Bangka Archipelago and Phyllidiidae

A diversity study on marine Heterobranchia in North Sulawesi (Indonesia), with a thorough assessment of the family Phyllidiidae (Nudibranchia, Gastropoda)

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Summary

Many members of marine Heterobranchia, usually also called sea slugs, are associated with the coral reef ecosystem and therefore in tropical marine waters. Sea slug diversity is reflecting the ecosystem and habitat diversity and also the health of these systems. North Sulawesi sea slugs diversity was rarely known. Four diversity publications from three localities have recently been published, with partly my involvement: Bunaken National Park (BNP) (Kaligis et al 2018, Eisenbarth et al. 2018), Sangihe Island (SI, Undap et al. 2019) and Lembeh Strait (LS, Ompi et al. 2020). Chapter 3 covers a new study location, Bangka Archipelago (BA), in which I investigated marine heterobranchs diversity as main author (Papu et al. 2020). BA is composed of small islands and is located in between BNP and LS. The substrate is dominated by biogenic reefs and volcanic rocks, providing highly diverse and strongly structured habitats. It is rich in nutrients and therefore provides good and diverse conditions for the benthic organisms, including sea slugs. In total 149 species are recorded from this area now. Approximately 78% of total species belong to the group of Nudibranchia. The rest, 22%, are comprised of Cephalaspidea, Aplysiida, Pleurobranchida and Eupulmonata. These extensive studies, including mine from BA, provide a baseline of diversity studies for the future when monitoring coral reef ecosystems. Especially of interest is the small overlapping of species in BA with the other rather close by areas of BNP and LS. With my results from BA, the total amount of known and in publications documented species from North Sulawesi now amounts to 259. 33 species recorded in my BA study are new to science. One of the most common families in all North Sulawesi sea slug studies are the Phyllidiidae

Members of the abundant tropical-subtropical nudibranch family Phyllidiidae are interesting with regard to the conspicuous colour (very popular for underwater photographers), chemical defense system (often investigated with regard to chemical compounds and of interest for pharmaceutical purposes) and the difficult taxonomic (therefore only few scientists have worked with this group). I combined molecular methods with thorough investigation of the external anatomy to tackle this challenge. Maximum likelihood tree based on a concatenated dataset with 598 CO1 and 16S mitochondrial genes resulted in a large phylogeny of Phyllidiidae, which covers 32 species. Specimens from our expeditions can be assigned to 11 species of *Phyllidia,* 7 species of *Phyllidiopsis,* and 11 species of *Phyllidiella*. Six other species were added

by extracting sequences from GenBank. Additionally a colleague applied chemical analyses for many species, which were identified using species delimitation tests and haplotype network analyses. With these integrative methods: external morphology, molecular and chemical analyses we could demonstrate unknown cryptic variation or identify paraphyly in groups that up to now were considered monophyletic. We were therefore able to identify subspecies, or external variation within the species, or similar color patterns in not closely related species due to mimicry. I was able to resurrect *Phyllidiella albonigra* Quoy & Gaymard 1831 and synonymize *Phyllidiopsis pipeki* Brunckhorst, 1993 with *Phyllidiopsis krempfi* Pruvot-Fol, 1957. Additionally, the chemical analyses provide further evidence for dietary preference within the Phyllidiidae.

The most problematic clade of Phyllidiidae is still *Phyllidiella.* Several other species were synonymized in the past with *P. pustulosa*, which was described with many external variations and many specimens with overlapping characters. All our specimens that clustered in a large clade, which we were able to assign to the “real” *Phyllidiella pustulosa* after re-analyses of type material, still split into subclades. Many other specimens assigned in our collection and also in former publications are not clustering in this clade, but form further 7 distinct clades, nested between the nominal species *Phyllidiella nigra, P. rudmani, P. zeylanica* auctt., and *P. hageni.* Two synonyms of *P. pustulosa* (*P. nobilis* and *P. spectabilis* holotype) are confirmed as synonyms of *P. pustulosa.* However, the paratypes of *P. nobilis* are different species. One was very similar to one of our unnamed clades, but the name *P. nobilis* is not available for this clade, because of the synonymy of the holotype with *P. pustulosa*. One of our unnamed clades is very similar to the description of *P. albonigra,* which was synonymized with *P. pustulosa* by Brunckhorst 1993. However, the chemical analyses of this clade clearly confirm my molecular data, they show a unique chemo type not found in any other phyllidiid species. During my studies of museum materials and especially literature and published sequences, I found misidentifications, e.g., Berg (1904) described from Siam one specimens as *P. nobilis*. The animals are very similar to our *Phyllidiella* sp. d and *P.* sp. e is their external appearance. In future analyses of Phyllidiidae, I advise to document all collected specimens from the dorsal and ventral side to preserve colour information, which is often lost in fixed material. Additionally, I advise always to barcode the specimens, because for some species the colour and colour pattern is not decisive, but is found in several species.

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Chapter 1

General Introduction

Heterobranchia

The Heterobranchia is a highly diverse group of Gastropoda. Their biological features are much more diverse than all other gastropods, from worm-like bodies, colorful appearances and life in the terrestrial, freshwater or marine habitats. The term "heterobranchs" mean "different-gilled" snails, which describes a typical character of this group (Ponder et al. 2020). Heterobranchia comprise groups that were united before as "Opisthobranchia", which is now an abandoned concept (Wägele et al. 2008). Nowadays, scientists accept opisthobranch taxa as part of Heterobranchia and are usually referred to as marine heterobranchs. Heterobranchia as a monophyletic taxon was originally proposed by Gerhard Haszprunar (1985b, 1988f). Heterobranchia comprise at the moment two infraclasses (see World Register of Marine Species): "lower Heterobranchia", and Euthyneura. 'Lower heterobranchs' comprise small groups, which often resemble typical shelled gastropods, and that are not well studied. Euthyneura comprises former opisthobranch groups, like the Nudibranchia, which completely lack a shell in the adult stage and which can have very large members, and Pulmonata (Wägele et al. 2013; Bouchet et al. 2017; Ponder et al. 2020). The name is now widely accepted. Heterobranchs occur in various habitats, from terrestrial environment (pulmonates), to freshwater (Hygrophila), but many live in the marine realm.

The size of marine heterobranchs ranges from one millimetre (*Philinoglossa* sp, *Acochlidium* sp) to about 70 cm (*Aplysia vaccaria).* Many heterobranch larvae are planktotrophic, but also lecithotrophic development is common (Purohit 2006, Gosliner et al. 2015). Especially in cold water, intracapsular development is also reported (Moles et al. 2017). Due to the lack of shells, many marine heterobranchs evolved other defense strategies such as camouflage, nocturnal behaviour, chemical compound, mimicry, autotomy, spicules, cnidosacs, in order to protect themselves from predators (Rudman, 2004).

Order Nudibranchia

Nudibranchia Cuvier, 1817 is the largest taxon with regard to species numbers within marine Heterobranchia, comprising around 3000 species. They are united by following

apomorphic characters: the first the reduction of a shell in the adult, and second 13 haploid chromosomes (Wägele & Willan 2000).

Nudibranchia comprises two suborders (Doridina and Cladobranchia). Doridina was known as Anthobranchia describing the primary gills around the anus. Cladobranchia do not have primary gills, but only dorsal appendages, sometimes also structures, called secondary gills.

Nudibranchs are distributed worldwide, from the polar to tropical areas, including Indonesia (GBIF Secretariat 2019b). They are living in shallow water down to the deep sea (https://ocean.si.edu/ocean-life/invertebrates/collage-nudibranch-colors). Nudibranchia are attractive for divers and underwater photographers because of their stunning colors and also sometimes shapes. Many divers are contributing as citizen scientists in recording nudibranch distribution in the field (Giglio et al. 2015; GBIF Secretariat 2019b; Sea Slug Forum Home page).

Despite nudibranchs known as the slimy sea slugs, many of them compose spicules in their body (mantle tissue, rhinophores and foot). Spicule forms differ, although the main compound of the spicules is calcite ($CaCO_3$), followed by brucite ($Mg(OH)_2$) and small percentage of fluorite (CaF_2), and collagen. The density of the spicules also varies within the different groups and can make them very hard (e.g., Phyllidiidae). Spicule types are good for describing the species, nevertheless it is not used for taxonomic information (Odum 1951; Cattaneo-Vietti et al. 1995; Penney 2008; Chang et al. 2013; Nikitenko et al. 2020, Neusser et al. 2011).

A very important defense system especially in the shell less nudibranchs are secondary metabolites Metabolomes of nudibranchs are well studied and differ to a great extent: e.g., naamidine, clathridine, latrunculin, mycothiazole, inorolide, aplyroseol, scalarane, bromelain, jorumycin, variabilin, trisoxazole macrolides, isocyanopupukeanane, many further terpenoids and alkaloids, as well as phenols and quinones are isolated from nudibranchs (Cimino, G. and Ghiselin 2006, Fisch et al. 2017). All these compounds are used for defending them against predators, but are probably also used for antifouling. Interestingly, these compounds are also of interest for humans. Greeks used the slugs already for the medical application. The Romans used slugs as repellents (Ponder et al. 2020). Recently, many studies have discovered the chemical compound might be useful for drug leads. This was one reason for the large project *IndoBio: Indonesian opisthobranchs – from biodiversity to drug lead detection*, financed by

the German Ministry of Education and Research and which my thesis is part of. Besides the potential for new drugs (Avila et al. 2018, Fisch et al. 2017), chemical compounds are also a potential for the chemotaxonomy (Hagadone et al. 1979; Wägele et al. 2008), which is here shown for Phyllidiidae (Bogdanov et al. 2020, Papu et al. submitted).

Sulawesi, Indonesia

Indonesia is located in the tropical area in between the Pacific and Indian Ocean and is in the heart of the Coral Triangle. As the biggest archipelago country in the world, Indonesia consists of 7000 Islands and around 70% of Indonesian territory is ocean. A large global current, the so called Indonesian ThroughFlow (ITF) brings the water masses from the Pacific Ocean to the Indian Ocean passing through the 'canals' of the many Indonesian seawater straits. Most of the water mass flows through Sulawesi Strait. This ITF is very important for the dispersal of larvae and thus for the biogeography of pelagic biota (Atmadipoera, 2017).

The Coral triangle is known as a hotspot of coral reefs and species diversity. Coral reefs play a big role in the intertidal ecosystems and are the most productive ecosystems in the intertidal to subtidal habitats. Coral reefs together with mangrove and seagrass ecosystems protect the coastal shorelines against wave actions. However, the coral reefs are threatened by natural phenomena and human activities. Climate change, global warming, storms, overfishing, bomb/dynamite fishing and further anthropogenic activities, plastic waste threaten the coral reef ecosystem to a great extent (https://www.worldwildlife.org/places/coral-triangle; Lasut et al. 2017; Poli, 2018). An important role in finding solutions for the coral reefs ecosystem protection is first of all the recording of the diversity of coral communities.

Indonesia is one of the mega-diverse countries; however, surveys covering marine invertebrates in particular are very rare, these documents are often not open-access, and they are frequently in Indonesian language (Kaligis et al. 2018). The largest open-access study on Indonesian marine life is the Rumphius Biohistorical Expedition to Ambon in 1990, which has been published as a series of reports on numerous marine taxa in Zoologische Mededelingen, e.g., Naturalis Biodiversity Center. This series also comprised studies on marine Heterobranchia, increasing our knowledge on this particular group considerably in an Indonesian region (Yonow 2001; 2011; 2017; 2018). Only recently, several further studies on marine Heterobranchia in North Sulawesi were published, increasing our knowledge on this group (Kaligis et al. 2018, Eisenbarth et al. 2018). Sea slugs are of special interest, because

they are specialist in feeding and very specific on food items. Therefore presence of many marine heterobranchs species indicate a high diversity of other marine metazoans, including sponges, tunicates and cnidarians. Nimbs et al. (2016) documented for the first time a correlation between the climate change in Australia and sea slug distribution. He recorded an extension of several warm water sea slug species into colder areas of Southern Australia.

Family Phyllidiidae

One family of nudibranchs was recorded in highest numbers in the published diversity studies of North Sulawesi, and comprises 30% of our total specimens collected in North Sulawesi, and this is the family Phyllidiidae, belonging to the Doridina. Phyllidiidae comprises 81 described species of genera *Phyllidia, Reticulidia, Phyllidiella, Ceratophyllidia* and *Phyllidiopsis*. Most of them are distributed in Indo-Pacific subtidal habitats followed by the Indian Ocean (GBIF Secretariat 2019e). Few of the species are distributed or even endemic in Persian Gulf, Caribbean Sea and Mediterranean Sea (Yonow, 1986, Gosliner 1992): *Phyllidia flava* is endemic in the Mediterranean Sea (Wägele 1985), *Phyllidiopsis dautzenbergi* (Vayssière, 1912) and *Phyllidiopsis sinaiensis* (Yonow, 1988) are Red Sea phyllidiids, and *Ceratophyllidia papilligera* (Bergh, 1890) is only found in Caribbean Sea (GBIF Secretariat 2019a; 2019g; 2019h).

Phyllidiidae are special within the Doridina in having no jaws and radula, and probably all members are sponge feeders. Very often Phyllidiidae were sitting on sponges when recorded. Some sponge species are known as food of phyllidiids, such as *Acanthella cavernosa, Acanthella acuta, Hymeniacidon, Axinella cannabina* etc. (Barletta & Melone 1976; Schulte 1982; Macri 1986; Brunckhorst 1993; Wägele 1985). The slugs have an extraoral digestion and swallow their food by sucking the dissolved sponge tissue into their mouth (suctorial feeder). The enzymes produced for the extraoral digestion are secreted from oral glands in the oral tube. The muscular pharynx allows the sucking of the digested food (Brunckhorst, 1993; Valdés & Gosliner 1999). The shape of these oral glands differ, and are characteristic for the various genera.

Layers of small and vertical leaflets (secondary lateral gills) are situated between the notum and foot. This is an apomorphic character of the Phyllidiidae. Only one other nudibranch group, the Arminidae (Cladobranchia), have gills situated in a similar position as in Phyllidiidae. However, the gills are not vertically oriented (Gosliner 1994).

Our knowledge on Phyllidiidae is usually restricted to dissection as the only method. Wägele (1985) for the first time described the endemic Mediterranean species *Phyllidia flava* using histological methods. Unfortunately, no further analyses on other species or genera using histology followed this unique investigation. Several thorough studies were performed by Yonow, who published several papers on the taxonomy of Phyllidiidae, with a focus on the Red Sea (1986; 1988; 2018). Yonow (1996) revised the phyllidiid systematic from Indian Ocean species. Valdés & Ortea 1995 discovered and described phyllidiids from the Atlantic Ocean. Brunckhorst (1993), Fahrner & Beck (2000), and Dominguez et al. (2007) focused on the Indo-Pacific. Brunckhorst (1993) published the most extensive paper about the morphology and systematics of Phyllidiidae and also described new species. Valdes (2001) discovered for the first time deep sea phyllidiids coming from depths down to 750 m. Valdes & Gosliner (1999) described for the first time the phylogeny of Phyllidiidae and their sister group, the Dendrodorididae, using morphology characters. Wollscheid et al. (2001) and Valdes (2003) started the first molecular phylogenies including a few of phyllidiid species, Stoffel et al. (2016) published for the first time a larger phylogeny only including members Phyllidiidae, but using only one molecular marker (CO1 gene), but provided many pictures of the phyllidiid species they collected from east of Indonesia. In the phylogenetic tree, they revealed incongruences within *Phyllidia babai*, the paraphyly of *Phyllidiella pustulosa*, and *Phyllidiopsis fissurata*. Several other phylogenies were published in the last years, dealing with dorids, but only included very few phyllidiids, like Hallas et al. (2017). Many of the published results based on molecular and morphological data are incongruent. The results therefore need to be re-evaluated and the taxa clarified.

