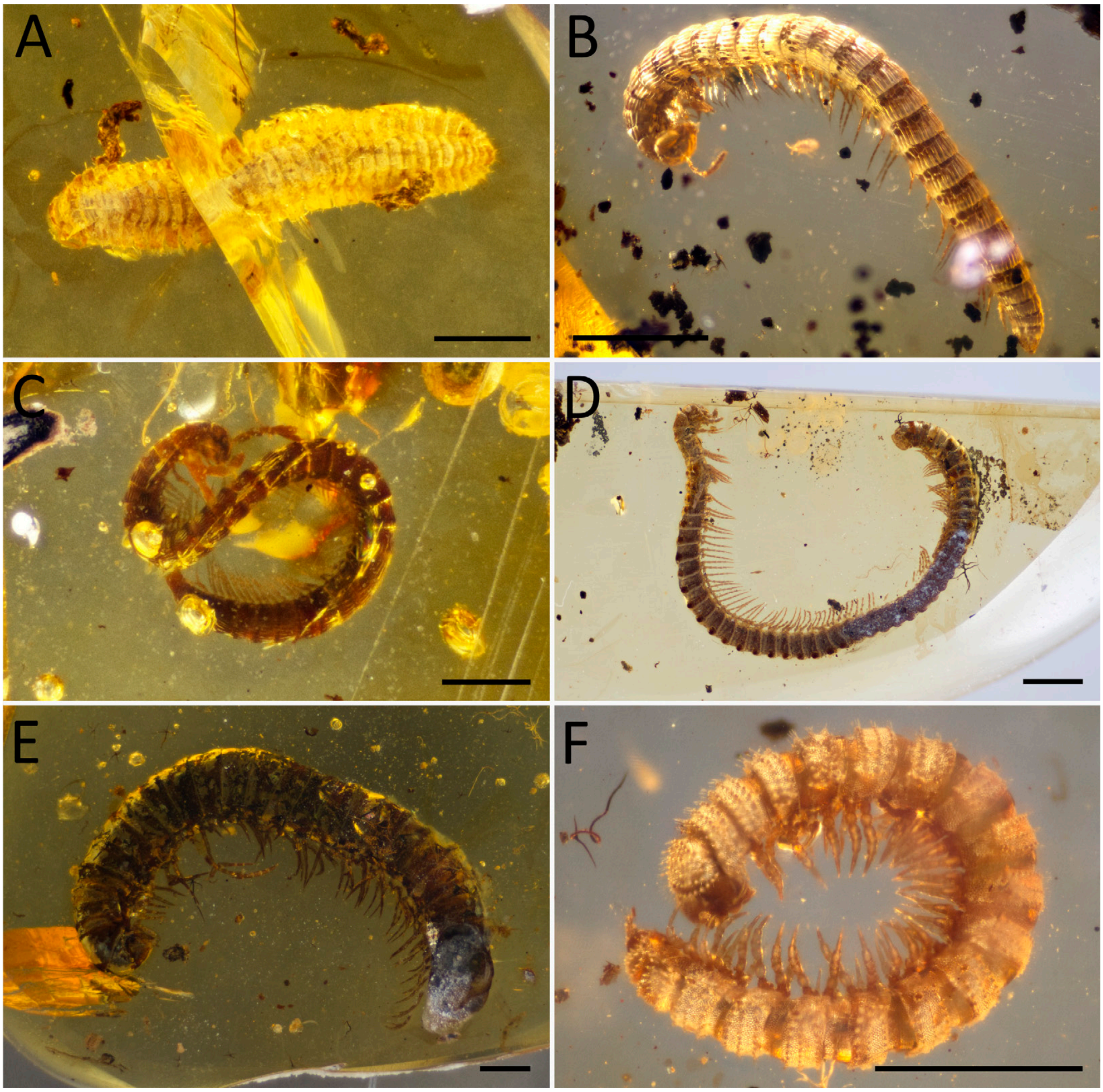

Figure 2. Nematophora, Juliformia and Polydesmida in Burmese amber. **A.** Chordeumatida: Heterochordeumatidae (BuB642), dorsal view. **B.** Stemmiulida (BuB907), lateral view. **C.** Callipodida (BuB1976-B), lateral view. **D.** Spirostreptida: Cambalidea (BuB1151), lateral view. **E.** Spirobolida (BuB1145), lateral view. **F.** Polydesmida (BuB1031-A), lateral view. Scale bars = 1 mm.

Order Chordeumatida Pocock, 1894

Family undetermined (3)

New records. 1 ?, BuB0974; 1 ?, BuB1978; 1 M, BuB2978.

Identification. Chordeumatida can be identified based on the presence of 25–31 tergites with a dorsal suture, an anal segment carrying 4 spinnerets, and tergites with 3+3 characteristic macrosetae (Enghoff et al. 2015). The order has many recent families, which in most cases can only be identified based on a careful examinations of the male copulatory legs.

Family Heterochordeumatidae Pocock, 1894 (20)

New records. 1 F,Wu F2806/Bu/CJW; 1 F, BuB0642; 2 M, BuB0833; 1 F, BuB0899; 1 M, BuB1141; 1 F, BuB1410; 1 F, BuB1411; 1F, BuB1412; 1 M, BuB1823; 1 ?, BuB1827; 1 M, BuB2685; 1 F, BuB3022; 1 ?, BuB3030; 1 F, BuB3051; 1 ?, BuB3056; 1 M, ZFMK MYR05545; 1 M, ZFMK MYR06123; 1 M, ZFMK MYR06624; 1 M, ZFMK MYR07367.

Figure 2A

Identification. Species of the family Heterochordeumatidae are among the most unusual Chordeumatida, with wide paranota resembling some species of Platydesmida (which also share the dorsal suture) and Polydesmida. The collum partly conceals the head.

Order Stemmiulida Pocock, 1894

Family undetermined (8)

New records. 1 M, BuB994; 1 F, BuB1961; 1 ?, BuB1968; 1 ?, BuB2998; 1 ?, BuB3009; 1 F, BuB3038; 1 ?, BuB3241; 1 M, ZFMK MYR07378.

Figure 2B

Identification. Members of the Stemmiulida have a very characteristic appearance. The body is cylindrical, carrying spinnerets at the telson. The head has laterally 1–3 large ommatidia. The order has only 1 recent family (Enghoff et al. 2015), but we hesitate to place these fossils in the recent family without a more detailed morphological investigation.

Order Callipodida Pocock, 1894

Family undetermined (1)

New record. 1 F, ZFMK MYR07366.

Figure 2C

Identification. Members of the order Callipodida have a characteristic habitus, the head has numerous ommatidia, the segments are cylindrical, with free sternites, lacking a dorsal suture. The telson is carrying spinnerets. The surface is often with characteristic crests (like in numerous Spirostreptida and Cambalidea, which, however, lack free sternites and spinnerets). The telson has divided anal valves (paraprocts), a unique character (Enghoff et al. 2015). The female vulva often extended into an elongated ovipositor, which is sometimes heavily modified resembling a clasping organ in recent species. The extended ovipositor is clearly visible in our single female specimen.

Order Spirostreptida Brandt, 1833

Suborder Cambalidea Cook, 1895

Family undetermined (19)

New records. 1 ?, BuB1115; 1 ?, BuB1144; 1 F, BuB1165; 1 ?, BuB1824; 1 F, BuB1825; 1 F, BuB1826; 1 F, BuB1990; 1 F, BuB1955; 1 M, BuB1962; 1 ?, BuB2981; 1 ?, BuB3005; 1 ?. BuB3012; 1 ?, BuB3250; 1 M, BuB3255; 1 M, ZFMK MYR06121; 1 M, ZFMK MYR07368; 1 M, ZFMK MYR07369; 2 F, ZFMK MYR07370.

Figure 2D

Identification. The tergites, pleurites and sternites are completely fused into body rings. The head is lacking a median suture. The anterior legs have a characteristic gap between leg 3 and 4, as legs 4 and 5 are on the posterior end of body ring 5. The specimens listed here are a bit unusual as they have frontal setae on their head, a character absent in all recent Spirostreptida. The presence of 2 pairs of gonopods in the males identify those specimens as members of the suborder Cambalidea, as species belonging to the other suborder, Spirostreptidea, only have one pair of gonopods (Enghoff et al. 2015).

Family Cambalidae Bollman, 1893 (1)

New record. 1 M, ZFMK MYR06696.

Identification. This 1 specimen among the numerous samples of Spirostreptida differs from the others in the absence of frontal setae on the head, in being more similar to recent Cambalidea. The specimen has the gonopods visible, showing anterior gonopods with a flagella and allowing us to place this specimen in a recent family, the Cambalidae (Enghoff et al. 2015).

Order Spirobolida Bollman, 1893

Family undetermined (9)

New records. 1 F, BuB830; 1 M (head missing), BuB916; 1 M, BuB1795; 1 F, BuB1840; 1 ?, BuB2616; 1 ?, BuB3020; 1 M, BuB3000; 1 F, BuB3260; 1 M, ZFMK MYR7373.