Role of molecular systematics

Traditional taxonomic methods were based on morphological characters. Since the end of the 20th century, molecular analyses have increased. Results of phylogenetic reconstruction, especially when based on molecular data indicate that many taxa considered as monophyletic in morphological analyses, are now paraphyletic. Although many morphologically defined groups are confirmed by molecular analyses, e.g., Nudipleura, which unite Nudibranchia and Cladobranchia (Wägele & Willan 2000), there is a lot of incongruence between the different methodologies, which is now a challenge in science. Molecular data can also help to find and interpret plesiomorphic and apomorphic characters and finally lead to

the identification of morphological characters that help in phylogenetic reconstructions (Wägele 2005).

Aims of the Study

A number of heterobranch diversity studies have been published from Indonesia (Yonow 2001; Yonow 2011; Yonow 2017; Yonow 2018; Gosliner 1992; Tonozuka 2003), and recently four publications from North Sulawesi were performed (Burghardt et al 2006; Kaligis et al. 2018; Eisenbarth et al. 2018; Ompi et al. 2019; Undap et al. 2019). Nevertheless, the species documented in these studies show that we have not reached the maximum possible amount of species numbers, and we also need to expand the localities. In my thesis one locality not studied before, was targeted. This first study from Bangka Archipelago aimed to expand the monitoring localities and continue our sea slug diversity monitoring in North Sulawesi (Chapter 3). The results of this study have been published in the international journal *Diversity* in 2020. From all slugs we collected, I focused on one family, the Phyllidiidae (Nudibranchia, Doridina), because it was the specimen richest family in the collections. My primary task was to identify the specimens. However, Phyllidiidae is a very difficult group and therefore I also aimed to clarify systematics by taking into consideration all species that were present in the collection. I therefore performed a taxonomic revision of some more common species, using external morphological characters, barcodes and chemotype descriptions (Chapter 5). This chapter will be submitted latest in June. Supplementary figures from my work are included in the appendix here. However, the supplementary work of the co-author A. Bogdanov on the chemical analyses is not attached. The most difficult group was the genus *Phyllidiella*, and therefore a separate chapter, Chapter 4, focused on this genus, also using an integrative approach. This chapter is already published with Bogdanov and myself contributing equally (Bogdanov, Papu, et al. 2020).

Chapter 2

Materials and Methods

Animal collections

Specimens were collected by diving, snorkelling or taken from the substrates we collected in the field and then sorted out in the laboratory. Before taken from the substrate, specimens were photo-documented underwater and *in situ*. Additional individuals were only recorded by photo-documentation without collecting the animal. In the laboratory, specimens were preliminary identified with help of the identification books and available literature (Yonow 2001, 2011, 2017; Burghardt et al. 2006, 2014; Haber et al. 2010; Martynov et al. 2012; Gosliner et al. 2015; Stoffels et al. 2016; Eisenbarth et al. 2018; Layton et al. 2018; and The Sea Slug Forum website www.seaslugforum.net and Epstein et al. 2019). The World Register of Marine Science (www.marinespecies.org) was used for taxa validity. Further photo documentations were performed in the laboratory using Olympus TG5 camera. The unique identifier (voucher number) was given for each specimen. Voucher numbers contain the abbreviations of species name, year of collection, locality and specimen number. All metadata were documented in an excel table, e.g. depth, animal size, time collection, preservation time, preservation type, etc. Most of the specimens were preserved in 95% EtOH. Few were preserved in formaldehyde for anatomical investigation (dissection, micro-CT scan and histology slides). All specimens which are not used up completely will be deposited as collections in the Sam Ratulangi reference collection and are already registered as collections there.

Diversity Workbench

Availability of metadata for future analyses and also future monitoring studies is essential. Therefore we deposited the extensive excel table in the Diversity Workbench, an integrative diversity database established in German Federation for Biological Data (GFBio). Additional to the meta data recorded (identifier name, species/author, family, size, substrate, when was collected, depth, collection date, location and area, latitude and longitude, and fixation), I also uploaded photographs of each specimen. All these data will be available in the webpage www.gbif.org.

DNA extraction, amplification, sequencing, and alignment

For the Phyllidiidae study, a tiny piece of the specimens were cut off for further molecular analyses (barcoding). DNA from the phyllidiid specimens were extracted successfully from the foot, notum, or whole body, depending upon the size of animals. DNA-isolation was carried out by means of QIAgen® DNeasy Blood and Tissue-Kit, following manufacturer's instructions. Partial sequences of mitochondrial CO1 (ca. 659bp) and ribosomal 16S (ca. 528-541bp) were amplified by polymerase chain reaction (PCR) using the primers LCO1490-JJ (5' –CHACWAAYCATAAAGATATYGG-3') and HCO2198-JJ (5'-AWACTTCVGGRTGVCCAAARAATCA-3') (Astrin and Stüben 2008) for CO1, and 16Sar-L (5′ -CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 2002) for 16S. Amplification of CO1 was performed by an initial step (95^{o} C for 15 min) followed by 40 touch-down cycles of denaturation (94^{o} C for 35 sec), annealing (55^{o} C for 90 sec) and extension (72^{o} C for 90 sec), with a final extension step 72^{o} C for 10 min. For 16S rRNA, the PCR started with an initial step (95^{o} C for 15 min), denaturation (94^{o} C for 45 sec), followed by 34 touch-down cycles, annealing (56^{o} C for 45 sec), extension (72^{o} C for 90 sec) and final extension step at 72^{o} C for 10 min. PCR products were sequenced by Macrogen Europe Laboratory (Amsterdam, Netherlands). GENEIOUS Pro 7.1.9 was used to extract the consensus sequence between the primer regions, and for editing and checking the quality of sequences. Consensus sequences were blasted against the NCBI database for evaluation of identification (NCBI Resource Coordinators 2018).

Additionally, 116 phyllidiid sequences available in GenBank were extracted, to increase coverage of species and genera (Table 2). These were all sequences available at that time, when I started with my analyses. As an outgroup, I selected members of the sister taxon of the Phyllidiidae, the Dendrodoridídae. These were also taken from GenBank (*Doriopsilla albopunctata*, *Doriopsilla bertschi*, and *Dendrodoris atromaculata).* Sequences were aligned using the web program MAFFT Alignment, by applying algorithm FFT-NS-2, scoring matrix 200PAM/k=2. The single gene data sets (635 bp of CO1, 538 bp of 16S) were analysed separately, but both genes were also concatenated using GENEIOUS Pro 7.1.9, resulting in a total length of 1173 bp.

Phylogenetic analyses and Species Delimitation Test

The single gene datasets as well as the concatenated data was processed in the online version of IQ-TREE in http://iqtree.cibiv.univie.ac.at/ using default settings for determination of the best-fit substitution model. For our tree reconstruction, I tried single substitution models HKY and GTR. For comparison, I run the reconstruction using an automodel. From three substitution models, the very make sense result (close to the morphology result) is the complex automodel, whether TVM+F+I+G4 was selected according to the Bayesian Information Criterion (BIC) (Burnham and Anderson 2009; Nguyen et al. 2015; Hoang et al. 2017; Kalyaanamoorthy et al. 2017). Phylogenetic reconstruction was carried out with Maximum likelihood algorithms implemented in the IQ tree web server. Following settings were applied: Ultrafast Bootstrap analyses, 1000 number of bootstrap, 1000max iterations, 0,99 min correlation coefficient (Minh et al. 2017). In the first analyses, it has become clear that some sequences were wrong. The reasons were the switch of samples during processing. I was able to reconstruct the switches and corrected these mistakes during the further analyses. For visualisation of the trees Dendroscope Vers. 3.5.10 was used (Huson et al. 2012). Collapsing of clades and tree editing was subsequently processed using FigTree v1.4.4 (Rambaut 2009).

To provide further evidences on species hypotheses, datasets of CO1 and 16S were separately analysed with the help of the ABGD (Automatic Barcode Gap Discovery) methodology (Puillandre et al. 2012), using the web server https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. This method helps to characterise barcode gaps between hypothetical species, especially when these are difficult to identify on morphological basis. Following default settings were used: Initial Partition prior maximal distance (P) is 1.29e-02, Distance K80 Kimura Min Slope is 1.000000. A second program, the bPTP delimitation test (https://species.h-its.org/) using Maximum likelihood and Bayesian algorithms, was used for the concatenated data to identify cryptic variabilities (Zhang et al. 2013). To visualise the relationship within DNA of several clades, TCS Network method (Clement et al. 2002) in popart http://popart.otago.ac.nz was used for haplotype network of *Phyllidiella* clades of interest.

Chemical analyses

This part was done separately by a colleague, Dr. Alexander Bogdanov from Pharmaceutical Biology Institute, Bonn University. For the analyses, I provided the alcohol from the first preservation. In some cases, I selected certain specimens which were also analysed completely for their metabolome.

Chapter 3

First Study on Marine Heterobranchia (Gastropoda, Mollusca) in Bangka Archipelago, North Sulawesi, Indonesia

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Abstract: As ephemeral, benthic, secondary consumers usually associated with sessile coral reef organisms, marine heterobranchs are good indicators of the health of marine tropical habitats. Thus, marine Heterobranchia have recently become a major target for monitoring programs. For this work, an extensive survey was made in Bangka Archipelago, the first of its kind in this area. Bangka Archipelago is composed of small islands and the adjacent coastline of North Sulawesi. The substrate is dominated by biogenic reefs and volcanic rocks, thus forming highly diverse habitats.

In total, 149 species were collected and/or photo-documented in September 2017 and September 2018, which represents the dry season. They can be assigned to the Cephalaspidea, Aplysiida, Pleurobranchida, Nudibranchia, and Eupulmonata. Thirty-three species are new to science, highlighting the lack of knowledge about the group and especially about this region. Our data provide a baseline for future monitoring surveys, as the anthropogenic pressures on Bangka Archipelago are increasing due to enhanced touristic activities, but also due to large scale environmental changes caused by previous mining operation activities.

1. Introduction

Indonesia is one of the mega-diverse countries; however, surveys covering marine invertebrates in particular are very rare, these documents are often not open-access, and they are frequently in the Indonesian language (Kaligis et al. 2018). The largest open-access study on Indonesian marine life is the Rumphius Biohistorical Expedition to Ambon in 1990, which has been published as a series of reports on numerous marine taxa in Zoologische Mededelingen, e.g., Naturalis Biodiversity Center (https://repository.naturalis.nl/). This series also comprised studies on marine Heterobranchia, increasing our knowledge on this particular group considerably in an Indonesian region (Yonow 2001; Yonow 2011, Yonow 2017, Yonow 2018, Gosliner 1992).

Marine heterobranchs are interesting in many ways. Many of them have reduced or lost their shells and developed alternative defense or antifouling systems, by taking up natural compounds from their food, or by de novo synthesis. These compounds are of interest as potential drug leads for medical application (Okino et al. 1996; Burghardt & Gosliner 2008; Böhringer et al. 2017; Fisch et al. 2017. The loss of the shell allows sea slugs to develop stunning body shapes and coloration, and they are therefore well-known to snorkelers, divers, and underwater photographers (Haber et al. 2010). The diversity of heterobranchs serves as an indication of the diversity of other metazoan life forms such as sponges, cnidarians, ascidians and bryozoans, as they provide nutrition with high food specificity on the species level. These filter feeders or consumers on lower levels are highly affected by changes in the environment, thus also affecting sea slug communities (e.g., Wells & Bryce 1993; Nimbs et al. 2016; Nimbs et al. 2018).

Burghardt et al. (2006) recorded molluscan diversity for the first time in North Sulawesi (Bunaken National Park, BNP), including heterobranch taxa. More recently, several

publications on heterobranch diversity have been published from North Sulawesi, including Bunaken National Park, Lembeh Strait and Sangihe Island (Kaligis et al. 2018; Eisenbarth et al. 2018; Ompi et al. 2019; Undap et al. 2019), which renders this area as one of the best investigated places in Indonesia with regards to this taxon. Our study in Bangka Archipelago increases our knowledge on the biodiversity of heterobranchs in North Sulawesi in an area that has never been documented before.

Bangka Archipelago comprises small islands located in the northeast of North Sulawesi, thus lying in between the recently well-studied area of Bunaken National Park, and the famous Lembeh Strait, whereas Sangihe Island lies approximately 200 km to the north. All studies have shown that these areas differ in heterobranch composition due to the differing habitat compositions (Eisenbarth et al. 2018; Ompi et al. 2019; Undap et al. 2019). Bangka Island exhibits biogenic reefs, volcanic rocks, and a few areas with mangroves and seagrass meadows. The mainland facing Bangka Island is of volcanic origin with smaller fringing reefs. The central area of the archipelago is shallow, while the outer slopes drop down to more than 1000 m depth. The islands are covered by lush vegetation and are framed by fringing reefs, alternating with few mangroves and volcanic cliffs. The islands are home to some villages and a few resorts, of which the oldest one was built in 1987. Bangka, the largest island of this archipelago (approximately 48 km2), has a resident population of nearly 2500 inhabitants and only six resorts Ponti et al. (2016). Therefore, this island is less affected by tourists than the more famous Bunaken National Park (BNP), Lembeh Strait, or other Indonesian islands, such as Bali. However, implementation of mining operations began in 2012 in coastal areas, leading to deforestation of mangroves and broad land erosion within only 5 years. Unfortunately, there is no detailed data available for the diversity of marine organisms and coral reef health in Bangka Island before mining started; therefore, the impacts of the mining in areas close by cannot be assessed. This study therefore aims to serve as a baseline for future monitoring surveys concerning further environmental changes within the Bangka Archipelago.

2. Materials and Methods

Material was collected during the dry season, in September 2017 and September 2018. In total, 28 dives were performed during the day and 10 during the early night, with 3–5 divers and 60–120 minutes per dive in 19 different localities around Bangka Island and the adjacent mainland (Figure 1A, B). A depth range from the eulittoral down to ca. 20 m was covered.

Table 1 summarizes the specific conditions of the various diving localities. Various substrates, such as macro algae and coral rubble, were collected for further analyses in the laboratory. Additionally, several hours were spent collecting and photo-documenting while snorkeling. In a few sites, only underwater photographs were taken of sea slugs for species recording. These species are included in our results on species numbers, but not in our overall specimen numbers.

Each collected specimen was documented with an Olympus TG5 under water (when possible) and additionally in the laboratory, provided with a unique identifier (abbreviation of name, year, location, and number of specimen), and preliminarily identified with the help of available literature (e.g., Yonow 2001; Burghardt 2006; Haber et al. 2010; Yonow 2011; Martinov et al. 2012; Burghardt & Wägele 2014; Gosliner et al. 2015; Stoffel et al. 2016; Yonow 2017; Layton 2018; Eisenbarth 2019; Seaslugforum.net;). Gosliner et al. (2015) depict many undescribed species. We used their species number for our internal identifier when we assigned one of our specimens to these undescribed species. However, we applied only a letter (e.g., sp. a, or sp. b) when a specific assignment to any undescribed species in this identification book was not possible and/or when our barcodes checked against the reference database in NCBI (National Center for Biotechnology Information) provided no hit. Validity of names was checked in the World Register of Marine Species (http://www.marinespecies.org/). Collected specimens were preserved in 96% EtOH and/or in formaldehyde/seawater.

Those families with documented cryptic speciation (e.g., Chromodorididae, Phyllidiidae) and a few other rare species of interest (e.g., *Moridilla)* were analyzed by using barcoding methods to verify identification. Additionally, small specimens that were difficult to identify (if at all) or where no clear assignment was possible were also barcoded.

Figure 1. Location of the study area: (**A**) depiction of the 19 diving sites at Bangka Archipelago and mainland. Insert showing the region of North Sulawesi with Bangka Archipelago. (**B**) Indonesia and Sulawesi, with the blue circle indicating the close-up area in **(A)**.

Table 1. Information about collection sites and dates in 2017–2018. *Batu Tiga is the only locality where no samples were collected, and specimens were recorded only by photo-documentation provided by Gianni Valenti.

Area of collection Site	Location	Characteristic of the Habitat	Year of Collection
Bangka Archipelago			
Batu Belah	1°46'22.20"N 125°10'57.38"E	sandy bottom with a deposit of large formation of stones richly covered with corals	2017-2018
Batu Tiga	1°46'07.6"N 125°10'35.7"E	Coral pinnacles on a deep coral sand bottom	2018*
Areng Kambing	1°46'07.8"N 125°10'34.6"E	Coral sand and coral rabble slope	2018
Sahaung	1°44'35.11"N 125 09'43.5"E	A shallow area made up of rocks and pinnacles of volcanic origin, richly covered with coral formations	2017
Tanjung Husi	1°44' 07.74"N 125° 09' 05.59"E	Volcanic rockslide, characterized by the presence of strong tidal currents and ocean swell	2017-2018
Busa Bora Timur	1°44'27.4"N 125°08'35.1"E	Fringing reef on coral sand slope	2018
Busa Bora Kampung	1°44'39.8"N 125°08'24.4"E	Fringing reef on coral sand slope in front of a little village	2018
Coral eye	1°45'03.61"N 125°07'58.85"E	Fringing reef in front of resort, 2012 was the last storm (Kaligis et al. 2018). Dominant branching coral and little rubble. Coral grown over the tide surface.	2017-2018
Mangrove	1°45'46.9"N 125°07'50.0"E	Fringing reef on coral sand slope that start from the lagoon in front of the mangrove	2017-2018
Sipi	1°47'02.8"N 125°07'42.4"E	River mouth: Mud / Fringing reef. site impacted by mining operations	2018
Pearl Farm	1°48'41.3"N 125°06'45.1"E	The former abandoned pear farm, fringing reef on coral sand slope	2018
Kinabuhutan	1°50'45.44"N 125° 5'55.26"E	Fringing reef on shallow coral sand slope	2018
Talisei	1°53'38.32"N 125°05'53.82"E	A vertical wall of volcanic rock that ended on a sandy bottom. The spot is subject to strong tidal currents and ocean swell, so the coral formations are small and compact	2018
Main Land			
Yellow Coco	1°40'21.67"N 125° 8'6.60"E	Fringing reef on a mix of volcanic and coral sand slope	2018
Sempini	1°41'4.83"N 125° 8'32.65"E	Fringing reef on coral sand slope	2018
Sempini 2	1°40'51.06"N 125° 8'22.45"E	Fringing reef on coral sand slope	2018
Efrata	1°40'52.34"N 125° 9'10.55"E	Volcanic rock beach and follow by volcanic sand.	2018
Batu Mandi	1°41' 16.71" N 125° 09' 42.79" E	Volcanic rock covered by coral reef, sponge and other invertebrates. Various topography	2017-2018
Tanjung Kusu-kusu	1°41'23.01"N 125°09'55.26"E	Volcanic rock wall on a backdrop of rock and coral, characterized by the presence of strong tidal currents and ocean swell	2017

All specimens are registered in the collection of Sam Ratulangi University according to the year under the numbers SRU2017/01 and SRU2018/01. Metadata of each individual are documented in the database Diversity Collection (Diversity Workbench) using the data brokerage service of the German Federation for Biological Data (https://www.gfbio.org/; Diepenbroek et al. 2014). Data are publicly available at www.gfbio.org for browsing and the archived data can be downloaded at https://doi.org/10.20363/heterobranchia-bangka-prj-1.1

3. Results

In total, 484 specimens comprising 149 species of marine heterobranchs were collected in the two sampling events in 2017 and 2018. Five species can be assigned to Cephalaspidea, two to Aplysiida, 15 to Sacoglossa, two to Pleurobranchida, and 124 to Nudibranchia. Additionally, one species is a member of the eupulmonate taxon Onchidiidae. Thirteen species of the 149 were only recorded by photo-documentation in 2018 without collection. Thirty-three species cannot be assigned to any described species and are considered new species. Table 2 summarizes available information all collected and photo-documented specimens, and also includes information about taxa authorities (these are therefore not mentioned in the text). Families and species are listed in the same order as in the text below. The table also provides information about the presence of the particular species in BNP, Sangihe, and Lembeh Strait according to the recently published surveys (Eisenbarth et al. 2018; Ompi et al. 2019; Undap et al; 2019) to highlight those species that are only recorded from our study area. All species listed in Table 2 are also depicted by at least one specimen, together with the specimen identifier, if the animal was collected, and are available for further investigations. In the following sections, species and specimens are discussed and compared to other studies from North Sulawesi (Kaligis et al. 2018; Eisenbarth et al. 2018; Ompi et al. 2019; Undap et al; 2019).

3.1. Animal Collections

3.1.1. Cephalaspidea (Five Species in Five Genera Belonging to Four Families)

The number of cephalaspidean species is low in comparison to the number from BNP (5 vs. 16).

Table 2. List of species, specimen identifiers, and further metadata of the material; (*) indicate species that were identified only by photo-documentation. The last three columns indicate the number of specimens or occurrence of the respective species in other study areas nearby (information is taken from Eisenbarth et al. (2018), Undap et al. (2019), and Ompi et al. (2019): BNP, Lembeh Strait, and Sangihe Island).

			Expedition in Bangka Archipelago and Main land nearby																			Other Expeditions		
						Bangka Archipelago											Mainland							
Higher Taxon Affiliation	Identifier	Species Name	Num of specimens	Size (mm)	Depth (m)	Batu Belah	Batu Tiga	Areng Kambing	Sahaung	Tanjung Husi	Busa Bora Timur	Busa Bora Kampung	Coral Eye	Mangrove	Sipi	Pearl Farm	Kinabuhutan	Talisei	Yellow Coco	Sempini	Efrata	Batu Mandi	Tanjung Kusu-kusu	Exp Bunaken	Exp Sangihe	Exp Lembeh
		Cephalaspidea (5 species)																							
Haminoeidae Pilsbry, 1895	Hasp18Ba-1	*Haminoea* sp. *(Haminoea* sp. 3 in Gosliner et al. (2015): 30)	1	2	7												1						x	-	-
Colpodaspididae Oskars, Bouchet & Malaquias, 2015		*Colpodaspis thompsoni* G. H. Brown, 1979	5	2-5	1.5-9.2					2		1	1			1							x	-	-
Aglajidae Pilsbry, 1895 (1847)		*Chelidonura amoena* Bergh, 1905	2	11-25	4-9.1								2										x	-	-
	Odsp.a18Ba-1	*Odontoglaja* sp. a	1	15	1.5								1										-	-	-
Gastropteridae Swainson, 1840		*Sagaminopteron psychedelicum* Carlson and Hoff, 1974	2	3	1								1										x	-	-
		Aplysiida (2 species)																							
Aplysiidae Lamarck, 1809		*Aplysia parvula* Mörch, 1863	4	4-10	1								4										x	-	-
		Stylocheilus striatus (Quoy & Gaimard, 1832)	2	5-15	7-7.5								1							1			x	-	-
		Sacoglossa (15 species)																							
Oxynoidae Stoliczka, 1868 (1847)	Losp1-18Ba1; Losp1-18Ba2	*Lobiger* sp.*(Lobiger* sp. 1 in Gosliner et al. (2015): 70)	2	2-11	8.4					2													x	-	-
Hermaeidae H. Adams & A. Adams, 1854		*Cyerce bourbonica* Yonow, 2012	2	3-4	1-1.5								2										x	-	-
		Cyerce nigra Bergh, 1871	1	4	8.6					1													-	-	-
Plakobranchidae Gray, 1840		*Elysia asbecki* Wägele, Stemmer, Burghardt and Händeler, 2010	1	3	8.5					1													x	-	-
		Elysia marginata (Pease, 1871)	1	3	11.6																	1	x	-	-
		Elysia cf *nigropunctata* (Pease, 1871)	1	21	2.6								1										-	-	-
		Elysia pusilla (Bergh, 1871)	2	7-11	1-1.5								1	1									x	x	-
	Elsp24-18Ba-1	*Elysia* sp. 24 (in Gosliner et al. (2015): 89)	1	7	7								1										-	-	-

	Elsp27-18Ba-1	*Elysia* sp. 27 (in Gosliner et al. (2015): 89)	1	5	7								1											-	-	-
	Elsp.a18Ba-1	*Elysia* sp. a	1	8	1									1										x	-	-
	Elsp.b18Ba-1	*Elysia* sp. b	1	9	20														1					x	-	-
		Thuridilla carlsoni Gosliner, 1995	2	10-17	7.6-8.3	1														1				-	-	-
		Thuridilla flavomaculata Gosliner, 1995	1	5	5.3					1														x	-	-
		Thuridilla vataae (Risbec, 1928)	2	7-9	1.5-3								2											x	-	-
		Thuridilla gracilis (Risbec, 1928)	12	8-24	1-12.8	1				2		3	4	1	1									-	x	-
		Pleurobranchida (2 species)																								
Pleurobranchidae Gray, 1827		*Pleurobranchus forskalii* Rüppell and Leuckart, 1828	2	23-25	4-12							1	1											x	-	-
		Pleurobranchus peronii Cuvier, 1804	2	19-140	2-12							1	1											-	-	-
		Nudibranchia, Doridina (82 species)																								
Hexabranchidae Bergh, 1891	Glsp1_17Ba-1	*Hexabranchus sanguineus* (Rüppell & Leuckart, 1830)	1	10	6.6					1														x	-	-
Polyceridae Alder and Hancock, 1845		*Nembrotha chamberlaini* Gosliner & Behrens, 1997	5	11-52	2-11.7				1			1	2			1								-	-	-
		Nembrotha cristata Bergh, 1877	5	48-65	1-8.1				3													2		x	-	-
		Nembrotha kubaryana Bergh, 1877	13	23-80	4.2-20				1	1			1		1	2			1	2	2		2	x	-	x
		Nembrotha lineolata Bergh, 1905	5	30-58	4-16.2								4									1		-	-	-
		Nembrotha milleri Gosliner & Behrens, 1997	1	80	6				1															-	-	-
		Nembrotha mullineri Gosliner & Behrens, 1997	2	17-20	7.2-11.7								2											-	-	-
	Nesp1_17Ba-1	*Nembrotha* sp. 1 (in Gosliner et al. (2015): 122)	1	11	8.7																	1		-	-	-
		Tambja gabrielae Pola, Cervera & Gosliner, 2005	1	51	11.8							1												-	-	-
		Tambja morosa (Bergh, 1877)	4	52-90	8-16.6						2		1									1		-	-	-
		Gymnodoris aurita (Gould, 1852)*	X										X											-	-	-
		Gymnodoris tuberculosa Knutson & Gosliner, 2014	1	3	1								1											x	-	-
	Gysp20-18Ba-1	*Gymnodoris* sp. 20 (in Gosliner et al. (2015): 156)	1	3	5					1														-	-	-
	Gysp25-18Ba-1-2	*Gymnodoris* sp. 25 (in Gosliner et al. (2015): 157)	2	4-5	1								2											-	-	-
Goniodorididae H. Adams and A. Adams, 1854	Gosp7-18Ba-1	*Goniodoris* sp. 7 (in Gosliner et al. (2015): 153)	1	6	10								1											-	-	-
		Trapania armilla Gosliner & Fahey, 2008	1	7									1											-	-	-
		Trapania safracornia Fahey, 2004	1	7	13.4							1												-	-	-

Aegiridae P. Fischer, 1883	Aesp7-18Ba-1	*Aegires* sp. 7 (in Gosliner et al. (2015): 149)	1	4	7										1													-	-	-
		Notodoris minor Eliot, 1904*	X					X																					-	-	-
Discodorididae Bergh, 1891		*Asteronotus cespitosus* (van Hasselt, 1824)*	X											X															-	-	-
		Asteronotus mimeticus Gosliner & Valdés, 2002	9	5-25	3-11.9									8									1						x	-	-
		Atagema intecta (Kelaart, 1859)	2	12-55	7-8									2															-	-	-
		Discodoris cebuensis Bergh, 1877	2	48-53	11.2									2															-	-	-
		Halgerda batangas Carlson & Hoff, 2000	10	32-57	6.2-19.2	1										1					2	5	1						x	-	x
		Halgerda carlsoni Rudman, 1978	2	33-40	11.8-12																			1	1				x	-	
		Jorunna funebris Kelaart, 1859	2	15-20	2										2														-	-	x
		Paradoris liturata (Bergh, 1905)	3	22-43	8-8.5								1										1		1				-	-	-
		Platydoris sanguinea Bergh, 1905	1	12	5.5									1															x	-	-
Chromodorididae Bergh, 1891		*Ceratosoma tenue* (Abraham, 1876)*	X																		X								-	-	-
		Chromodoris dianae Gosliner & Behrens, 1998	2	16-20	13.6-15						1		1																x	x	-
		Chromodoris annae Bergh, 1877	46	18-50	1.5-12.1	3		4	10	6			19				1					1		2				x	x	x
		Chromodoris elisabethina Bergh, 1877*	X																			X							-	x	-
		Chromodoris lochi Rudman, 1982	15	17-47	4-20.4	3	1		1	1	2	2											4		1				x	-	x
		Chromodoris magnifica (Quoy & Gaimard, 1832)	2	37-60	2-5.9								1				1												-	-	-
		Chromodoris quadricolor (Rüppell & Leuckart, 1830)	1	38	15				1																				-	-	-
		Chromodoris strigata Rudman,1982	2	22-28	7.4-13.6							1	1																x	x	-
		Chromodoris willani Rudman, 1982	2	37-40	8					2																			x	-	-
		Doriprismatica atromarginata (Cuvier, 1804)	4	17-74	7.1-10				1							2									1				-	-	-
		Doriprismatica sibogae Berg, 1905	1	85	2								1																-	-	-
		Glossodoris cincta (Bergh, 1888)	2	34-60	1-6								2																x	x	x
		Glossodoris rufomarginata (Bergh, 1890)*	X																				X						-	-	-
		Goniobranchus coi (Risbec, 1956)	2	30-40	4								2																-	-	-
		Goniobranchus fidelis (Kelaart, 1858)	1	13	17																			1					x	-	x
		Goniobranchus geometricus (Risbec, 1928)	6	613-22	5-14.8	1										3		1			1								x	x	x
		Goniobranchus kuniei (Pruvot-Fol, 1930)	1	46	12																1								-	-	-
		Goniobranchus reticulatus (Quoy & Gaimard, 1832)	2	48-66	10.3																	1		1					x	x	x
		Goniobranchus verrieri (Crosse, 1875)	1	3	20																1								-	-	-
		Hypselodoris apolegma (Yonow, 2001)	1	33	16.4																			1					x	-	-
		Hypselodoris bullockii (Collingwood, 1881) *	X																					X					-	-	-
	Hysp1_17Ba-1	*Hypselodoris cerisae* Gosliner & Johnson, 2018	1	16	6.6								1																-	-	-