Figure 2E

Identification. The tergites, pleurites and sternites are completely fused into body rings. The head has a median suture, a character allowing for these specimens to be distinguished from the 2 other recent orders with complete body rings: Julida and Spirostreptida. A detailed analysis of the male gonopods (Enghoff et al. 2015) is necessary to determine those specimens even to suborder level (e.g. the absence or presence of a tiny sternite connecting the posterior telopods, which are hidden within the anterior telopods), which is beyond the scope of this checklist.

Order Polydesmida Leach, 1815

Family undetermined (161)

New records. 1 ?, BuB600; 1 ?, BuB672; 1 ?, BuB818; 6 ?, BuB902; 2 ?, BuB909; 1 M, BuB911; 4 ?, BuB912; 1 ?, BuB915; 1 ?, BuB966; 1 M, BuB975; 1 M, BuB976; 1 M, BuB980; 1 F, BuB983; 1 M, BuB993; 1 ?, BuB995; 1 M, BuB1029; 1 M, BuB1031-A; 1 M, BuB1035; 1 F, BuB1084; 1 F, BuB1085; 1 M, BuB1146; 1 F, BuB1148; 1 M, BuB1149; 2 M, 5 F, BuB1154; 1 F, BuB1155; 1 F, BuB1156; 1 ?, BuB1414; 1 F, BuB1548; 1 F, BuB1794; 2 F, BuB1830; 1 F, BuB1832; 1 F, BuB1836; 1 M, BuB1844; 1 M, BuB1847; 1 M, BuB1848; 1 M, BuB1849; 3 ?, BuB1850; 1 M, BuB1852; 1 ?, BuB1954A; 3 F, BuB1957; 1 ?, BuB1958; 2 F, BuB1964; 1 ?, BuB1967; 1 M, BuB1975; 1 F, BuB1985; 1 ?, BuB1986; 1 F, BuB1987; 1 ?, BuB1989B;1 ?, BuB1992; 1 ?, BuB1993; 1 ?, BuB2436; 1 ?, Bub2437; 1 ?, BuB2613; 1 ?, BuB2622; 1 ?, BuB2624; 1 ?, BuB2631; 2 F, BuB2632; 1 ?, BuB2639; 1 ?, BuB2640; 1 ?, BuB2645; 1 ?, BuB2646; 1 F, BuB2647; 1 F, BuB2648; 1 ?, BuB2653; 1 F, BuB2672; 1 F, BuB2683; 1 ?, BuB2684; 1 ?, BuB2686; 1 F, BuB2687; 1 F, BuB2688; 1 ?, BuB2960; 1 ?, BuB2967; 1 ?, BuB2968; 1 F, BuB2969; 1 ?, BuB2970; 2 ?, BuB2972; 1 M, BuB2976; 1 F, BuB2982; 2 F, BuB2983; 1 ?, BuB2987; 1 F, BuB2988; 1 M, BuB2992; 1 M, BuB2994; 1 ?, BuB2999; 1 F, BuB3001; 1 F, BuB3002; 1 M, BuB3003; 1 F, BuB3004; 1 M, BuB3008; 1 ?, BuB3011; 1 ?, BuB3021; 1 ?, BuB3023; 1 F, BuB3025; 1 ? BuB3029; 1 ?, BuB3032; 1 M, BuB3034; 1 M, BuB3039; 1 F, BuB3040; 1 ?, BuB3028; 1 M, BuB3049; 1 M, BuB3055; 1 M, BuB3238; 1 F, BuB3246; 1 F, BuB3251; 1 F, BuB3252; 1 F, BuB3253; 1 F, BuB3254; 1 M, BuB3256; 1 ?, BuB3265; 2 F, BuB3267; 1 ?, BuB3269; 1 ? (posterior half), BuB3270; 1 ?, BuB3274; 2 ?; BuB3275; 1 ?, BuB3276; 1 F, BuB3277; 1 ?, BuB3278; 1 ?, BuB3279; 1 ?, BuB3285; 1 F, BuB3286; 1 M, BuB3293; 1 M, ZFMK MYR06118; 1 F, ZFMK MYR06120; 1 F, ZFMK MYR07374; 1 M, 1 ?, ZFMK MYR07377; 1 ?, ZFMK MYR07375; 1 M, ZFMK MYR07379; 1 F, Wu F2817/Bu/CJW; 1 ?, Wu F3385/Bu/CJW; 1 ?, Wu F3396/Bu/CJW; 1 ?, Wu F3397/Bu/CJW;

1 F, RO my249; 1 F, RO my301; 1 F, RO my304.

Figure 2F

Identification. These millipedes are flat-backed; the tergites, pleurites and sternites are completely fused into body rings, triangular shaped with extended side wings (paranota). The head is lacking eyes, the tergites are lacking a dorsal suture. The body consists of 18 or 19 rings, the telson carries more or less evident spinnerets (Enghoff et al. 2015). Suborders and families are only determinable after a careful analysis of the male gonopods, which is beyond the scope of this study.

Class CHILOPODA Latreille, 1817

Order Scutigeromorpha Leach, 1814

Family undetermined (7)

Scutigeromorpha spp.

Literature records. 2 ? (Zhang 2017).

New records. 1 ?, BuB63; 1 ?, BuB120; 1 ?, BuB625; 1 ? RO my111; 1 ?, RO my168.

Identification. With 15 legs, all of them long. The stigma openings are located dorsally, and the large eyes consist of numerous ommatidia (Bonato and Zapparoli 2011).

Order Lithobiomorpha Newport, 1844

Family undetermined (2)

New records. 1 ?, RO my340; 1 ?, BuB3289.

Identification. With 15 legs The stigma openings are located laterally, and the eyes consist of few ommatidia (Bonato and Zapparoli 2011).

Order Scolopendromorpha Leach, 1814

Family Cryptopidae Kohlrausch, 1881 (1)

Cryptopidae sp.

Literature record. 1 ? (Zhang 2017).

Family Scolopendridae Leach, 1814 (1)

Scolopendridae sp.

Literature record. 1 ? (Zhang 2017).

Family undetermined (7)

Literature record. 1 ?, NMS G.2010.41.40 (Ross et al. 2010).

New records. 1 ? BuB834; 1 ?, BuB2661; 1 ?, BuB3064; 1 ?, BuB3240; 1 ?, Wu F3395/Bu/CJW; 1 ?, RO my111.

Identification. With 21/23 legs. The stigma openings are located at the pleura, eyes absent or are constisiting of few ommatidia (Bonato and Zapparoli 2011).

Order Geophilomorpha Leach, 1815

Family undetermined (9)

New records. 1 ?, BuB1997; 1 ?, BuB2660; 1 ?, BuB3063; 1 ?, BuB3065; 1 ?, BuB3287; 1 ?, BuB3288; 1 ?, BuB3290; 1 ?, Wu F3402/Bu/CJW; 1 ?, RO my35.

Identification. Legs 30 to more than 100. The stigma openings are pleural, the animals are blind (Bonato and Zapparoli 2011).

Family Geophilidae Leach, 1815 (3)

***Kachinophilus pereirai* Bonato et al., 2014**

Literature records. 1 M, AMNH Bu-Ba41a; 1 ?, AMNH Bu-Ba50a; 1 ?, AMNH Bu-Ba63a.

Class SYMPHYLA Ryder, 1880

Family Scolopendrellidae Bagnall, 1913 (1)

***Symphylella patrickmuelleri* Moritz & Wesener, 2017**

Literature record. 1 ?, ZFMK MYR6269 (Moritz and Wesener 2018).

Family Scutigerellidae Bagnall, 1913 (2)

New records. 1 ?, BuB3292; 1 ?, BuBXY.

Identification. The antennae are consisting of more than 20 articles, the scuta of the tergites are well-rounded, the leg bases carry styli (see Moritz and Wesener 2018).

Corrections of misidentifications by Zhang (2017)

Zhang (2017), in his large book about inclusions in amber, listed numerous interesting arthropod specimens, rivaling most public museum collections in the diversity of the presented inclusions. He also listed several myriapod specimens with impressive preservation quality. Unfortunately, many of the records were determined to the level of a Recent genus, and some of these identifications are clearly wrong, which we correct below:

Chilopoda, Lithobiomorpha

“Lithobiomorpha sp.” (Zhang 2017: 132–133) = Chilopoda sp. This is clearly a juvenile that cannot be determined.

Polyxenidae

The mentioned specimens can only be determined to genus level based on detailed head characteristics that cannot be examined from the photographs. Therefore, the figured specimens only show characters of the family, not of any specific genus.

“*Unixenus* sp.“ (Zhang 2017: 144) = Polyxenidae sp. The figure shows a polyxenid. Diagnostic characters of the genus *Unixenus* are not visible.

“*Propolyxenus* sp.“ (Zhang 2017: 144) = Polyxenidae sp. The figure shows a polyxenid. Diagnostic characters of the genus *Propolyxenus* are not visible.

“*Polyxenus* sp.“ (Zhang 2017: 145) = Polyxenidae sp. The figure shows a polyxenid. Diagnostic characters of the genus *Polyxenus* are not visible. In fact, the single tuft of caudal setae is more of an indication of *Unixenus*.