		Hypselodoris decorata Gosliner & Johnson, 2018	1	20	8.1	1	1							1							1	x	-	-
		Hypselodoris emma Rudman, 1977*	X															X				-	-	-
	Chromos18Ba-1	*Hypselodoris iacula* Gosliner & R. F. Johnson, 1999	1	50	15														1			-	-	-
	Hysp19_17Ba-1-2	*Hypselodoris lacuna* Gosliner & Johnson, 2018	2	6-8	6.8														2			-	-	-
		Hypselodoris maculosa (Pease, 1871)	4	18-23	3-10.3	1																-	-	-
		Hypselodoris maridadilus Rudman, 1977	1	10	6.5				1													-	-	-
		Hypselodoris zephyra (Eliot, 1904)	1	9	14.2	1																-	-	-
		Hypselodoris tryoni (Garret 1873)	7	26-54	4-14.1						2		2					2	1			x	x	x
	Thsp1-18Ba-1	*Hypselodoris* sp. a	1	8	11.5													1				-	-	-
		Mexichromis aurora (R. F. Johnson & Gosliner, 1998)	1	11	8.6									1								-	-	-
		Mexichromis trilineata (A. Adams & Reeve, 1850) *	X						X													-	-	-
Doridoidea Rafinesque, 1815	Dorid18Ba-1	*Miamira magnifica*	1	2.5	20												1					x	-	-
	Misp17Ba_1	*Miamira* sp.a	1	10	4							1										-	-	-
		Noumea simplex Pease, 1871	1	6	5.2				1													-	-	-
	Thfu18Ba-1-2	*Thorunna furtiva* Bergh, 1878	2	14-16	13.8												2					x	-	-
Dendrodorididae O'Donoghue, 1924 (1864)		*Dendrodoris nigra* (Stimpson, 1855)	1	6	1							1										-	-	-
Phyllidiidae Rafinesque, 1814		*Phyllidia* cf *babai* Brunckhorst, 1993*	X			X																-	-	-
		Phyllidia coelestis Bergh, 1905	8	12-43	1.5-20	X		1		1		1				1	3	1				x	x	-
		Phyllidia elegans Bergh, 1869	3	34-38	2										1		2					x	-	x
		Phyllidia exquisita Brunckhorst, 1993	2	30-32	4.5-12.4			1				1										-	-	-
		Phyllidia ocellata Cuvier, 1804	8	14-40	2-13.4	1				1		2					1	2	1			x	x	-
		Phyllidia picta Pruvot-Fol, 1957	5	18-35	4-12	2				1		1						1				-	x	-
		Phyllidia varicosa Lamarck, 1801	18	23-85	2-20		1		5			1		2		1	5	1		1		1	x	x	x
	Phsp18Ba-2-3	*Phyllidia* sp. a	2	33-34	6-13.5														1	1			-	x	-
		Phyllidiella annulata (Gray, 1853)	1	30	10												1					x	-	-
		Phyllidiella lizae Brunckhorst, 1993	4	15.26	5-20												1	1	2			-	-	-
		Phyllidiella nigra (van Hasselt 1824)	1	56	1								1									-	x	-
		Phyllidiella pustulosa (Cuvier, 1804)	39	12-75	1.5-20	5	2	2	1		2	7		1	3	3	2	2	6	1	2	x	x	x
		Phyllidiopsis annae Brunckhorst, 1993	4	3-12	9.2-11.9						2							2				-	-	-
		Phyllidiopsis cf. *burni* Brunckhorst, 1993	1	39	20												1					-	-	-
		Phyllidiopsis krempfi Pruvot-Fol, 1957	3	17-56	9.2-16.2		1											1		1		-	x	-
		Phyllidiopsis xishaensis (Lin,1983)	4	9-17	3.9-16.8				1					2						1		x	-	-

Nudibranchia, Cladobranchia (42 species)																			
Arminidae Iredale and O'Donoghue, 1923		*Dermatobranchus caeruleomaculatus* Gosliner & Fahey, 2011*	X										X			-	-	-
		Dermatobranchus rodmani Gosliner & Fahey, 2011	2	3-4	1				2							-	-	-
		Dermatobranchus pustulosus van Hasselt, 1824	1	70	9.1	1										-	-	-
	Desp.a18Ba-1-2	*Dermatobranchus* sp. a	2	8-11	1				2							-	-	-
	Desp18Ba-1	*Dermatobranchus* sp. b	1	6	20.4									1		-	-	-
	Desp17Ba-1	*Dermatobranchus* sp. c	1	3	3				1							-	-	-
Proctonotidae Gray, 1853		*Janolus* sp. 1 (in Gosliner et al. (2015): 305)*	X										X			-	-	-
Bornellidae Bergh, 1874		*Bornella anguilla* S. Johnson, 1984	2	25-40	7.8-8.5	2										-	-	-
		Bornella dotoides Pola, Rudman, Gosliner 2009	1	8	1				1							-	-	-
Tethydidae Rafinesque, 1815	Mesp.a18Ba-1	*Melibe bucephala* Bergh, 1902	1	9	1				1							-	-	-
		Melibe engeli Riscec, 1937	1	34	7			1								-	-	-
Dotidae Gray, 1853		*Doto ussi* Ortea, 1982	3	4-12	4-6.5				2		1					x	-	-
Tritoniidae Lamarck, 1809	Masp2.18Ba-1	*Marionia* sp. 2 (in Gosliner et al. (2015): 324)	1	40	2				1							-	-	-
	Trsp3_17Ba-1	*Tritonia* sp. 3 (in Gosliner et al. (2015): 320)	1	13	4				1							-	-	-
Flabellinidae Bergh, 1889		*Coryphellina exoptata* (Gosliner and Willan, 1991)	3	16-32	2-12.4	1		1			1					x	-	-
		Coryphellina rubrolineata O'Donoghue, 1929	8	20-40	6.3-20.4	1	2				2		1	1	1	x	-	x
	Flsp2-18Ba-1	*Flabellina* sp. 2 (in Gosliner et al. (2015): 333)	1	16	14.5					1						-	-	-
	Flsp3_17Ba-1	*Flabellina* sp. 3 (in Gosliner et al. (2015): 333)	1	11	13.6									1		-	-	-
Samlidae Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017		*Samla riwo* (Gosliner and Willan, 1991)	3	9-13	4-8.6				2					1		x	-	-
Eubranchidae Odhner, 1934		*Eubranchus* sp. 22 (in Gosliner et al. (2015): 341)	1	6	5.1		1									x	-	-
Trinchesiidae F. Nordsieck, 1972		*Trinchesia yamasui* (Hamatani, 1993)	X										X			-	-	-

Cuthonidae Odhner, 1934		*Cuthona* sp. 57 (in Gosliner et al. (2015): 353)	2	17-25	12.7								2												-	-	-
Facelinidae Bergh, 1889	Aeol18Ba-1	*Aeolidia* sp. a	1	8	1.5								1												-	-	-
	Ansp17Ba-1	*Antonietta* sp. a	1	2	6.4																		1		-	-	-
		Caloria indica (Bergh, 1896)	15	2-38	5-13.4	5				2			3			1				1	2	1			x	-	-
	Crsp17Ba-1	*Cratena* sp. a	1	8	4								1												-	-	-
		Favorinus japonicus Baba, 1949	2	10-16	8.9-20											1			1						x	-	-
	Fasp1-18Ba-1-2	*Favorinus* sp. 1 (in Gosliner et al. (2015): 363)	2	13-15	20															2					-	-	-
		Favorinus tsuruganus Baba and Abe, 1964	1	11	2								1												-	-	-
	Mojo18Ba-1	*Moridilla* sp. a	2	12	6.8-11.2	1				1															-	-	-
		Noumeaella sp. 2 (in Gosliner et al. (2015): 367)	1	11	4								1												-	-	-
		Noumeaella sp. 3 (in Gosliner et al. (2015): 367)	6	8-12	4-9								6												x	-	-
		Noumeaella sp. 13 (in Gosliner et al. (2015): 369)	7	7-16	3-9								7												-	-	-
		Phyllodesmium briareum (Bergh, 1896)	17	15-60	3-20								16						1						x	-	-
		Phyllodesmium cf *crypticum* Rudman, 1981	5	8-22	4								5												-	-	-
		Phyllodesmium lizardense Burghardt, Schrödl & Wägele, 2008	3	10-13	1-10							1	2												-	-	-
		Phyllodesmium longicirrum (Bergh, 1905)	1	125	25																1				-	-	-
		Phyllodesmium magnum Rudman, 1991	2	50	5.5-11.5					1			1												-	-	-
		Phyllodesmium parangatum Ortiz & Gosliner, 2003	5	8-15	1-7								5												-	-	-
		Phyllodesmium pecten Rudman, 1981	1	15	1								1												-	-	-
		Phyllodesmium poindimiei (Risbec, 1928)	2	14-21	4-8.9								1								1				x	-	-
		Pteraeolidia semperi (Bergh, 1870)	25	13-54	5-8.9	2		2		1										15	2	3			x	-	-
Eupulmonata (1 species)																													
	Onchidiidae	*Peronia* sp. a	1	39	1									1											-	-	-
Total			**149**			**34**	**0**	**11**	**14**	**40**	**14**	**23**	**162**	**8**	**13**	**16**	**7**	**12**	**23**	**41**	**30**	**23**	**14**		-	-	-

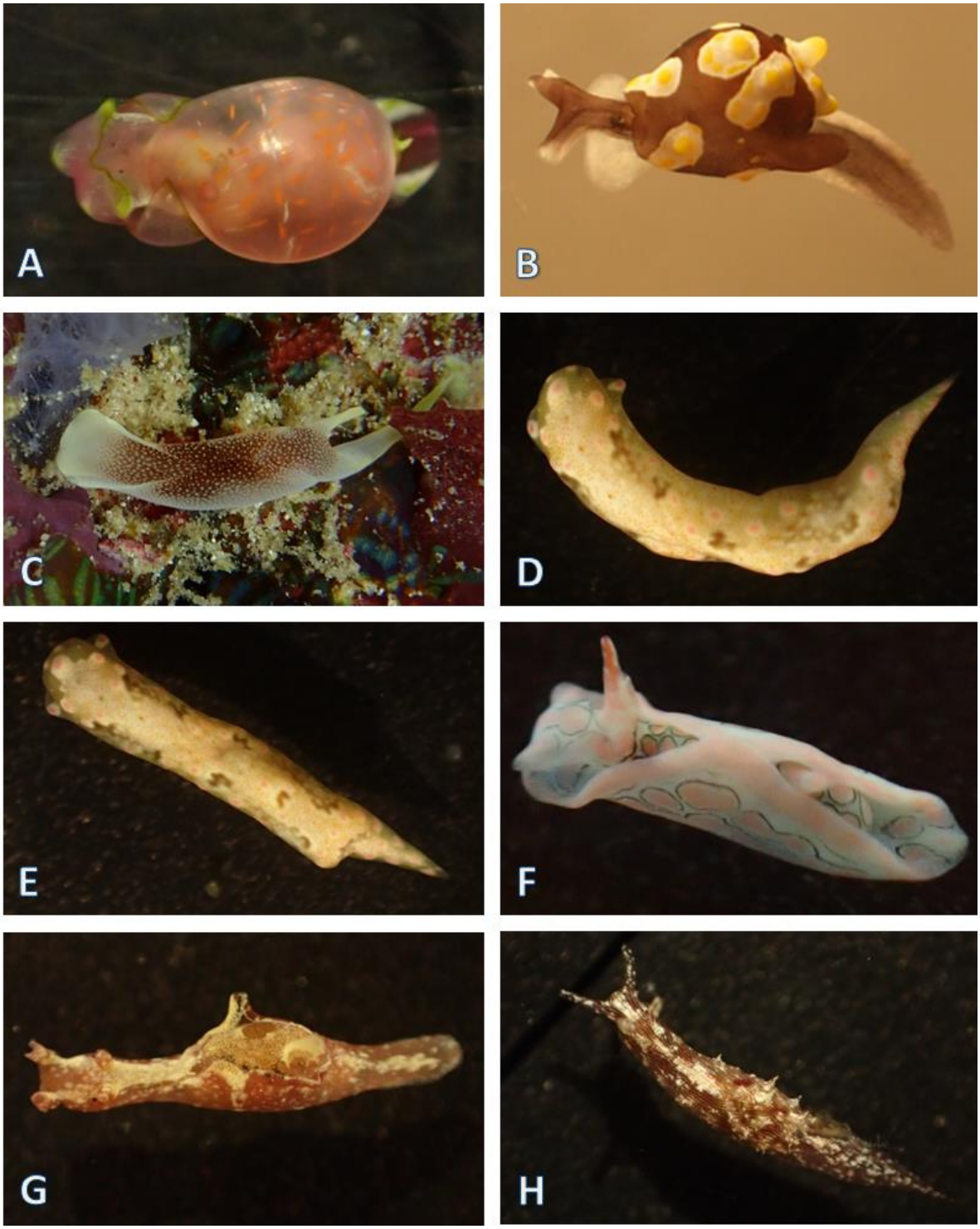


Figure 2. Cephalaspidea and Aplysiida: (**A**) *Haminoea* sp. (*Haminoea* sp. 3 in Gosliner et al. (2015): 34), Hasp18Ba-1; (**B**) *Colpodaspis thompsoni*, Coth18Ba-2; (**C**) *Chelidonura amoena*, Cham17Ba-1*;* (**D**, **E**) *Odontoglaja* sp. (*Odontoglaja* sp. a), Odsp.a18Ba-1; (**F**) *Sagaminopteron psychedelicum*, Saps18Ba-2; (**G**) *Aplysia* cf. *nigrocincta*, Appa18Ba-1*;* (**H**) *Stylocheilus striatus*, Stst18Ba-2.

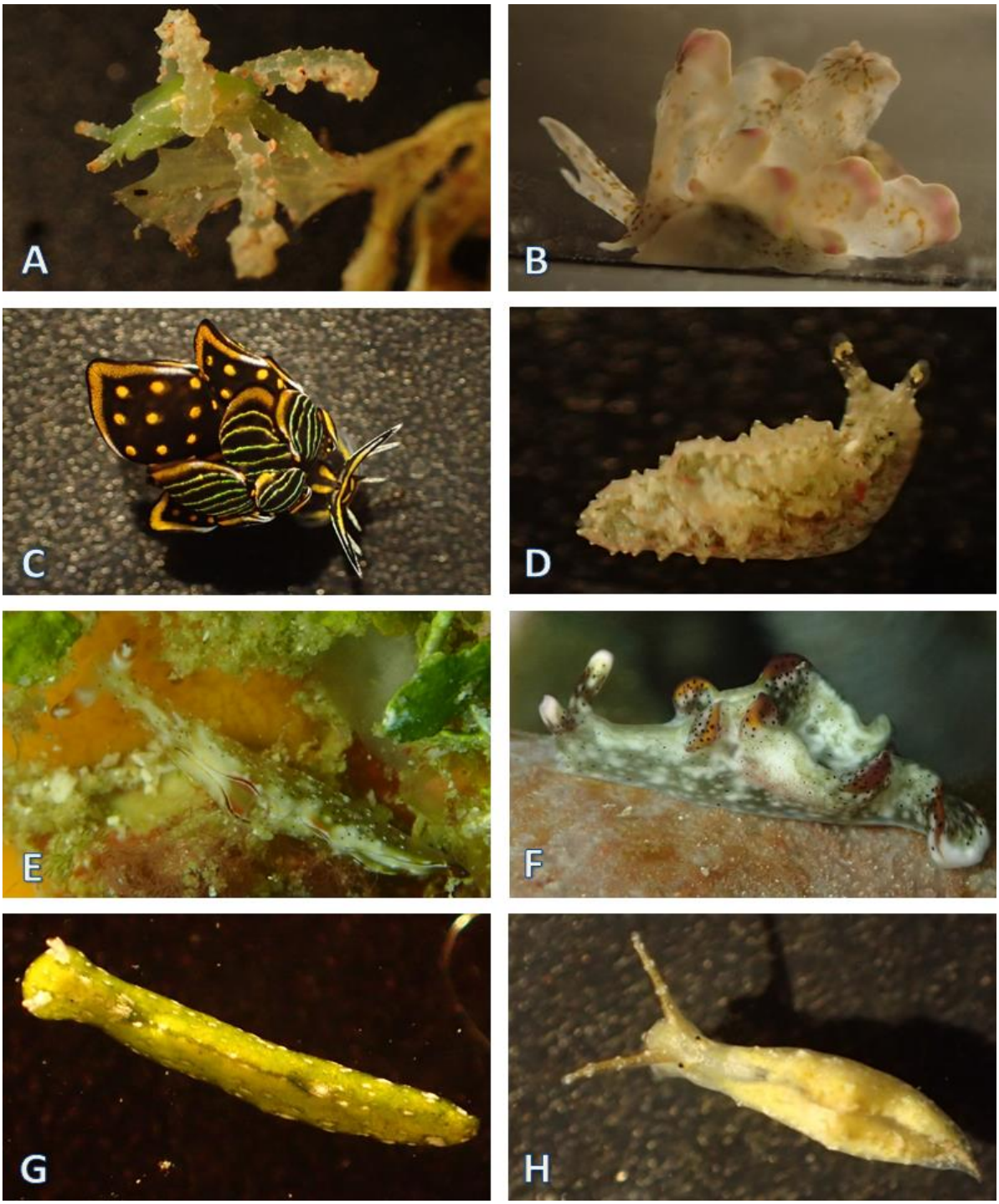


Figure 3. Sacoglossa: (**A**) *Lobiger* sp. (*Lobiger* sp. 1 in Gosliner et al. (2015): 70), Losp1-18Ba1; (**B**) *Cyerce bourbonica*, Cybo18Ba-1; (**C**) *Cyerce nigra*, Cyni18Ba-1*;* (**D**) *Elysia asbecki*, Elas18Ba-1; (**E**) *Elysia marginata*, Elma17Ba-1*;* (**F**) *Elysia* cf. *nigropunctata*, Elni18Ba-1*;* (**G**) *Elysia pusilla*, Elpu18Ba-2*;* (**H**) *Elysia* sp. (*Elysia* sp. 24 in Gosliner et al. (2015): 89), Elsp24-18Ba-1.

Haminoeidae: *Haminoea* sp. (Figure 2A), collected at a depth of 7 m, has a translucent orange shell with orange and yellow marks, resembling *Haminoea* sp. 3 from Gosliner et al. (2015).

Colpodaspidae: *Colpodaspis thompsoni* (Figure 2B; length 2–5 mm) was most often found crawling in the coral rubble at 1.5–9.2 m depth. With only five specimens, it was not as common as in BNP.

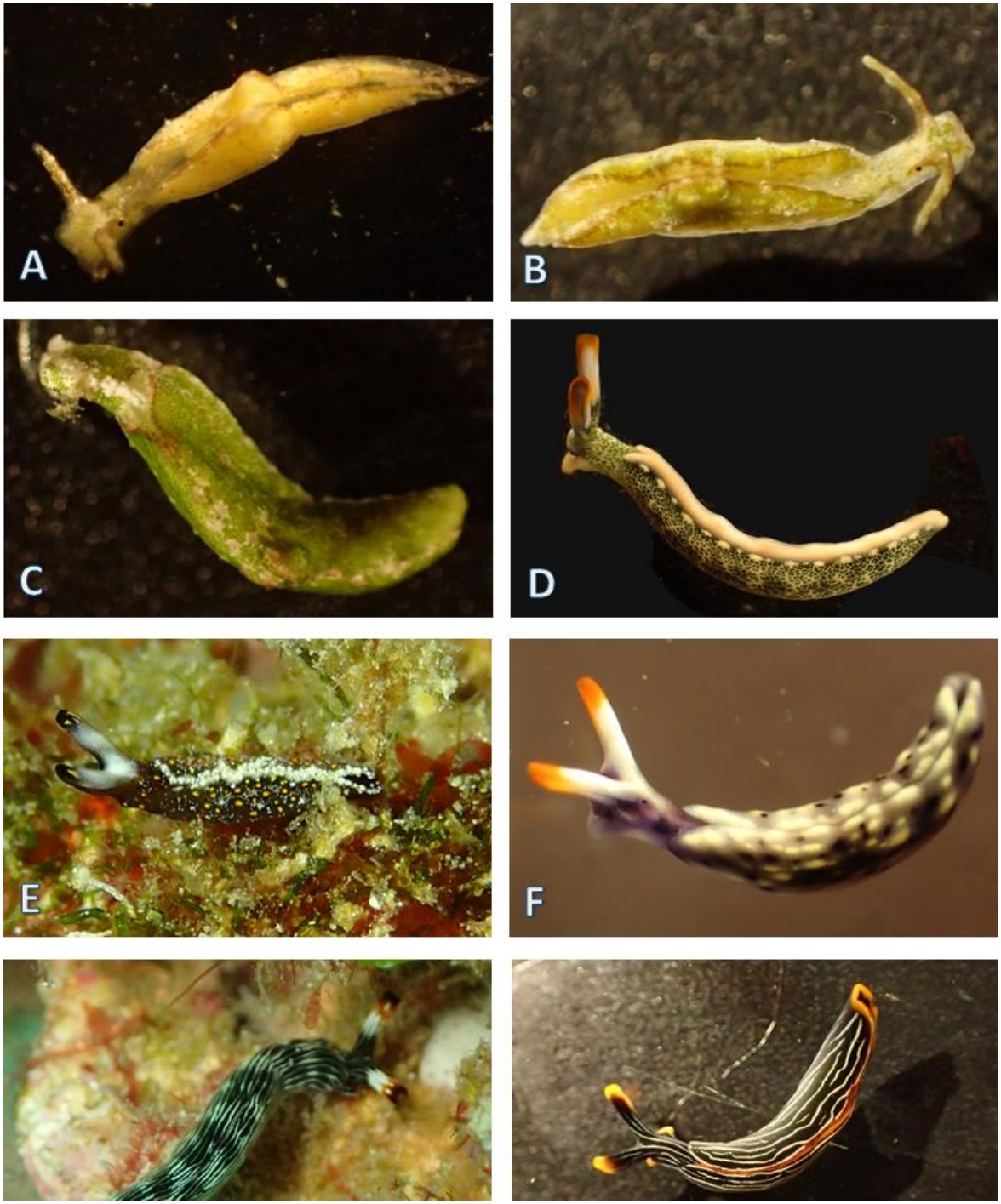


Figure 4. Sacoglossa: (**A**) *Elysia* sp. *(Elysia* sp. 27 in Gosliner et al. (2015): 89), Elsp27-18Ba-1; (**B**) *Elysia* sp. (*Elysia* sp. a), Elsp.a18Ba-1; (**C**) *Elysia* sp. (*Elysia* sp. b), Elsp.b18Ba-1; (**D**) *Thuridilla carlsoni*, Thca18Ba-1*;* (**E**) *Thuridilla flavomaculata*, Thfl17Ba-1*;* (**F**) *Thuridilla vataae*, Thva18Ba-3*;* (**G**, **H**) *Thuridilla gracilis*, Thgr18Ba-1, Thgr18Ba-4.

Aglajidae: Only two specimens of *Chelidonura amoena* (Figure 2C), a common species in sandy reefal habitats, were found in one site only. One specimen of *Odontoglaja* (Odsp.a18Ba-1, Figure 2D, E) was found in the coral rubble. It looks similar to *Odontoglaja guamensis* but lacks the distinct color pattern on the dorsum. Gosliner et al. (2015) depict several *Odontoglaja* spp. with similar background patterns; however, the dorsal color patterns and especially the intensity of the color differ in all of these. We cannot assign our specimen to any of these taxa.

Gastropteridae: Two specimens of *Sagaminopteron psychedelicum* (Figure 2F) were found in coral rubble.

3.1.2. Aplysiida (= Anaspidea) (Two Species in Two Genera Belonging to One Family)

Aplysiidae: Members of this family mainly forage on algae in shallow water. Four specimens of probably *Aplysia* cf. *nigrocincta* (Figure 2G) (Appa18Ba-3, with a black line along the parapodia) were found under coral rubble. Recently, Golestani et al. (2019) revised *Aplysia parvula* and identified 10 different lineages within this species. They resurrected the name *A. nigrocincta* Martens, 1880 for specimens from the Philippines and Papua New Guinea. However, molecular analyses still need to be performed on our material for correct assignment. The same holds true for the specimens described in Eisenbarth et al. (2018) as *A. parvula*. Two specimens of *Stylocheilus striatus* (Figure 2H) were found under coral rubble.

3.1.3. Sacoglossa (15 Species in Four Genera Belonging to Three Families)

Oxynoideae: *Lobiger* sp. 1 (Figure 3A), mimicking the green alga *Caulerpa*, has elongate leaf-like cerata and a green bubble-like shell. Our two specimens lack the blue lines typical for *L. viridis* (as depicted in Gosliner et al. 2015) and seem to be undescribed.

Hermaeidae: According to Bouchet et al. (2017), the family Caliphyllidae is united with this family, and we follow their systematics. Two *Cyerce* species were collected: one specimen of *C. bourbonica* (Figure 3B) shows the characteristic opaque white body, dark brown marks along the cerata and rhinophores, and violet to pink color at the tip of cerata. The other species can be assigned to *C. nigra* (Figure 3C), based on the typical color and color pattern of the cerata.

Plakobranchidae: Only the genera *Elysia* and *Thuridilla* are present in our collections. *Elysia asbecki* (Figure 3D), *E. marginata* (Figure 3E), *E.* cf. *nigropunctata* (Figure 3F), *E. pusilla* (Figure 3G), and four unidentified *Elysia* species were sampled from

various algae in the coral rubble or highly structured micro-habitats in the coral reef. *E.* cf. *nigropunctata* (Elni18Ba-1) was collected from a sponge, probably feeding on its epiphytic green algae. Four undescribed *Elysia* spp. (*Elysia* sp. 23, *Elysia* sp. 27, *Elysia* sp. a, and *Elysia* sp. b) in our collection indicate the need for further investigation with regard to this group: *Elysia* sp. 24 (Elsp24-18Ba-1; Figure 3H) and *Elysia* sp. 27 (Elsp27-18Ba-1; Figure 4A) are depicted in Gosliner et al. (2015), whereas *Elysia* sp. a (Elsp.a18Ba-1; Figure 4B) and *Elysia* sp. b (Elsp.b18Ba-1; Figure 4C) are featured here for the first time. These species were not found previously in North Sulawesi. *Elysia* sp. 24 has numerous white dots on its body, the rhinophores have three white bands, and the light green parapodia are without a line at the edge. *Elysia* sp. 27 (Elsp27-18Ba-1), collected from *Halimeda,* is pale green with brown dots and numerous opaque white spots on the dorsum and rhinophores; an orange line runs along the edge of the parapodia. *Elysia* sp. a (Figure 4B) looks like *Elysia* sp. 24 (Gosliner et al. 2015) but has a red line along the mantle margin. *Elysia* sp. b (Figure 4C) has an opaque white body color with small green dots covering the dorsal notum and parapodia. The rhinophores are relatively short, with papillae, and a brown line running along the margin of the parapodia crosses the foot on the ventral side. Four *Thuridilla* species were collected, including *T. carlsoni* (Figure 4D), *T. flavomaculata* (Figure 4E), *T. vataae* (Figure 4F), and *T.* cf. *gracilis* (Figure 4G, H), the latter being the most common *Thuridilla* species in the study area. According to Martín-Hervás et al. (2019), this species consists of a complex of 14 species. We cannot assign our specimens to any of these cryptic species, but we can provide more details on the coloration especially of the rhinophores that might help in future assignment of our material. Our specimens from Bangka have a black to dark brown background with narrow white to light green longitudinal lines, which are broader in certain areas, implicating transversal white interrupted patches. The parapodial edge exhibits a narrow orange line, but no blue spots on the outside of the parapodia. Some specimens have white rhinophores with a black tip apical to an orange band, and others have rhinophores only with orange tips (Figure 5A–E).

3.1.4. Pleurobranchida (Two Species in One Genus Belonging to One Family).

Pleurobranchida (Pleurobranchomorpha) are carnivorous, nocturnal animals usually large in size. We mainly observed the specimens during night dives.

Pleurobranchidae: *Pleurobranchus forskalii* (>200 mm) was only found during the night. The tubercles of this species (Figure 6A) are compound and brownish in color. The

specimens of *P. peronei* (Figure 6B), also only found during night dives, have only simple tubercles.

Figure 5. Variability of rhinophores in the *Thuridilla gracilis* species complex from Bangka Archipelago: (**A**) Thgr18Ba1; (**B**) Thgr17Ba-3; (**C**) Thgr18Ba4; (**D**) hgr18Ba6; (**E**) Thgr18Ba-5.