Order Glomerida

The pictured specimens are clearly Polyzoniida.

“*Glomeridella* sp.” (Zhang 2017: 150–151) = Polyzoniida sp. The figured specimen is clearly not a member of *Glomeridella*, a European genus of dwarf pill millipedes (Enghoff et al. 2015, Oeyen and Wesener 2018).

Order Sphaerotheriida

The specimens figured by Zhang (2017) are quite interesting, as there is no fossil record of the order Sphaerotheriida yet (Wesener and VandenSpiegel 2009, Wesener et al. 2010, Wesener 2016) and we also did not recover any specimens in the vast amount of material studied by us.

"Zephroniidae sp." (Zhang 2017: 154–155) = Glomerida. The figures clearly show Glomerida, as visible on the exposed head in 1 of the specimens and the striae at the thoracic shield.

Order Platydesmida

This order also is unknown as fossils. Unfortunately, the alleged specimens figured by Zhang (2017) belong to other orders.

"*Brachycybe* sp. 1" = Siphonophorida. The head morphology and the absence of a dorsal suture clearly identify the pictured specimen as a member of the Siphonophorida and not a Platydesmida (Blanke and Wesener 2014, Enghoff et al. 2015).

"*Brachycybe* sp. 2" = undetermined. This specimen lacks a distinct dorsal suture, and can therefore not be a Platydesmida (Blanke and Wesener 2014, Enghoff et al. 2015). The head is missing, which makes a clear identification impossible, but this specimen otherwise shows characters observed in species of the Siphonophorida.

Order Polyzoniida

"*Bdellozonium* sp." (Zhang 2017: 182–183) = Siphonotidae sp. This specimen was determined to the wrong family. The telson, head and legs clearly show that this is a member of the Siphonotidae, not Polyzoniidae. We are unsure how Zhang determined this specimen to the genus *Bdellozonium*, a recent genus with 2 species in California (Enghoff et al. 2015), which are much larger and can only be determined by a study of the gonopods, which are absent in the pictured specimen.

Order Siphonophorida

Here, 1 of the 5 specimens is determined to the wrong order, while the other 4 are in the wrong family. Nevertheless, Zhang's record confirms our observation that this order is frequently found in Burmese amber.

"Siphonorhinidae sp. 2" (Zhang 2017: 167), "Siphonorhinidae sp. 3" (Zhang 2017: 168), "Siphonorhinidae sp. 4" (Zhang 2017: 168), "Siphonorhinidae sp. 5" (Zhang 2017: 169), all = Siphonophoridae. All these Siphonophorida are members of the family Siphonophoridae, which can be easily distinguished from Siphonorhinidae (also not uncommon in Burmese amber, see above) based on the shape of the head and antennae characteristics (Enghoff et al. 2015). Species of Siphonophoridae are very difficult to determine to the genus or even species because the taxonomy is chaotic (Jeekel 2001, Read and Enghoff 2009). Careful descriptions of the tiny gonopods are a necessity in order to not create more chaos.

Order Chordeumatida

Unfortunately, all alleged specimens are members of the order Polydesmida, not Chordeumatida as is evident from the body-ring number and the absence of ommatidia. Chordeumatida sometimes lack ommatidia, mainly in cave taxa (Liu et al. 2017b). We do not know how Zhang could determine the exclusively female specimens to family, or even genus, as a careful observation of the male gonopods would have been necessary.

Anthroleucosomatidae sp. (Zhang 2017: 176) = Polydesmida.

"*Tingupa* sp. 1" (Zhang 2017: 176) = Polydesmida.

"*Tingupa* sp. 2" (Zhang 2017: 177) = undetermined, possibly Polydesmida or Platydesmida.

Order Spirostreptida

"*Cambala* sp." (Zhang 2017: 172) = Cambalidea. Even most families of the Spirostreptida, and especially those belonging to the suborder Cambalidea, can only be determined after a careful examination of the gonopods (Enghoff et al. 2015). The figured specimen is female, so it cannot be determined below the level of suborder.

Order Polydesmida

"Paradoxosomatinae sp." (Zhang 2017: 179–180) = Polydesmida. Families and subfamilies of Cretaceous Polydesmida can only be determined after a careful observation of the male gonopods. Unfortunately, the gonopods are not visible in the specimen, and therefore, it might be any Polydesmida. Elongated legs are often found in Recent species of the family Paradoxosomatidae, but it is entirely possible that 100 mya other families of Polydesmida had species with elongated legs.

"*Scytonotus* sp." (Zhang 2017: 180) = Polydesmida. *Scytonotus* is a genus of Polydesmida currently endemic to North America. Species can only be assigned to this genus based on gonopod characteristics. Families and subfamilies of Cretaceous Polydesmida can only be determined after a careful observation of the male gonopods. Unfortunately, the gonopods are not visible in the specimen, and therefore, it might be any Polydesmida.

Abundance of Myriapoda in Burmese amber

In the studied Burmese amber, 13 of the 16 extant orders of millipedes are represented. Only the orders Sphaerotheriida, Julida and Siphonocryptida are missing. In the studied collections, the Colobognatha are dominant, although several specimens (15–20) could not be determined to order level and are therefore not included in our list. Among the specimens determined to order, the Polydesmida are dominant with 30.55% of all recorded fossils. The abundances of the remaining orders are as follows: Polyxenida = 15.37%, Glomeridesmida =

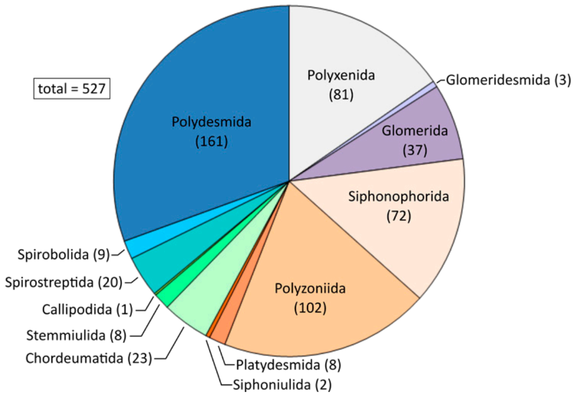

Figure 3. Relative abundance of millipede orders preserved in the studied Cretaceous Burmese amber and reported in the literature. In total 527 millipedes are listed in this publication and the literature. The orders Sphaerotheriida, Julida and Siphonocryptida are not known from Burmese amber.

0.57%, Glomerida = 7.02%, Siphonophorida = 13.66%, Polyzoniida = 19.36%; Platydesmida = 1.52%, Siphoniulida = 0.38%, Chordeumatida = 4.36%, Stemmiulida = 1.52%, Callipodida 0.19%, Spirostreptida = 3.8%, and Spirobolida = 1.71% (Fig. 3). In centipedes, 4 of the 5 extant orders are present in the studied Burmese amber.

Discussion

Present knowledge of the Myriapoda of Myanmar is quite limited. Most records are more than 120 years old and a Recent species list of the Diplopoda lists only 8 orders: Glomerida, Sphaerotheriida, Siphonophorida, Chordeumatida, Polydesmida, Julida, Spirostreptida, and Spirobolida (Likhitrakarn et al. 2017), of which 6, all but Sphaerotheriida and Julida, are also recorded from Cretaceous amber. Species of 7 orders, Polyxenida, Glomeridesmida, Polyzoniida, Platydesmida, Siphoniulida, Stemmiulida and Callipodida, are present in the Cretaceous fauna, but they have no records yet from Myanmar. However, given the known worldwide distribution of the groups (Shelley and Golovatch 2011), all 7 of those millipede groups can be expected from Myanmar once extensive faunal inventories are conducted.

Burmese amber contains the oldest fossils and/or first fossils for 9 of the 16 orders of Diplopoda: Polyxenida, Glomerida (Fig. 1A), Glomeridesmida (Fig. 1B), Siphonophorida (Fig. 1C), Polyzoniida (Fig. 1D), Platydesmida (Fig. 1E), Chordeumatida (Fig. 2A), Stemmiulida (Fig. 2B) and Spirostreptida (Fig. 2D). At the family level, the oldest/first fossils of 9 representatives are known from Burmese amber: Synxenidae and Polyxenidae (both Polyxenida), Glomeridesmidae, Siphonophoridae, Siphonorhinidae, Siphonotidae, Siphoniulidae, Heterochordeumatidae and Cambalidae. The minimum age of these order- and family-level taxa is therefore 99 mya. As shown by Shelley and Golovatch (2011), these orders most likely evolved long before the Cretaceous, based on the fossil record and biogeographic data.

For Chilopoda, the 4 orders represented in the fossil records, Scutigeromorpha, Lithobiomorpha, Scolopendromorpha and Geophilomorpha, have a worldwide distribution and several recent species occur in Myanmar. Here, family determinations, which are beyond the scope of this checklist, might provide more input on potential faunal change that occurred since the Cretaceous in Southeast Asia.

The high abundance of Polyzoniida and the presence of polyzoniid juveniles with as few as 4 leg pairs indicates a strong sampling bias towards this group, of which 1 or several species probably lived and reproduced on the amber-producing trees.

With this list the Cretaceous Diplopoda fauna of Myanmar is now better known than the Recent one, which is an indication of the need of more inventories and taxonomic studies on the fauna of this megadiverse country. This first checklist of the millipede fauna preserved in Cretaceous Burmese amber will serve as a starting point for further research. More detailed descriptions of families, genera and species, based on the reconstruction of the gonopods and telopods, will unfortunately take years to accomplish.

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Authors' Contributions

TW and LM sorted and determined the studied specimens. TW wrote the text.

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Appendix Chapter 8

Leif Moritz & Thomas Wesener (2019) The first known fossils of the Platydesmida—an extant American genus in Cretaceous amber from Myanmar (Diplopoda: Platydesmida: Andrognathidae). *Organisms Diversity & Evolution* 19: 423–433.

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ORIGINAL ARTICLE

The first known fossils of the Platydesmida—an extant American genus in Cretaceous amber from Myanmar (Diplopoda: Platydesmida: Andrognathidae)

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Abstract
Millipedes have been inhabiting the earth for more than 400 my and show a great diversity regarding their morphology and ecology. For a better understanding of the timing and pattern of millipede evolution, Burmese amber offers a unique window into the Cretaceous period, ca. 99 Ma. Here, we describe the first known fossil of the colobognathan order Platydesmida, the species *Andrognathus burmiticus* n. sp. based on 15 specimens from Cretaceous Burmese amber. We combine classical light-microscopy and modern micro-computer tomography (μCT) with computer aided 3D-reconstructions. These non-invasive techniques allow us to describe the fossil millipedes as detailed as is general practice for extant species, and to grant the scientific community open access to the deposited "Cybertypes". Based on the combination of unique morphological characters such as surface structures, body type, the unique size and shape of tergite 5, the absence of a hypoproct at the anal segment, and detailed gonopod characteristics, the studied fossils can be placed in the family Andrognathidae and the extant genus *Andrognathus*, which nowadays is restricted to the eastern USA and Mexico with three extant species. Therefore, the minimum age of the genus *Andrognathus* is pushed to the Cenomanian, 99 Ma. It can be assumed that the genus was much more diverse and wider distributed in the past and migrated between Asia and America via one of the once existing land bridges. These unique fossils prove the unusual relictual distribution of *Andrognathus* and can serve as key-fossils for the dating of the diplopod phylogeny.

Keywords Burmese amber · Southeast Asia · Migration · μCT · Virtual paleontology

Abbreviations

BuB#	Burmese amber collection of Patrick Müller (Käshofen Germany)
F#/BU/CJW	Burmese amber collection of Jörg Wunderlich (Hirschberg, Germany)
ZFMK-MYR#	Myriapoda collection of the Zoological Research Museum Alexander Koenig (Bonn, Germany)

Introduction

Among the 16 orders and more than 12,000 described species of the Diplopoda, the four orders belonging to the Colobognatha are unusual in many aspects. The Colobognatha comprise the four extant orders Platydesmida, Siphonocryptida, Siphonophorida, and Polyzoniida and are unique within the class Diplopoda by, among other characters, bearing reduced mouthparts, which are in some groups adapted to a piercing-sucking feeding style (Blanke and Wesener 2014; Enghoff et al. 2015). Because of their small body size, their allegedly unspecific male copulation legs (gonopods, but see Read and Enghoff 2018), and uniform morphology, the Colobognatha are taxonomically largely understudied and in need of further attention. The internal relationship of the Colobognatha still remains unresolved (Blanke and Wesener 2014) and none of the present molecular studies includes all colobognathan orders, usually lacking the Siphonocryptida (e.g., Sierwald and Bond 2007; Rodriguez et al. 2018).

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The order Platydesmida shows fewer derived characters in comparison to the other Colobognatha groups. In platydesmids, the mouthparts are only slightly modified. Platydesmida share with the Siphonocryptida a fusion of the tergites and pleurites, which are free in the Polyzoniida and Siphonophorida (Blanke and Wesener 2014; Enghoff et al. 2015). Before the first phylogenetic analyses were conducted, a sister-group relationship of the Platydesmida to the Spirobolida was suggested (e.g., Hoffman 1982), but all phylogenetic analyses (e.g., Enghoff 1984; Blanke and Wesener 2014) place the Platydesmida firmly within the Colobognatha. The order Platydesmida shows a Holarctic distribution (North and Central America, Southern Europe, the Mediterranean and Caucasus, SE and northern Asia (Shelley and Golovatch 2011)), and is species-poor with 63 described species in 14 genera (Enghoff et al. 2015). Interordinal phylogenetic relationships of the Platydesmida have not been explored yet. The Platydesmida exhibit two major body types: flat-bodied forms with long paranota and long and slender forms with short paranota, appearing like a string of pearls. The latter can be found in the family Andrognathidae, which is characterized by a knob-like extension of the sternites, and to which family the fossils described here belong. The Andrognathidae count 37 extant species in 12 genera, which are known from Europe as well as North and Central America with two genera, *Brachycybe* Wood, 1864 and *Pseudodesmus* Pocock, 1887 also known from Asia (Enghoff et al. 2015). Not a single fossil representative of the order Platydesmida has been described. In general, the fossil record of the millipedes (Diplopoda) is quite poor (for a review see Edgecombe 2015). This is especially true for the Mesozoic, but recently Mesozoic Burmese amber revealed a great diversity of Myriapoda (Liu et al. 2017; Moritz and Wesener 2018; Wesener and Moritz 2018). Burmese amber originates from the Hukawng Valley, Kachin State, Northern Myanmar in Southeast Asia and is dated to the Cretaceous Albian-Cenomanian boundary ca. 99 Ma based on U-Pb dating of zircons (Shi et al. 2012). For a detailed review of the history and geology of Burmese amber deposits, see the pertinent literature (Zherikhin and Ross 2000; Grimaldi et al. 2002; Cruickshank and Ko 2003). The history of the study of inclusions in Burmese amber since 1916 is summarized by Ross (2019). So far, 1192 species, including 1117 species of arthropods, have been described. Most of the species known from Burmese amber have been described in the last decade, 320 species have been described in 2018 alone (Ross 2019). Among the more than 500 millipede inclusions in Burmese amber listed by Wesener and Moritz (2018) and Grimaldi et al. (2002) are the extant Colobognathan orders Platydesmida, Siphonophorida, and Polyzoniida, the latter two are also known from Cenozoic amber deposits (Bachofen-Echt 1942; Shear 1981; Santiago-Blay and Poinar 1992). The Platydesmida are represented by eight fossils known from Burmese amber (Wesener and Moritz 2018).

Here we describe the first known fossils of the Platydesmida. Excessive morphological studies of the well-preserved 15 Platydesmida specimens, including the utilization of micro-CT scanners and digital 3D reconstructions, allow us an exact systematic placement of the fossils based on diagnostic and apomorphic characters.

Material and methods

Specimens and data deposition

The 15 studied specimens are from the private collections of Patrick Müller (Käshofen, Germany), Jörg Wunderlich (Hirschberg, Germany), and from the Zoological Research Museum Alexander Koenig, Bonn (ZFMK) (Table 1). The male holotype of *Andrognathus burmiticus* n. sp. (ZFMK-MYR08421) and the five paratypes, one male (ZFMK-MYR08689), three females (ZFMK-MYR08617, ZFMK-MYR08679, ZFMK-MYR08709), and the only known juvenile specimen (ZFMK-MYR08710) are deposited at the ZFMK. All legal exportation permits are present and available upon request. The authenticity of the amber was tested using UV-light, under which amber is known to fluoresce blue. Micro-computer tomography (μCT)-data and photographs, including additional pictures, are deposited on MorphoBank (O'Leary and Kaufman 2012) under project number 3277 (http://morphobank.org/permalink/?P3277).

Light microscopy and photography

Photographs were obtained with a Canon EOS 7D camera equipped with magnifier lenses and with an Olympus TG-5 digital compact camera. Images were manipulated in Fiji/Image J 1.51f (Schindelin et al. 2012). Drawings were made with a camera lucida mounted to an Olympus BX51 light microscope with × 200 magnification.

Micro-computer tomography

μCT-Scans of ten specimen were acquired with a SKYSCAN 1272 (Bruker microCT, Kontich, Belgium) at the Zoological Research Museum A. Koenig. Thermal-drift correction, ring artifact reduction, and digital section reconstruction was done in NRecon 1.7 (Bruker microCT, Kontich, Belgium). For scanning and reconstruction parameters, see the accompanying media notes on Morphobank. Volume rendering and measurements were performed in Drishti Version 2.6.3 (Limaye 2012).