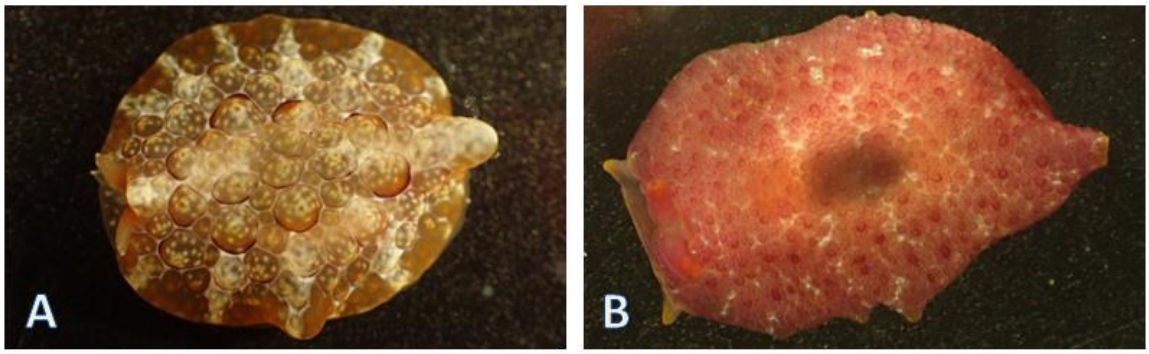


Figure 6. Pleurobranchida: (**A**) *Pleurobranchus forskalii*, Plfo18Ba-1; (**B**) *Pleurobranchus peronii*, Plpe18Ba-1.

3.1.5. Nudibranchia (124 Species)

a. Nudibranchia, Doridina (83 species in 29 genera belonging to eight families)

Doridina are represented in our study by nine families of the 17 listed in WoRMS (WoRMS Editorial Board 2021).

Hexabranchidae: One specimen of *Hexabranchus sanguineus* (Figure 7A) was found in coral rubble. It was a juvenile mimicking a *Hypselodoris maculosa* in coloration. Molecular analyses confirmed its assignment to *H. sanguineus.*

Polyceridae: The family is represented by 13 species in three genera. Compared to BNP and Sangihe, the genus *Nembrotha* is relatively common in Bangka Archipelago, with seven species records. Five specimens of *N. chamberlaini* (Figure 7B) and five specimens of *N. cristata* (Figure 7C) were collected at different sites and depths. *N. kubaryana* (Figure 7D), encountered quite often, is represented in our samples by 13 specimens, collected at 10 different sites. It exhibits some variation with regard to the presence of orange patterns or lines along the body. Two mating *N. lineolata* (Figure 7E) were only recorded by photographs. *N. milleri* (Figure 7F) was crawling on brown sponges near green tunicates. Two

specimens of *N. mullineri* (Figure 7G) were collected only during two night dives, one in 2017 and one in 2018. In both cases, the animals were crawling on sand. One undescribed *Nembrotha* (Nesp1_17Ba-1; Figure 7H), similar to *Nembrotha* sp. 1 (Gosliner et al. 2015), was collected on a greenish tunicate; however, our specimen has bluish rhinophores and gills. *Tambja gabrielae* (Figure 8A) and *T. morosa* (Figure 8B) were collected at 8–17 m depth. The monotypic genus *Gymnodoris* within the Gymnodorididae comprises many similar species with at least 60 undescribed species (Gosliner et al. 2015). The single specimen of *G. aurita* (Figure 8C) was identified only by photo-documentation. *Gymnodoris tuberculosa* (Figure 8D; 3 mm long) was crawling in coral rubble at 1 m depth. Two specimens of two *Gymnodoris* spp. (Figure 8E, F) are undescribed and depicted in Gosliner et al. (2915) as *Gymnodoris* sp. 20 and *Gymnodoris* sp. 25, respectively.

Goniodorididae: Members of this family are quite rare and not represented by many species in North Sulawesi (Eisenbarth et al. 2018). Some undescribed *Goniodoris* were recorded by Gosliner et al. (2015). We collected only one specimen (Figure 8G), which is very similar to *Goniodoris* sp. 7 in Gosliner et al. *Trapania armilla* (Figure 8H) was collected on a gray sponge in shallow water, whereas *T. safracornia* (Figure 9A) was collected in deeper areas. **Aegiridae**: Both acknowledged aegirid genera were present in our collections: *Aegires* sp. (Figure 9B), depicted in Gosliner et al. (2015) as *Aegires* sp. 7, and the "banana sea slug" *Notodoris minor* (Figure 9C) (the latter only by photo-documentation).

Discodorididae: The large and easily identifiable *Asteronotus cespitosus* (Figure 9D) was only recorded by photo-documentation. *Asteronotus mimeticus* (Figure 9E) is always associated with a phyllospongian sponge. It usually sits underneath and was more often observed during the night. The coloration of the sea slug matches perfectly with the color and structure of the sponge surface (Figure 9F). Two specimens of *Atagema intecta* (Figure 9G) were collected during night dives, one from sand substrate, the other on a sponge. Two specimens of *Discodoris cebuensis* (Figure 9H) were also collected during a night dive. *Halgerda batangas* (Figure 10A) is a common species in this location, while in other areas (e.g., BNP) the species is quite rare. The same is true for *Halgerda c.* (Figure 10B), represented by two specimens. *Jorunna funebris* (Figure 10C) was common in mangroves, and not all of the about 20 specimens seen were collected. *Paradoris liturata* (Figure 10D) is a member of the Discodorididae that mimics *Phyllidiella pustulosa,* illustrated in Figure 10E. *Paradoris liturata* has tubercles on the notum with black lines in between, as well as black rhinophores, similar to *P. pustulosa*. When the gills in *P. liturata* are retracted into the gill

pockets, the opening looks like the anus of a *Phyllidiella* species. *Platydoris sanguinea* (Figure 10F) was found on coral rubble at 5.5 m depth.

Figure 7. Nudibranchia Doridina: (**A**) *Hexabranchus sanguineus*, Glsp1_17Ba-1; (**B**) *Nembrotha chamberlaini*, Nech18Ba-1; (**C**) *Nembrotha cristata* Necr17Ba-2; (**D**) *Nembrotha kubaryana*, Neku17Ba-4; (**E**) *Nembrotha lineolata**; (**F**) *Nembrotha milleri*, Nemi17Ba-1; (**G**) *Nembrotha mullineri*, Nemu17Ba-1; (**H**) *Nembrotha* sp. (*Nembrotha* sp. 1 in Gosliner et al. (2015): 122), Nesp1_17Ba-1. *Specimen not collected.

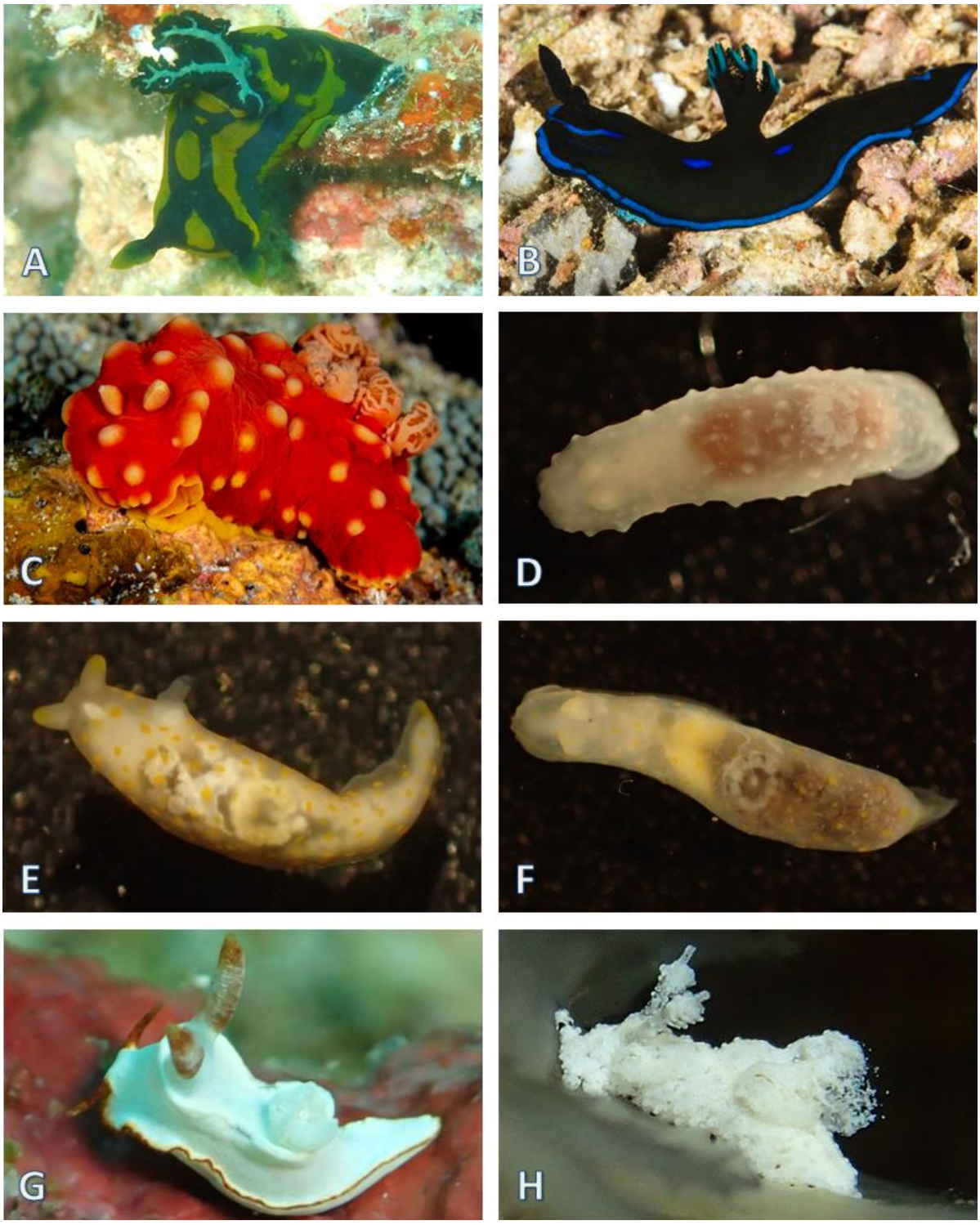


Figure 8. Nudibranchia Doridina: (**A**) *Tambja gabrielae*, Taga18Ba-1; (**B**) *Tambja morosa*;* (**C**) *Gymnodoris aurita*;* (**D**) *Gymnodoris tuberculosa*, Gytu18Ba-1; (**E**) *Gymnodoris* sp. (*Gymnodoris* sp. 20 in Gosliner et al. (2015): 156), Gysp20-18Ba1*;* (**F**) *Gymnodoris* sp. (*Gymnodoris* sp. 25 in Gosliner et al. (2015): 157), Gysp25-18Ba1; (**G**) *Goniodoris* sp. (*Goniodoris* sp. 7 in Gosliner et al. (2015): 153), Gosp7-18Ba1; (**H**) *Trapania armilla*, Trar17Ba-1. *Specimen not collected.

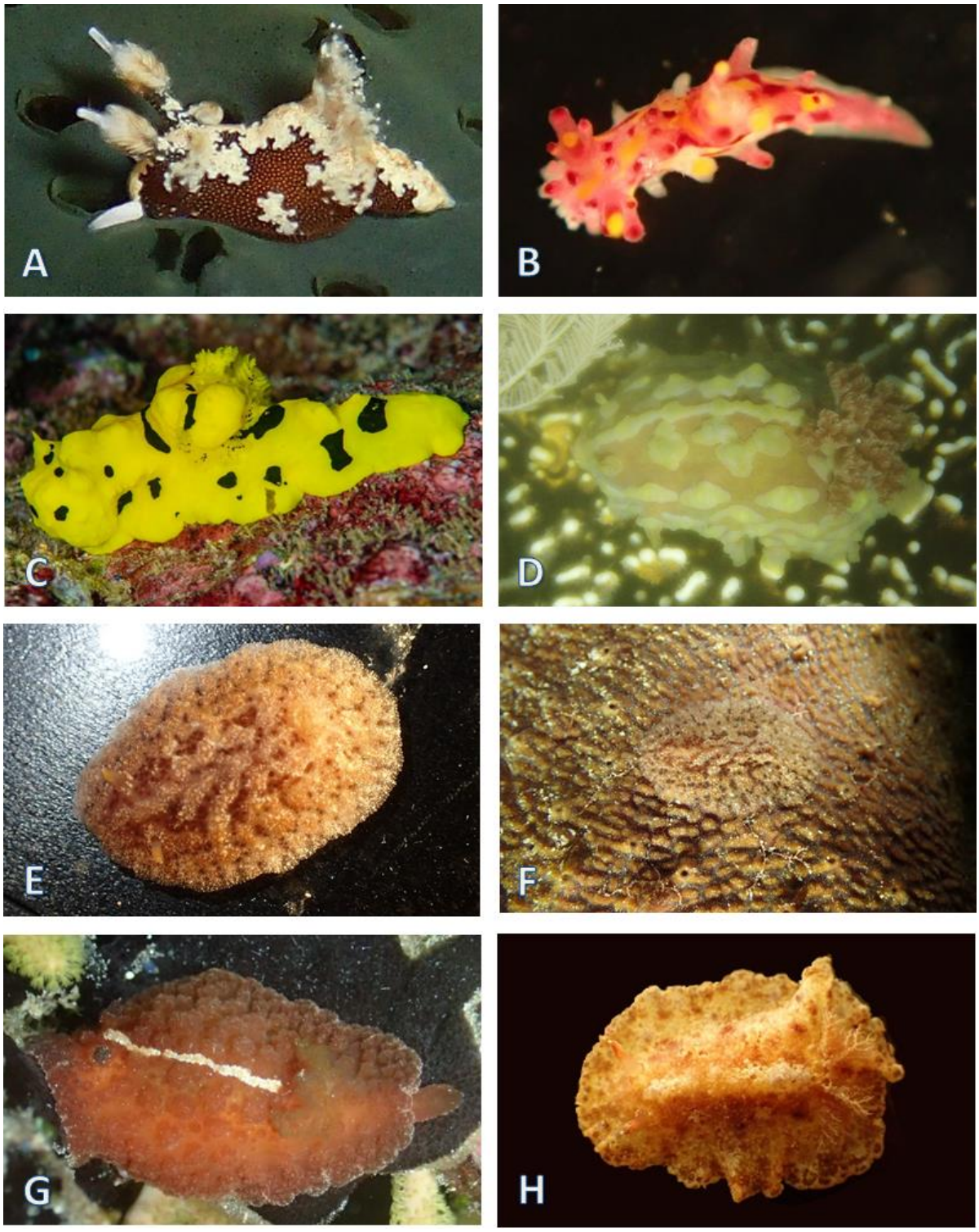


Figure 9. Nudibranchia Doridina: (**A**) *Trapania safracornia*;* (**B**) *Aegires* sp. (*Aegires* sp. 7 in Gosliner et al. (2015): 149), Aesp7-18Ba-1; (**C**) *Notodoris minor*;* (**D**) *Asteronotus cespitosus*;* (**E**) *Asteronotus mimeticus*, Asmi18Ba-2; (**F**) *Asteronotus mimeticus* substrate, Asmi18Ba3S; (**G**) *Atagema intecta*, Atin18Ba-2; (**H**) *Discodoris cebuensis*, Dice18Ba-1. *Specimen not collected.