Table 1 Material examined. f = female; juv = juvenile; m = male; $^+$ = μCT-scans have been acquired; * = the exact number of body-rings cannot be determined due to artifacts

Specimen	Type	Sex	Body-rings (apodous rings)	Length (mm)	Width (mm)	Length/width ratio	Fig.
ZFMK-MYR08241$^+$	Holotype	m	44 (1)	8.8	0.4	22.00	1a
ZFMK-MYR08689	Paratype	m	> 32*	> 5.8	0.4	–	–
BuB997	–	m	> 38*	9.9	0.4	24.75	–
BuB1413$^+$	–	m	30 (3)	4.4	0.3	14.67	1b
BuB2991$^+$	–	m	30 (3)	7.3	0.4	18.25	1c
BuB3005$^+$	–	m	> 18(?)*	> 3	0.4	–	–
BuB3237$^+$	–	m	38 (3)	9.3	0.5	18.60	1d
BuB3308$^+$	–	m	41(3)	7.2	0.3	24	1e
F3391/BU/CJW	–	m	> 40(2)*	9.0	0.4	22.5	–
ZFMK-MYR08617$^+$	Paratype	f	40(2)	8.0	0.3	26.67	–
ZFMK-MYR08679	Paratype	f	> 38*	> 14	0.5	–	–
ZFMK-MYR08709	Paratype	f	38 (2)	9.1	0.4	–	–
BuB3291$^+$	–	f	> 42(?)*	10.2	0.4	25.50	1f
BuB3307$^+$	–	f	54(2)	18.2	0.5	36.4	1g
ZFMK-MYR08710$^+$	Paratype	juv	17(3)	3.9	0.3	13	–

Results

Paleontological Systematic

Class **Diplopoda** de Blainville in Gervais, 1844

Subclass **Chilognatha** Latreille, 1802/1803

Infraclass **Helminthomorpha** Pocock, 1887

Subterclass **Colobognatha** Brandt, 1834

Order **Platydesmida** de Saussure, 1860

Remarks: The specimens can be placed in the order Platydesmida based on the following character combination: habitus long, slender (Figs. 1 and 2a, b); head small pyriform, posteriorly wide; ommatidia absent (Figs. 2c and 3a, b); gnathochilarium lacking palpi (Fig. 3d); mandible partly reduced, not visible externally; eight legs anterior of the gonopods (Fig. 3a); tergites and pleurites fused, knob-like sternites free (Fig. 2e); tergites with prominent median suture and tubercles; ozopores starting from body-ring 5 and located lateral on paranota (Figs. 2d and 3c; compare to Enghoff et al. 2015).

Family **Andrognathidae** Cope, 1869

Remarks: The specimens can be placed in the family Andrognathidae based on the following character combination: Sternites narrow; coxae almost in touch but separated by a knob-like extension of sternites (Fig. 2e). Furthermore, specimens are slender and long with short paranota (Figs. 1 and 2a), a habitus-type found in extant representatives of the family Andrognathidae (compare to Enghoff et al. 2015).

Genus ***Andrognathus*** Cope, 1869

Remarks: The specimens can be assigned to the genus *Andrognathus* based on the following characters: Tergite 5 uniquely modified, enlarged with paranota pointing anteriorly (Figs. 2d and 3a, c, purple), the main diagnostic character of the genus (Cook and Loomis 1928; Gardner 1974; Shear and Marek 2009; Enghoff et al. 2015; Shorter et al. 2018); tergites with small round tubercles (Figs. 2d and 3c); from third leg pair onwards prominent coxal-sacs (Fig. 3b); hypoproct absent (Figs. 2g and 3i; compare to Shear and Marek 2009; Enghoff et al. 2015; Shorter et al. 2018).

Type-species: *Andrognathus corticarius* Cope, 1869 (eastern USA);

Other species included:

Andrognathus grubbsi Shorter, Hennen, & Marek, 2018 (New Mexico, USA)

Andrognathus hoffmani Shear & Marek, 2009 (Mexico);

Andrognathus burmiticus **† n. sp.** Moritz & Wesener

Material examined (Table 1):

Male holotype (ZFMK-MYR8241) from the collection of Patrick Müller (transferred to ZFMK), adult male, Myanmar, Kachin State, Hukawng Valley, Noije Bum amber mine, N 26° 15′, E 96° 34′. (In Wesener & Moritz (2018) listed as BuB2670).

Paratypes: 1M (ZFMK-MYR08689), 3F (ZFMK-MYR08617, ZFMK-MYR08679, ZFMK-MYR08709), 1 juvenile (ZFMK-MYR-08710) all in the collection of the ZFMK, same data as holotype.

Additional specimens: 6M (BuB997, BuB1413, BuB2991, BuB3005, BuB3237 & BuB3308) in the collection of Patrick Müller, same data as holotype; 1M (F3391/BU/CJW) in the private collection of Jörg Wunderlich, same data as holotype; 2F (BuB3291 & BuB3307) in the collection of Patrick Müller, same data as holotype.

Comments: ZFMK-MYR08617, ZFMK-MYR08679, ZFMK-MYR08689, ZFMK-MYR08709, ZFMK-MYR08710

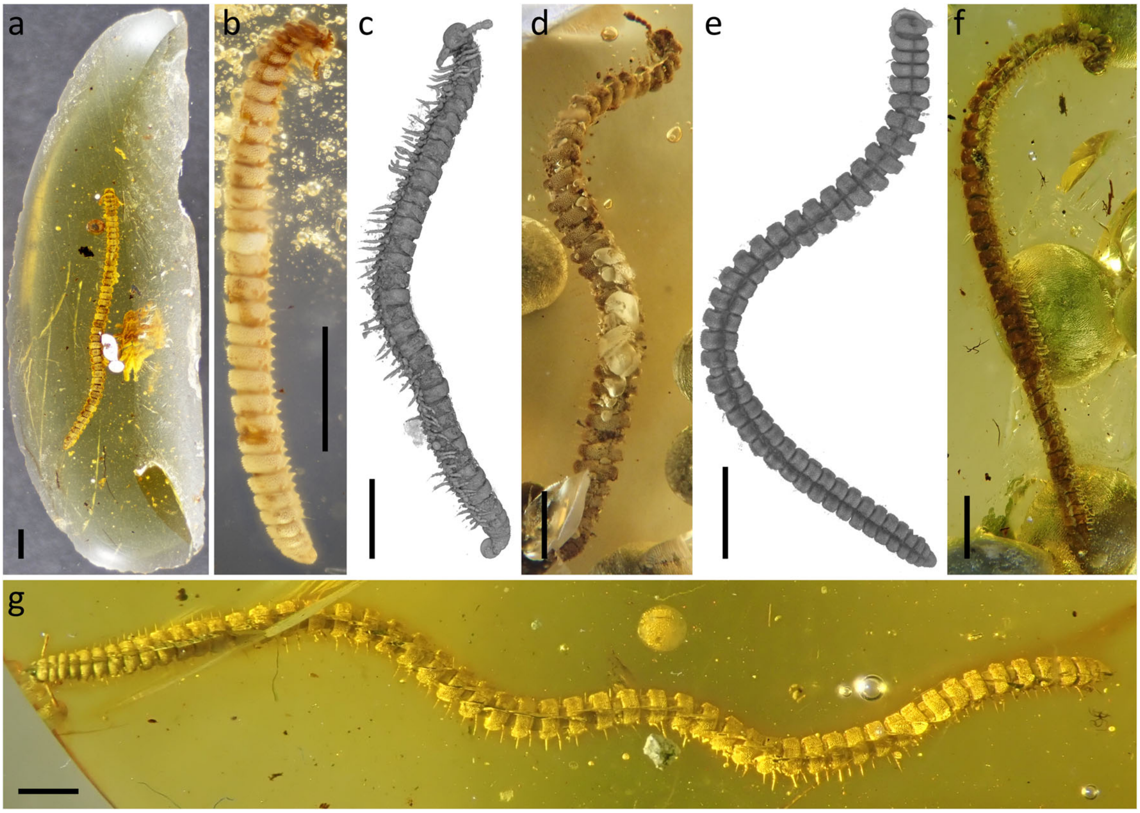

Fig. 1 *Andrognathus burmiticus* n. sp.. Material examined. **a** Male holotype (ZFMK-MYR08241), photograph, dorsal view. **b** Male (BuB1413), photograph, lateral view. **c** Male (BuB2991), volume rendering, ventro-lateral view. **d** Male (BuB3237), photograph, dorsal view. **e** Male (BuB3308), volume rendering, dorsal view. **f** Female (BuB3291), photograph, lateral view. **g** Female (BuB3307), photograph, dorsal view. Scale = 1 mm

and BuB3005 are not listed by Wesener and Moritz (2018). BuB997 is erroneously listed as representative of the Siphonorhinidae (Siphonophorida) by Wesener and Moritz (2018).

Locality and horizon: Burmese amber, early Cenomanian, 98.79 ± 0.62 Ma (Shi et al. 2012) from the Noije Bum amber mine Hukawng Valley, Kachin State Northern Myanmar.

Etymology: *burmiticus*, noun in apposition, refers to the origin of the studied specimens, Burmese amber from Myanmar.

Diagnosis*:* Body long and slender (Fig. 2a). Paranota short, rectangular, tergites and paranota highly pilose with small round tubercles (Fig. 2d). Distinct from *A. corticarius* by single lobe on tergite 5 as in *A. hoffmani* and *A. grubbsi* (Figs. 2d and 3a). Differing from all other *Andrognathus* species by presence of ventral pleurotergal lobes laterally to legs, especially well developed laterally of gonopods on body-ring 7 in males (Figs. 2e and 3b, blue), metazonite with numerous dorsal rows of tubercles. A transverse tergite division, as present in other *Andrognathus* species, is missing.

Description

Measurements: Body elongated, slender, at least 14–26 times longer than wide. Up to at least 18.2 mm long, mid-body-rings ca. 0.4 mm wide. 30–44 body-rings, including collum, 1–3 penultimate apodous rings and telson consisting of epiproct (Table 1; Figs. 1a–g and 2a, b).

Head rounded, slightly triangular, labrum without teeth, frontal setae absent (Figs. 2c and 3a, b). Epicranium laterally with round apical depression on each side (Fig. 3a). Head highly pilose. Antennae inserting laterally in deep grooves, tip extending up to body-ring 5, distally enlarged. Size of antennomeres: 1 = 7 < 3 = 4 = 5 < 2 = 6. Antennomer 2 ca. 2 times as long as wide, antennomere 6 ca. 1.3 times as long as wide, antennomere 7 with 4 apical cones (Figs. 2c and 3b). Tömösváry organ absent. Mandible not visible, Gnathochilarium as typical for the order (Fig. 3d).

Collum as wide as head, not covering head, not as wide as following body-rings, highly pilose, median suture inconspicuous (Figs. 2c, d and 3a).

Body-rings with tergites and pleurites fused to pleurotergites, sternites free. Metazonite 1.7 times as wide as prozonite (Figs. 2d, e and 3c). Prozonites with inconspicuous elliptical ornamentation, lacking setae (Fig. 2d). Metazonites highly pilose with 8–12 irregular arranged rows of 11–14 round cone-shaped tubercles with setae on each side of the median suture, covering the dorsal and ventral surface of the pleurotergites and the paranota (Fig. 2c, d).

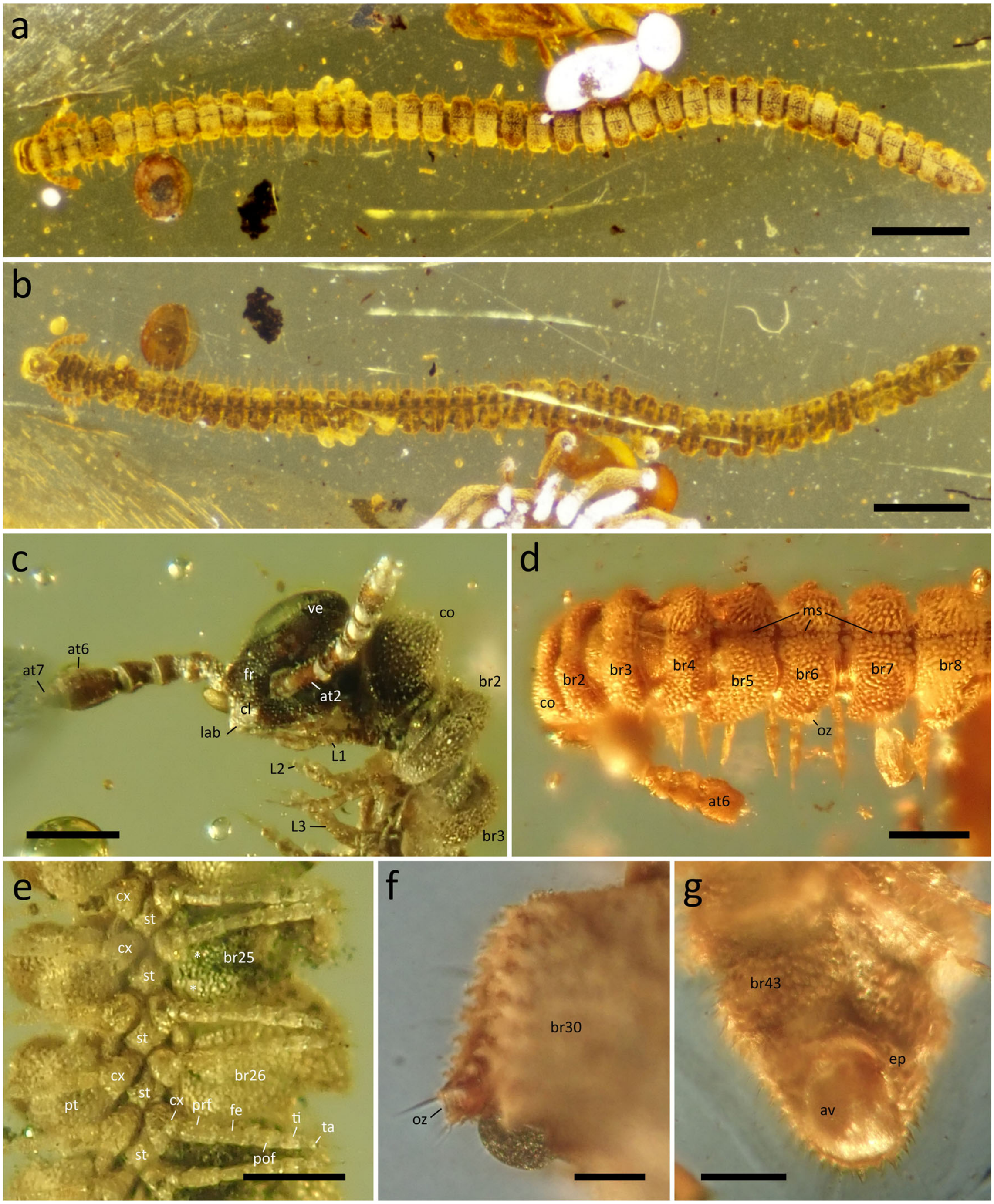

Fig. 2 *Andrognathus burmiticus* n. sp.. Photographs. **a, b, d, g** Male holotype (ZFMK-MYR08241). **c, e, f** Male (BuB3237). **a** Habitus, dorsal view. **b** Habitus ventral view. **c** Head, fronto-lateral view. **d** Anterior body-rings, dorsal view. **e** Mid-body-rings (24–27), ventral view. **f** Pranota of body-ring 30, dorsal view. **g** Posterior body-rings, ventral view. Scale: **a**, **b** = 1 mm, **c**–**e** = 200 μm, **f** = 50 μm, **g** = 100 μm. Abbreviations: at# = antennomere #; av. = anal valve; br# = bodyring #; cl = clypeus; co = collum; cx = coxa; ep = epiproct; fe = femur; fr = frons; lab = labrum; L# = leg #; ms = median suture; oz. = ozopores; pof = postfemur; prf = prefemur; pt. = pleurotergite; st = sternites; ta = tarsus; ti = tibia; ve = vertex; Asterisk (*) marks ventral pleurotergal lobes

Pleurotergites with prominent median suture. Body-rings 2–4 as wide as head, wider than collum. Body rings 2–4 with an inconspicuous median suture in shape of a median depression, paranota shaped as small lateral swellings. From body-ring 5 onwards pleurotergites distinctly longer with a distinct median suture. Posterior margin with median embayment. Body-ring 5 ca. 1.5 times as long as preceding body-ring, with a single lobe pointing anteriorly. From body-ring 6 onwards body rings slightly shorter than body-ring 5, rings equal in length, pleurotergites with short rectangular paranota pointing