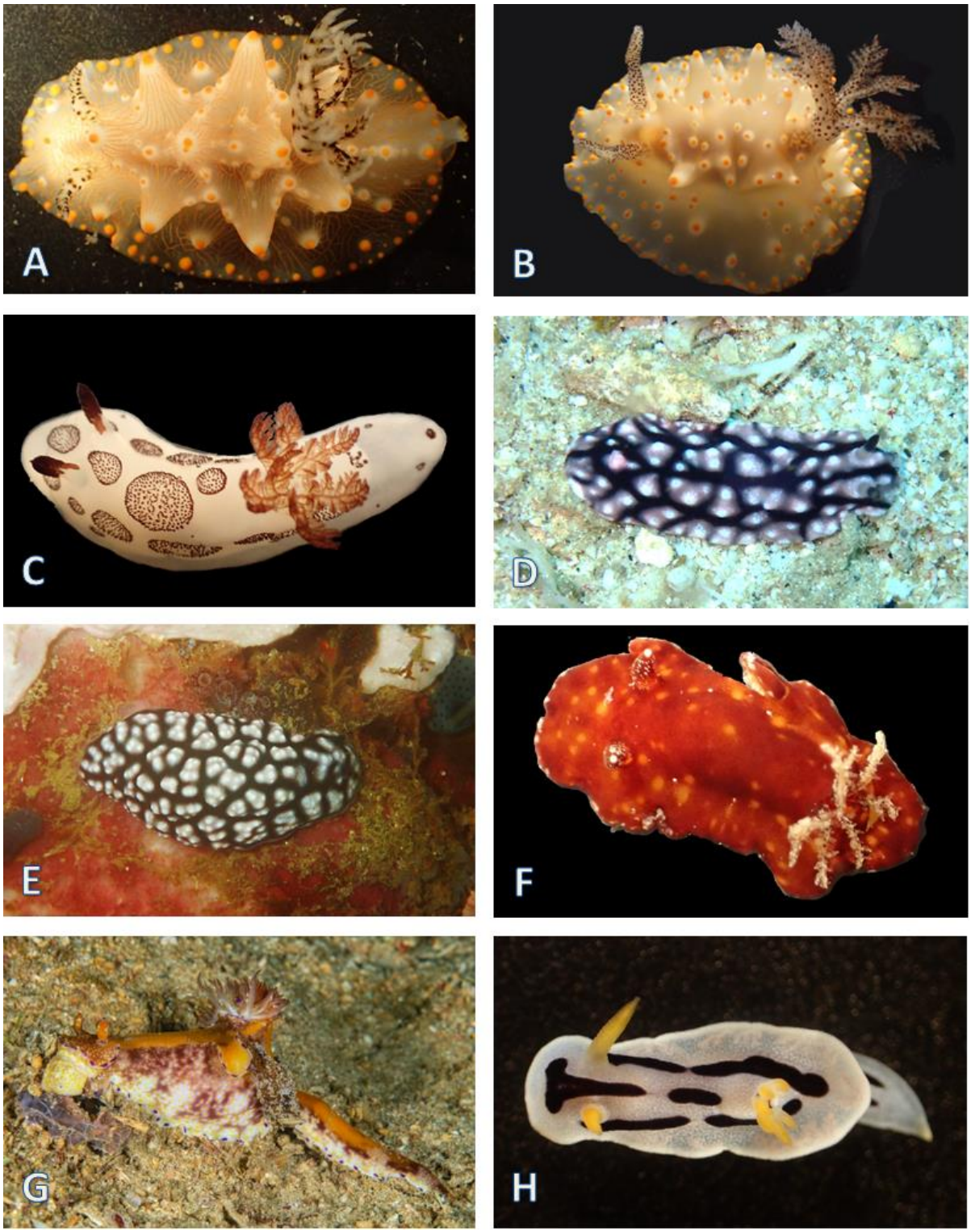


Figure 10. Nudibranchia Doridina: (**A**) *Halgerda batangas*, Haba18Ba-8; (**B**) *Halgerda carlsoni*, Haca17Ba-2; (**C**) *Jorunna funebris*, Jofu17Ba-1; (**D**) *Paradoris liturata* in situ, Phpu17Ba-6; (**E**) *Phyllidiella pustulosa* mimicked by *Paradoris liturata*; (**F**) *Platydoris sanguinea*, Plsa17Ba-1; (**G**) *Ceratosoma tenue**; (**H**) *Chromodoris dianae*, Chdi18Ba-1. *Specimen not collected.

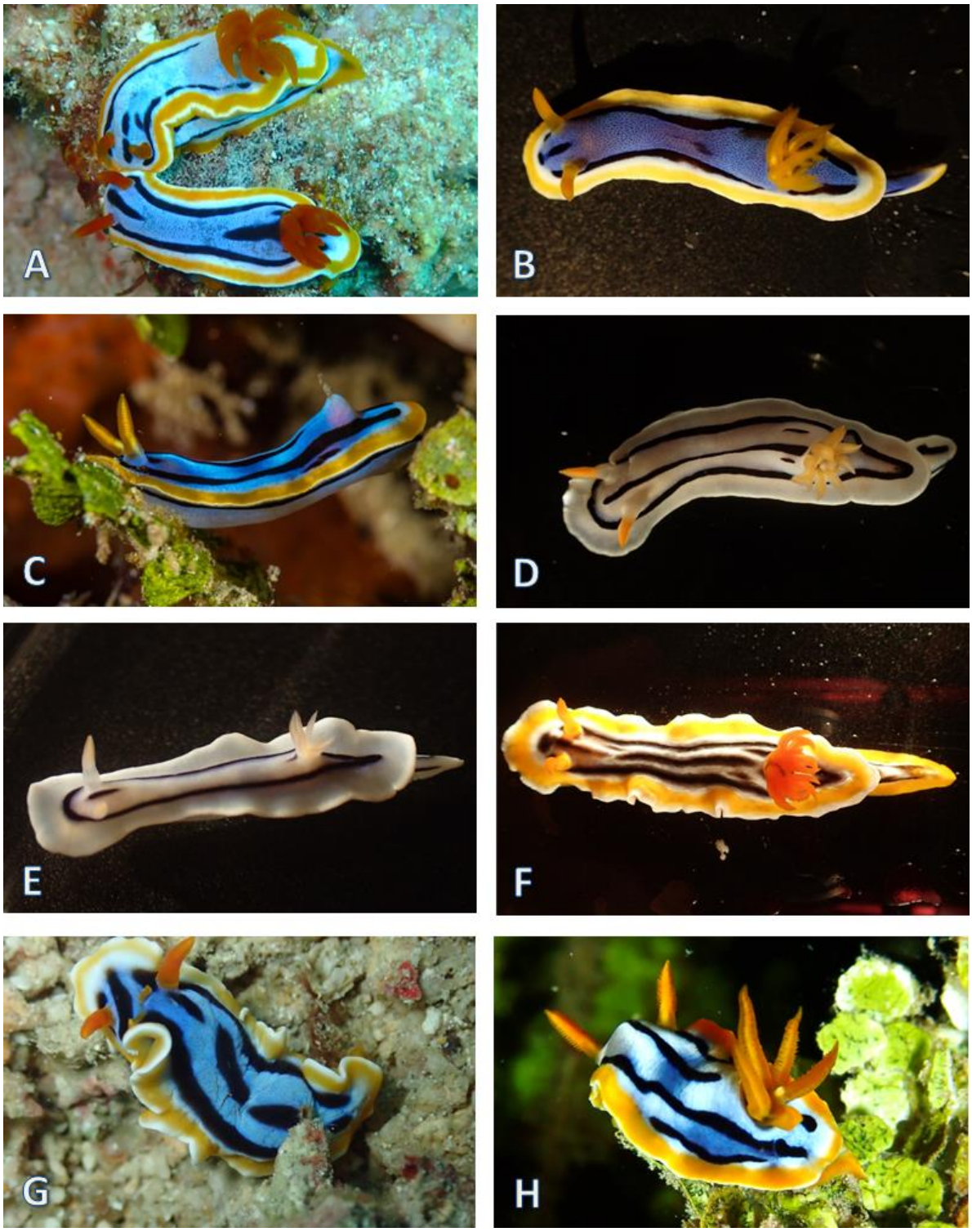


Figure 11. Nudibranchia Doridina: (**A**, **B**) *Chromodoris annae*, Chan18Ba-11-12, Chan18Ba-7; (**C**) *Chromodoris elisabethina*;* (**D**, **E**) *Chromodoris lochi*, Chlo18Ba-6, Chlo18Ba-1*;* (**F**) *Chromodoris magnifica*, Chma18Ba-1*;* (**G**) *Chromodoris quadricolor*, Chma18a-2; (**H**) *Chromodoris strigata*, Chst17Ba-1. *Specimen not collected.

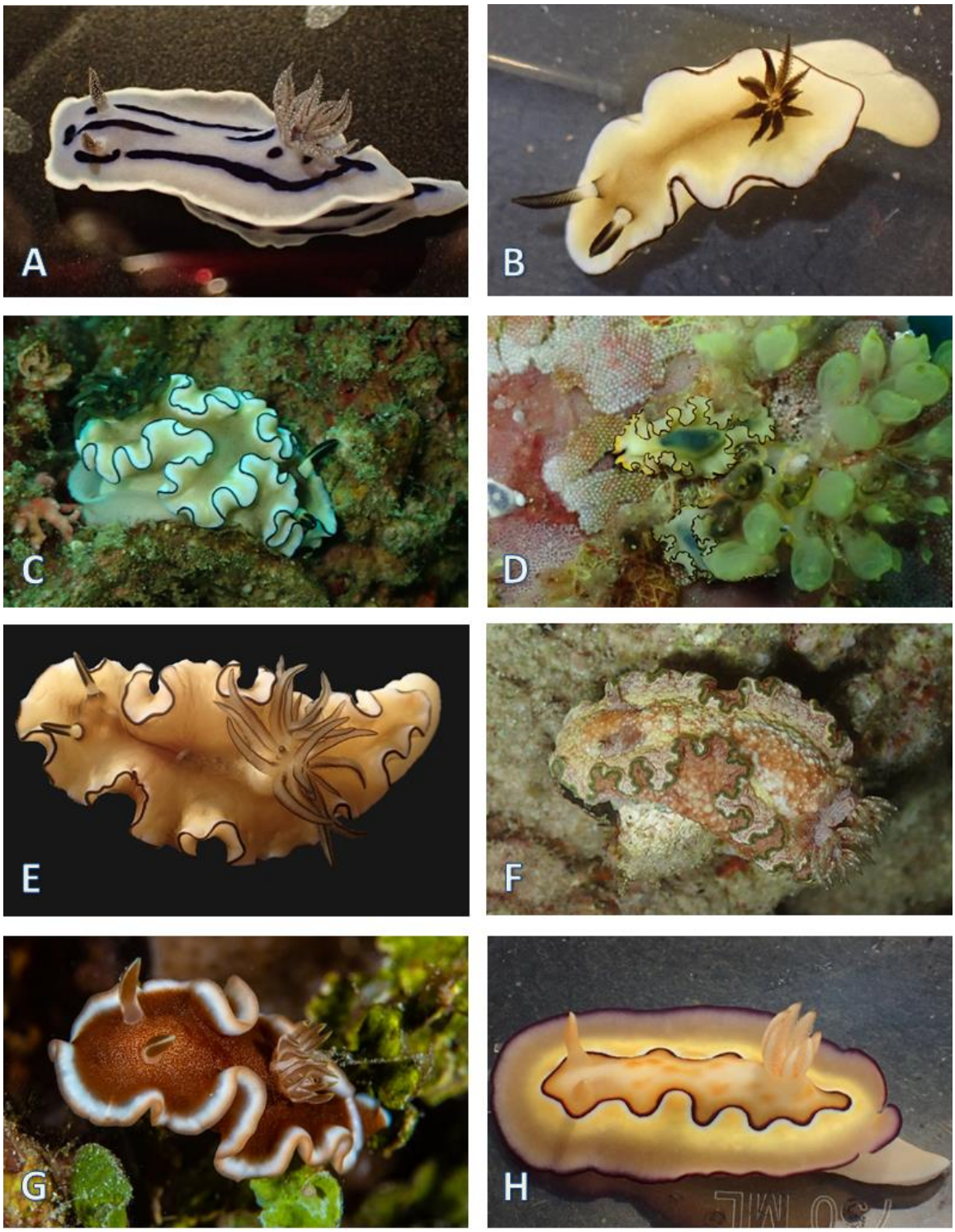


Figure 12. Nudibranchia Doridina: (**A**) *Chromodoris willani*, Chwi18Ba-1; (**B**) *Doriprismatica atromarginata*, Doat17Ba-1; (**C**) Doriprismatica atromarginata in situ; (**D**) Flatworm mimicking *D. atromarginata*; (**E**) *Doriprismatica sibogae*, Dosi17Ba-1; (**F**) *Glossodoris cincta*, Glci18Ba-1; (**G**) *Glossodoris rufomarginata**; (**H**) *Goniobranchus coi*, Gocho17Ba-1. *Specimen not collected.

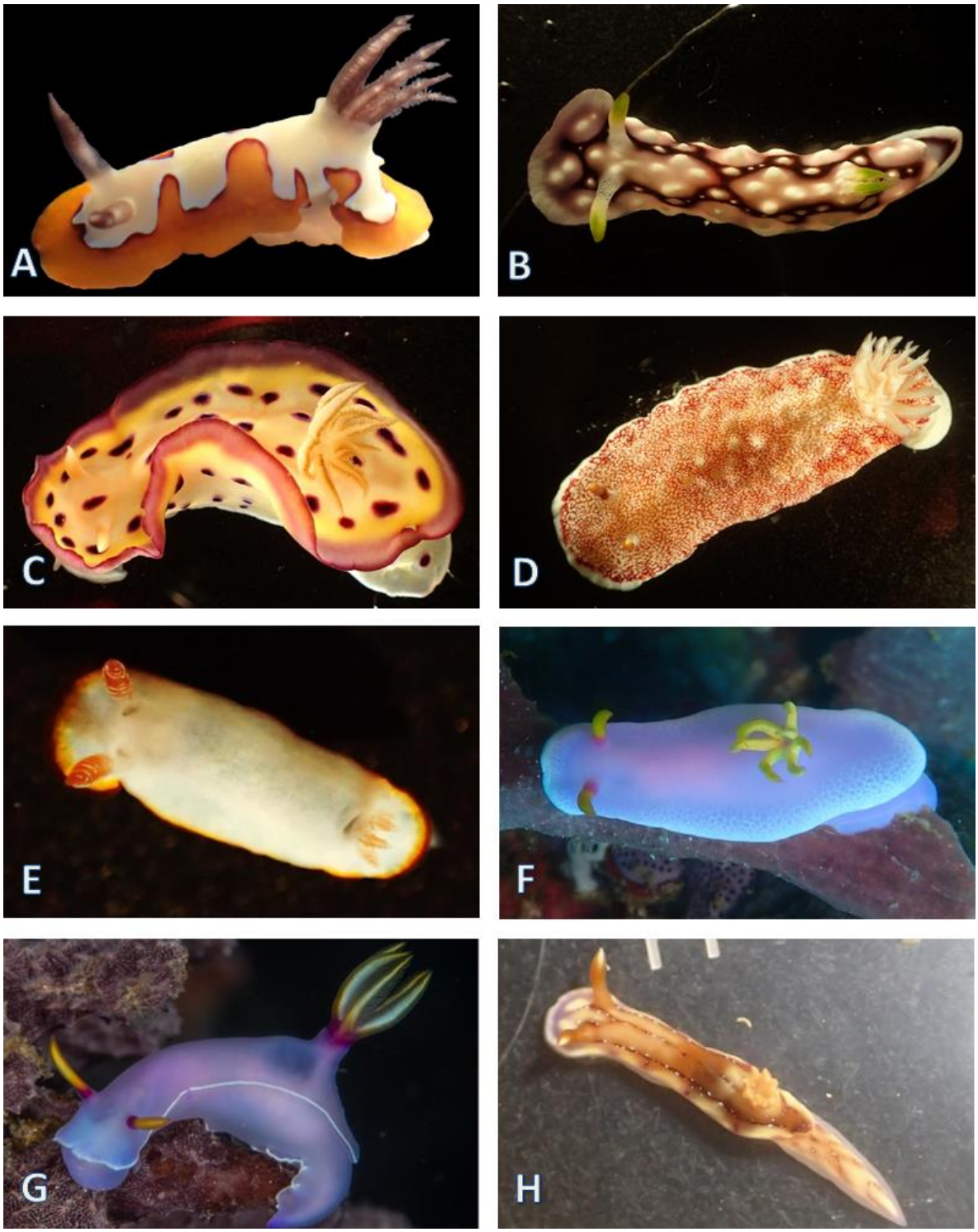


Figure 13. Nudibranchia Doridina: (**A**) *Goniobranchus fidelis*, Gofi17Ba-1; (**B**) *Goniobranchus geometricus*, Goge18Ba-5; (**C**) *Goniobranchus kuniei*, Goku18Ba-1; (**D**) *Goniobranchus reticulatus*, Gore18Ba-1; (**E**) *Goniobranchus verrieri*, Thho18Ba-1; (**F**) *Hypselodoris apolegma*, Hyap17Ba-1; (**G**) *Hypselodoris bullockii**; (**H**) *Hypselodoris cerisae*, Hysp1.17Ba-1. *Specimen not collected.