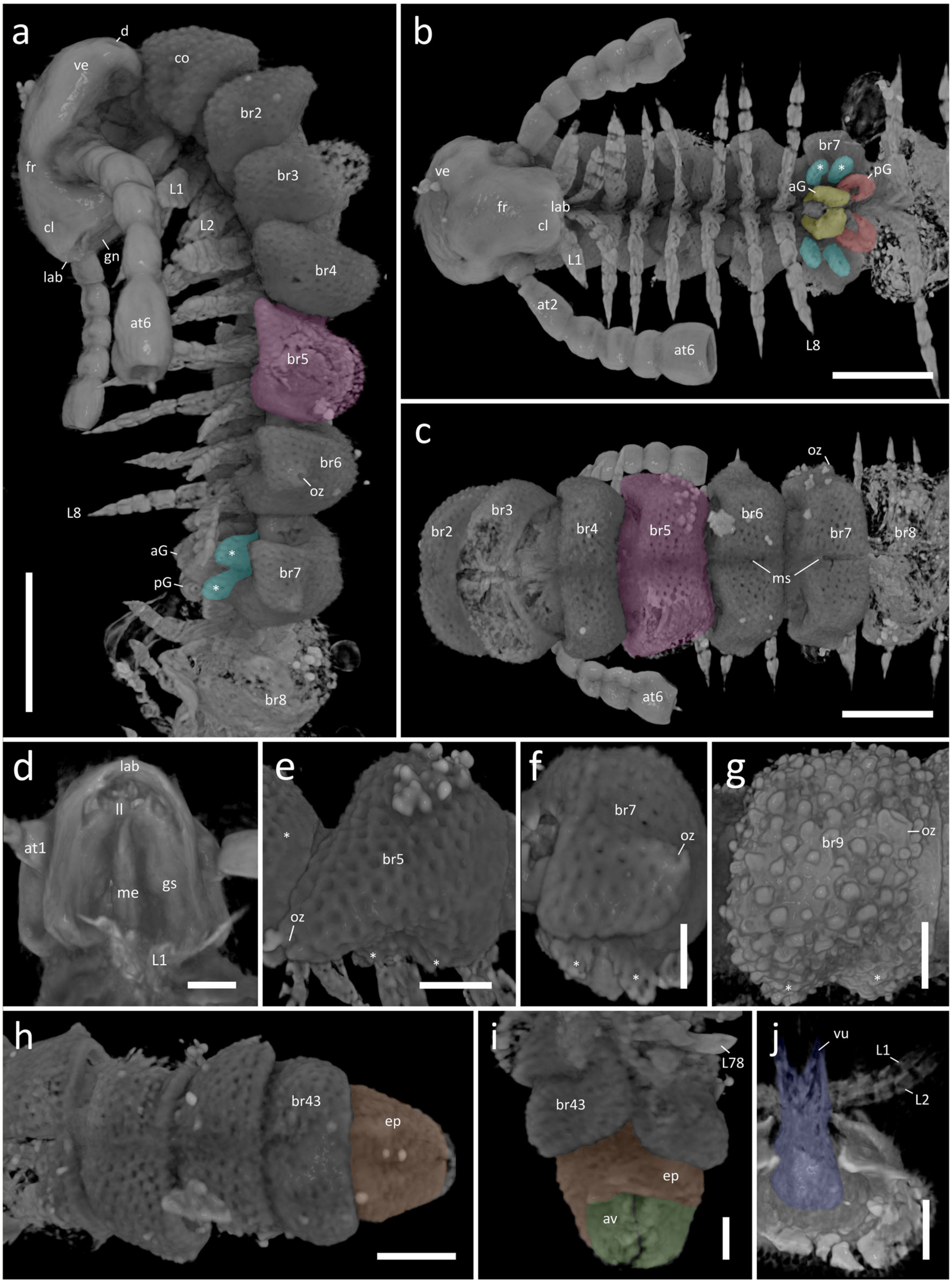

Fig. 3 *Andrognathus burmiticus* n. sp., volume rendering. **a–f, h, i** male holotype (ZFMK-MYR08241). **a** Front, lateral view. **b** Front, ventral view. **c** Front, dorsal view. **d** Gnathochilarium. **e** Body-ring 5, lateral view. **f** Body-ring 7, lateral view. **g** Female paratype ZFMK-MYR08617, body-ring 9, lateral view. **h** Posterior body, dorsal view. **i** Posterior body, ventral view. **j** Female (BuB3307), vulva posterior view. Scale: **a**–**c** = 200 μm, **d**–**i** = 50 μm, **j** = 100 μm. Abbreviations: aG = anterior gonopod; at# = antennomere #; av. = anal valve; br# = bodyring #; cl = clypeus; co = collum; d = depression on vertex; ep = epiproct; fr = frons; gp = gonopore; gs = gnathochilarial stipes; lab = labrum; L# = leg #; ll = lamella lingualis; me = mentumms = median suture; oz. = ozopores; pG = posterior gonopod; pt. = pleurotergite; ve = vertex; vu = vulva; Asterisk (*) marks ventral pleurotergal lobes

anteriorly ca. twice as long as paranota on body-ring 4 (Figs. 2d and 3c), paranota pointing posteriorly on more caudal body-rings (Fig. 3h). Paranota arising from anterior part of metazonite. From body-ring 6 onwards pleurotergites ventrally with two paired lobes lateral of legs, decreasing in size posteriorly (Figs. 1c, 2e, and 3b, f, g).

Ozopores present from body-ring 5 onwards, on body-ring 5 located apically on tip of paranota (Figs. 3e and 4a). Ozopore elevated, pointing anteriorly. On body-rings 6 and onwards ozopores located at posterior corner of paranota, slightly elevated, pointing posteriorly (Figs. 2f, 3f, g, and 4b). Ozopore opening elevated surrounded by torus (peritremata?), only poorly preserved and visible on few body-rings in few specimens (BuB997, BuB1413, BuB3237, F3391/Bu/CJW).

Sternites free, with knob-like projection extending between coxae (Fig. 2e).

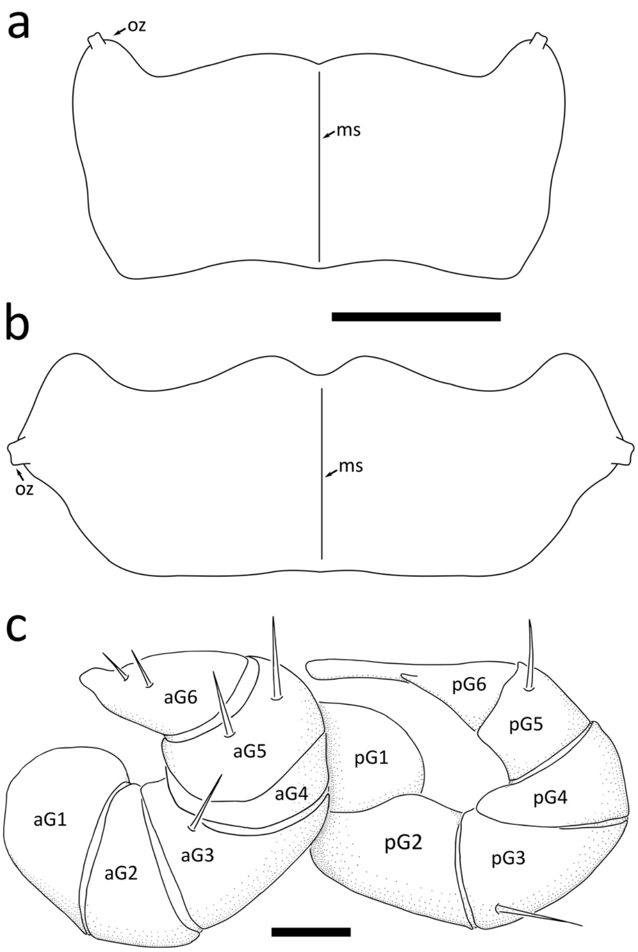

Fig. 4 *Andrognathus burmiticus* n. sp., schematic drawings. **a** Reconstruction of Body-ring 5, dorsal view. **b** Reconstruction of mid-body-ring, dorsal view. **c** Gonopods of male holotype (ZFMK-MYR08241) reconstructed from light-microscopy combined with µCT-data. Scale: **a** and **b** = 100 µm, **c** = 20 µm**.** Abbreviations: aG # = podomere # of anterior gonopod; ms = median suture; oz. = ozopores; pG # = podomere # of posterior gonopod

Telson: 1–3 apodous penultimate body-rings anteriorly of epiproct (Fig. 3h, j, marked brown) without visible sternite, tips of pleurotergites overlapping (Figs. 2g and 3i). Epiproct cylindrical without tail and without hypoproct, ca. 1.5 times as long as wide (Fig. 3h). Epiproct with six long setae surrounding anal valves (Fig. 3i, marked green). Anal valves covered by few short setae (Fig. 2g).

Legs consisting of six podomeres (coxa, prefemur, femur, postfemur, tibia, tarsus), covered by several short and few long setae (Fig. 2e). Leg 1 short and flattened, leg 2 ca. 2/3 as long as following legs. Following legs long, protruding laterally above paranota (Fig. 3b); size of podomeres: tarsus > femur > prefemur > coxa > postfemur = tibia. Tarsus 2 times as long as wide, Femur 2 times as long as wide. Coxa globular with anterior-median pit and prominent coxal-sacs from leg-pair 3 onwards. Tarsus with single long claw, no apical spine and no paronychium (Fig. 2e).

Male sexual characters: Paired penis behind leg 2 adjacent to coxa, with broad base and apically tube-like (bottle shaped), extending above coxa when everted, nearly protruding as far as femur. Leg-pair 9 and 10 on body-ring 7 modified to leg-like gonopods (Figs. 3b and 4c). Anterior (marked yellow) and posterior gonopod (marked red) similar in length, both with several setae. Ventral pleurotergal lobes most prominent on body-ring 7 lateral to gonopods, pointing posteriorly (Fig. 3a, b, marked blue).

Anterior gonopod with stout podomeres, distal podomere with short membranous lobe.

Posterior gonopod more slender than anterior gonopod, distal podomere long with tapered tip, apically with long membranous lobe, slightly spatulate (Fig. 4c).

Female sexual characters: Females larger than males (Table 1, Fig. 1f, g). Paired eversible vulva (Fig. 3j, marked blue) behind coxa of leg 2, reaching to postfemur of leg 2. Ventral paratergal lobe on body-ring 7 less prominent in females.

Intraspecific variation: The studied specimens vary in size, and the number of body-rings varies between 30 and 54 (Table 1, Fig. 1). The number of rows of tubercles on the tergites varies between 8 and 12. Variations in the proportions of podomeres and antennomeres are due to artifacts based on the preservation of the specimens in amber.

Ecology: Only adult and subadult and a single juvenile representatives of *A. burmiticus* were found so far in Burmese amber. The three extant species of the genus *Andrognathus* are mainly found on dead, decaying wood, where they sometimes aggregate in groups, a typical behavior in the order Platydesmida (Shorter et al. 2018). Our 15 specimens were all found in separate pieces of amber, never in groups. We record the following syninclusions: Pollen (ZFMK-MYR08241, ZFMK-MYR08679); stellate hairs (BuB997, BuB1413, BuB2991, BuB3291, BuB3005, BuB3307, BuB3308, ZFMK-MYR08617); leaf and bark fragments of unidentified plants (BuB1413, BuB3291, F3391/Bu/CJW, ZFMK-MYR08689, ZFMK-MYR08679, ZFMK-MYR0879, ZFMK-MYR-08710); fern sporangia (BuB2991, ZFMK-MYR-08710); fungal (?) sporangia (BuB3237, ZFMK-MYR08617, ZFMK-MYR08679); fragments of unidentified arthropod legs and cuticle (ZFMK-MYR08241, BuB997, BuB3237, TW/ZFMK-MYR, ZFMK-MYR-08710); Pseudoscorpiones (ZFMK-MYR08617); Archaeognatha (BuB3307: 1); Coleoptera (BuB3005: 2 adults, 1 larvae; ZFMK-MYR08617: 1); Auchenorrhyncha (BuB3005: 1, ZFMK-MYR0879: 1); Hemiptera (ZFMK-MYR08689: 8, ZFMK-MYR08679: 1); Hymenoptera (BuB3005: 1); Diptera (ZFMK-MYR0879: 1); unidentifiable millipede, possibly Platydesmida (ZFMK-MYR08679).

Discussion

The fossil record of the Platydesmida

Although Zhang (2017) reported fossils of *Brachycybe* sp. as representatives of the Platydesmida from Burmese amber, the depicted specimens are clearly not Platydesmida as corrected by Wesener and Moritz (2018). Furthermore, Shelley and Golovatch (2011) suggested that the Carboniferous *Hexecontosoma* Hannibal, 2000 resembles a Platydesmida despite the absence of convincing characters. Thus Hannibal (2000) stated that the only characters *Hexecontosoma* shares with the order Platydesmida are the presence of many segments and of paranota, while it shares a greater number of characters with several eugnathan orders. Mundel (1981) noted a Carboniferous Platydesmida from Mazon Creek in a conference abstract without further description. Therefore, *Andrognathus burmiticus* n. sp. from Cretaceous Burmese amber is the first definite and described fossil record of the order Platydesmida and we can expand the number of known Platydesmida from Burmese amber from eight (Wesener and Moritz 2018) to 15.

Systematic placement

Based on the somatic character, which were described and documented in great detail by Shear and Marek (2009) and Shorter et al. (2018), the studied fossils can be placed in the extant family Andrognathidae and the extant genus *Andrognathus*. Thus the enlarged fifth body-ring, which is overlapping the fourth body-ring in *Andrognathus*, is an unique character within the Platydesmida which can otherwise only be seen in the Siphonocryptida genus *Siphonocryptus*, with which it is clearly not homologous (Enghoff and Golovatch 1995). Despite the remarkable preservation of the fossils, not all details of the gonopods could be accessed, thus especially membranous structures which could be species-specific cannot be studied in as much detail as is possible for extant specimens utilizing scanning electron microscopy (e.g., Shear and Marek 2009; Shorter et al. 2018). Nevertheless, we do not restrain from describing a new species within the genus *Andrognathus*, because the somatic characters in *A. burmiticus* n. sp. are clearly distinct from those reported from any extant representative (which also come from a different continent). The great variation of the number of body-rings in *A. burmiticus* n. sp., ranging from 30 to 44 body-rings is not surprising, as Shorter et al. (2018) reports for *A. corticarius* great intraspecific variation of 34–57 body-rings.

The potential of micro-computer tomography and “Cybertypes”

Micro-computer tomography proved to be a valuable tool for the study and description of millipedes from amber and enables us to study structures in detail which would otherwise not be accessible, as already demonstrated by Riquelme et al. (2014), Liu et al. (2017) and Stoev et al. (2019). It is possible to perform exact measurements of structures in the μm-range. Furthermore, μCT data allows us to deposit so called “Cybertypes”, as shown for example by Akkari et al. (2015, 2018) for extant myriapods, which are free to use by every interested scientist for re-examination or further studies. Nevertheless, μCT cannot replace but only complement classical light-microscopy. Characters like the setation and in this case the detailed structure of the gonopods cannot be documented solely with μCT-methods. Although, using light-microscopy, all specimens seem to be equally well-preserved, they greatly vary in their contrast using μCT, which could be due to variations in the fossilization process.

The age and origin of Andrognathus

The minimum age of the genus *Andrognathus* is ca. 99 Ma, as Shi et al. (2012) determined the age of Burmese amber to ca. 98.79 ± 0.62 Ma, although it is still under debate whether the amber is Cenomanian or Albian in age (Ross et al. 2010; Smith and Ross 2018). The Albian/Cenomanian boundary lies at 100.5 Ma (International Chronostratigraphic Chart v2016.04, www.stratigraphy.org), and is therefore more than 1 million years older than the age of the Burmese amber deposit after Shi et al. (2012). This age of the Platydesmida fossils described here only broadly fits to the interpretation of the present molecular data. Thus, Rodriguez et al. (2018) present a dated phylogeny of the Diplopoda based on genomic data, including the three platydesmidans *Platydesmus* sp., *Brachycybe lecontii* and *Andrognathus corticarius*. Based on the molecular data the split between *Andrognathus corticarius* and the other studied Platydesmida is dated to a median age of

75.8 Ma with a 95% highest posterior density spanning almost 100 Ma (36.84–128.55 Ma, supplement figure 1.4 in Rodriguez et al. 2018). Therefore, we conclude that the genus *Andrognathus* originated in the lower Cretaceous between 98.79 ± 0.62 Ma and 128.55 Ma, with the minimum age given by the fossil data and the maximum age given by the 95% interval of the molecular data (Rodriguez et al. 2018). It can be assumed that the family Andrognathidae and the order Platydesmida most likely evolved long before the Cretaceous. We are convinced that the described fossils will be of great value for future calibrations of the molecular clock and the dating of molecular phylogenies especially within the Platydesmida, because of (1) the precise dating of the Noije Bum amber deposit by Shi et al. (2012) and (2) the exact placement of the fossils in the genus *Andrognathus* based on apomorphies. Thus, the Platydesmida fossils meet the requirements pointed out by Parham et al. (2011) for the fossil calibration of molecular clocks.

The extant genus *Andrognathus* is today restricted to the eastern USA, New Mexico, and Mexico and only known from the three species *Andrognathus corticarius* Cope, 1869, *A. hoffmani* Shear & Marek, 2009, and *A. grubbsi* Shorter, Hennen, & Marek, 2018. Therefore, representatives of the genus *Andrognathus* must have migrated between North America and Asia. Based on the proposed age of the genus *Andrognathus* (ca. 99–128.55 Ma), we assume that *Andrognathus* originated from Asia and migrated to North America. It is not possible to determine at which time, how often and via which route migration events of *Andrognathus* between Asia and North America took place. Migration could have taken place via (1) Beringea or via (2) a North Atlantic land bridge. Via Beringea migrations could have taken place during Late Cretaceous and several times during the Cenozoic, as the distribution of extant plants, fossil, and geological data suggest a connection of North America and Asia during these periods (Fiorillo 2008; Wen et al. 2016). Considering that *Andrognathus* is mainly reported from the eastern USA and Mexico, a migration via Europe and the North Atlantic land bridges, as suggested for several plant and vertebrate taxa (Tiffney 1985a, 1985b; Milne 2006; Brikiatis 2016), seems plausible as well. North Atlantic land bridges probably connected eastern North America and Eurasia around the Jurassic-Cretaceous boundary, during the late Cretaceous (Brikiatis 2016) and in the early Eocene (Tiffney 1985a; Graham 1993). For the genus *Brachycybe* Wood, 1864, which has an extant North American-Asian distribution, Brewer et al. (2012) estimated an ancient origin ca. 50 Ma based on molecular data. Brewer et al. (2012) assumed for *Brachycybe* a migration between North-America and Asia ca. 20 Ma via Beringia and ancient extinction events. A similar scenario is possible for *Andrognathus*, which probably had a much greater distribution as well. Thus the genus went extinct (or is still undiscovered) in Southeast Asia, leaving the three American representatives just as a remnant of a once greater diversity of the genus.

So far, not a single Platydesmida is recorded from Myanmar (Likhitrakarn et al. 2017). It can be expected that further collecting in Southeast Asia and especially Myanmar will potentially result in the discovery of further representatives of the order Platydesmida and might even yield extant representatives of the genus *Andrognathus*.

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