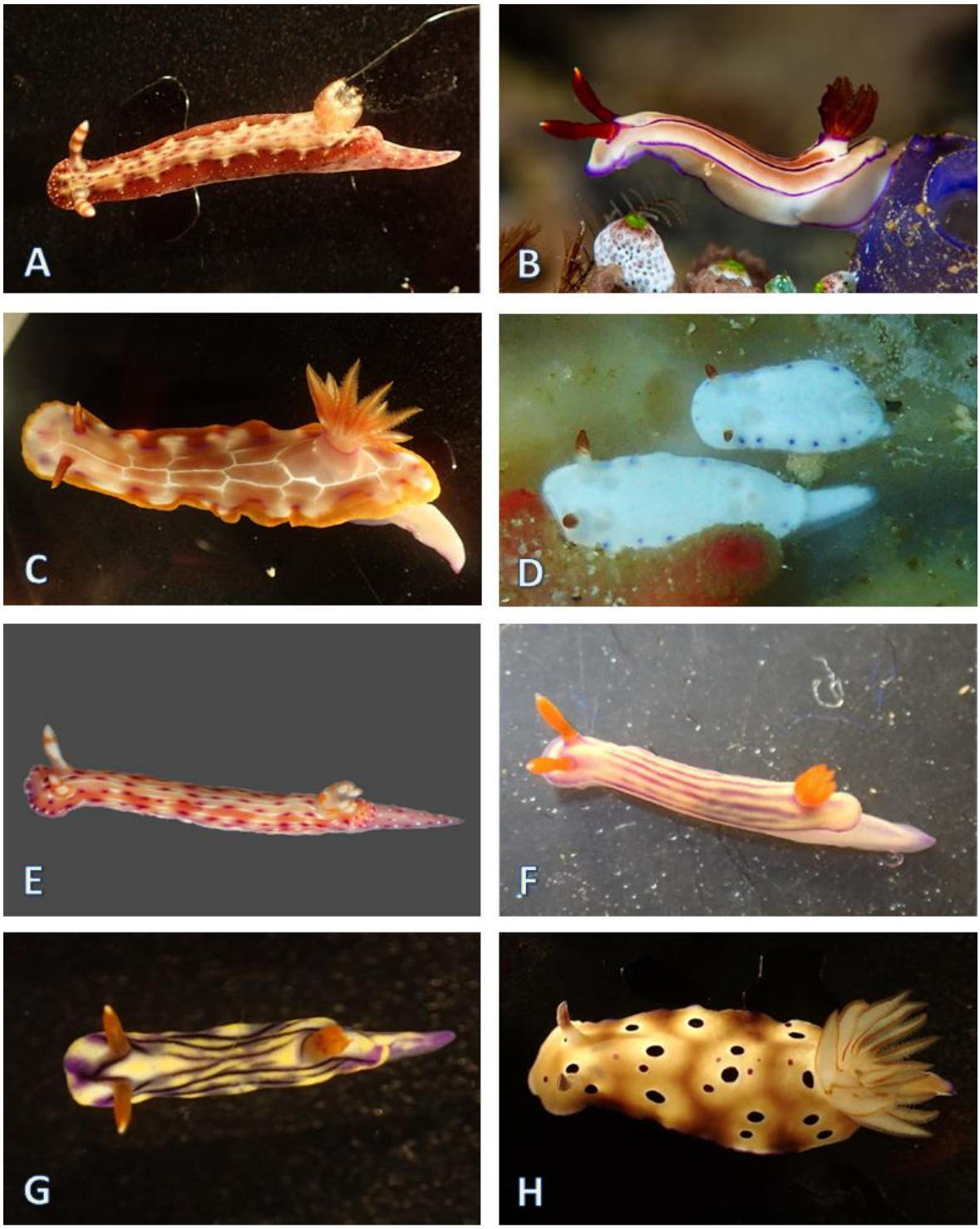


Figure 14. Nudibranchia Doridina: (**A**) Hypselodoris decorata, Hyma18Ba-1; (**B**) *Hypselodoris emma**; (**C**) *Hypselodoris iacula*, Chromos18Ba-1; (**D**) *Hypselodoris lacuna*, Hysp19_17Ba-1-2; (**E**) *Hypselodoris maculosa*, Hyma17Ba-1; (**F**) *Hypselodoris maridadilus*, Hymari17Ba-1; (**G**) *Hypselodoris zephyra*, Hyni18Ba-1; (**H**) *Hypselodoris tryoni*, Hytr18Ba-5. *Specimen not collected.

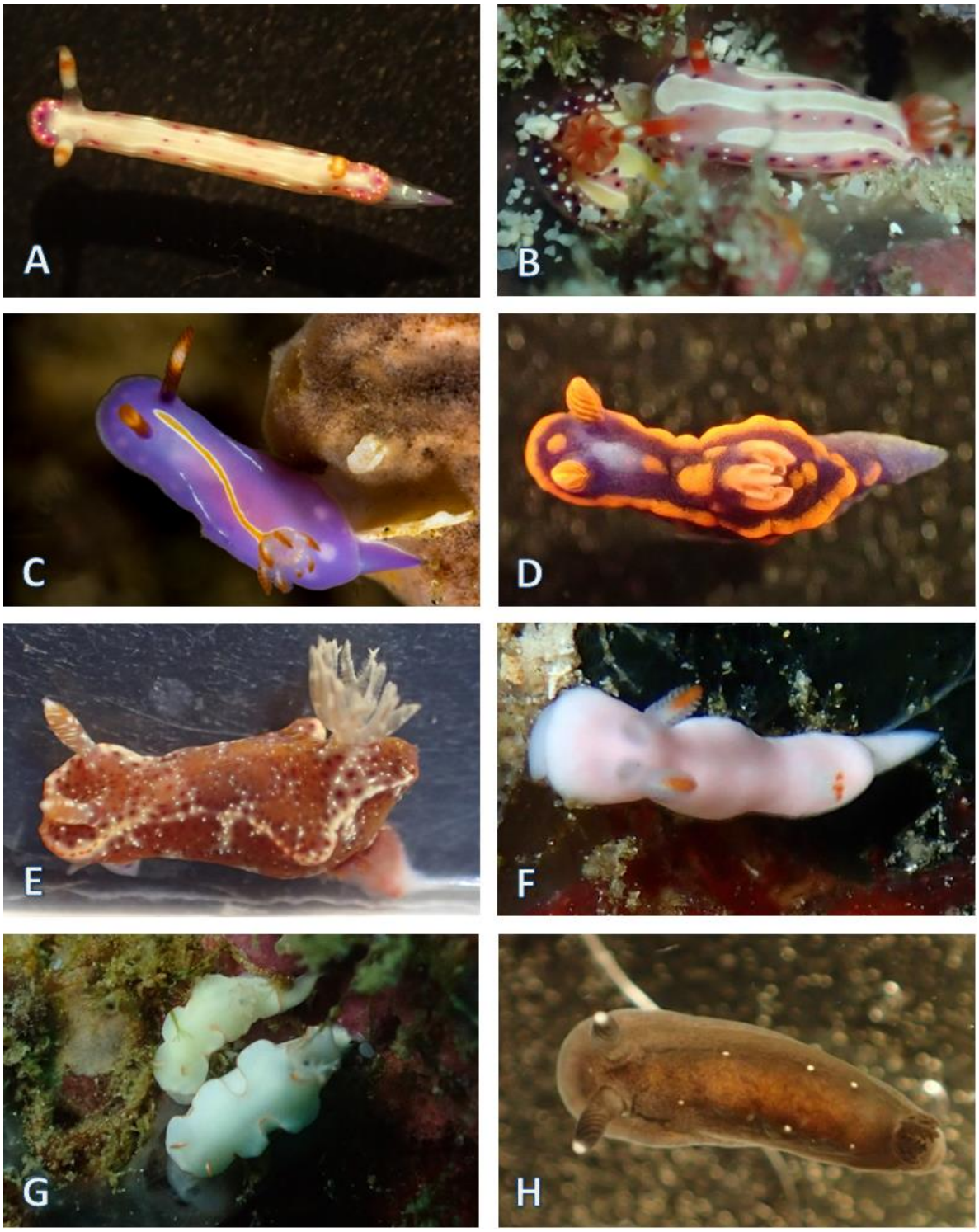


Figure 15. Nudibranchia Doridina: (**A**) *Hypselodoris* sp. a, Thsp1-18Ba-1; (**B**) *Mexichromis aurora*, Meau18Ba-1; (**C**) *Mexichromis trilineata**; (**D**) *Miamira magnifica*, Dorid18Ba-1*;* (**E**) *Miamira* sp. a, Misp17Ba-1*;* (**F**) *Verconia simplex*, Nosi17Ba-1; (**G**) *Thorunna furtiva*, Thfu18Ba-1-2*;* (**H**) *Dendrodoris nigra*, Deni18Ba-1. *Specimen not collected.

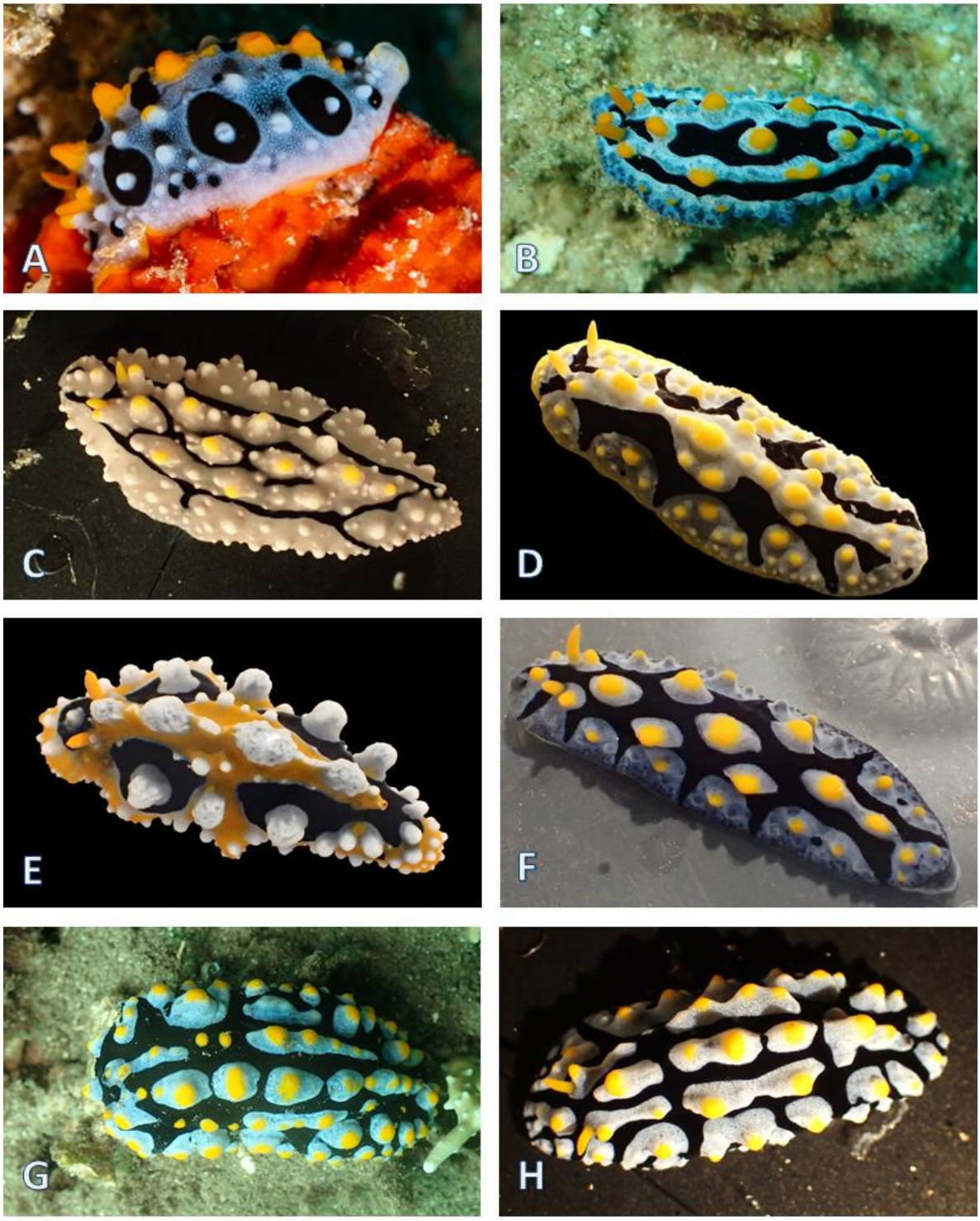


Figure 16. Nudibranchia Doridina: (**A**) *Phyllidia* cf. *babai**; (**B**) *Phyllidia coelestis*, Phco18Ba-1; (**C**) *Phyllidia elegans*, Phel18Ba-1; (**D**) *Phyllidia exquisita*, Phex17Ba-1; (E) *Phyllidia ocellata*, Phoc17Ba-1; (**F**) *Phyllidia picta*, Phpi17Ba-1; (**G**, **H**) *Phyllidia varicosa*, Phva18Ba-11, Phva18Ba-6. *Specimen not collected.

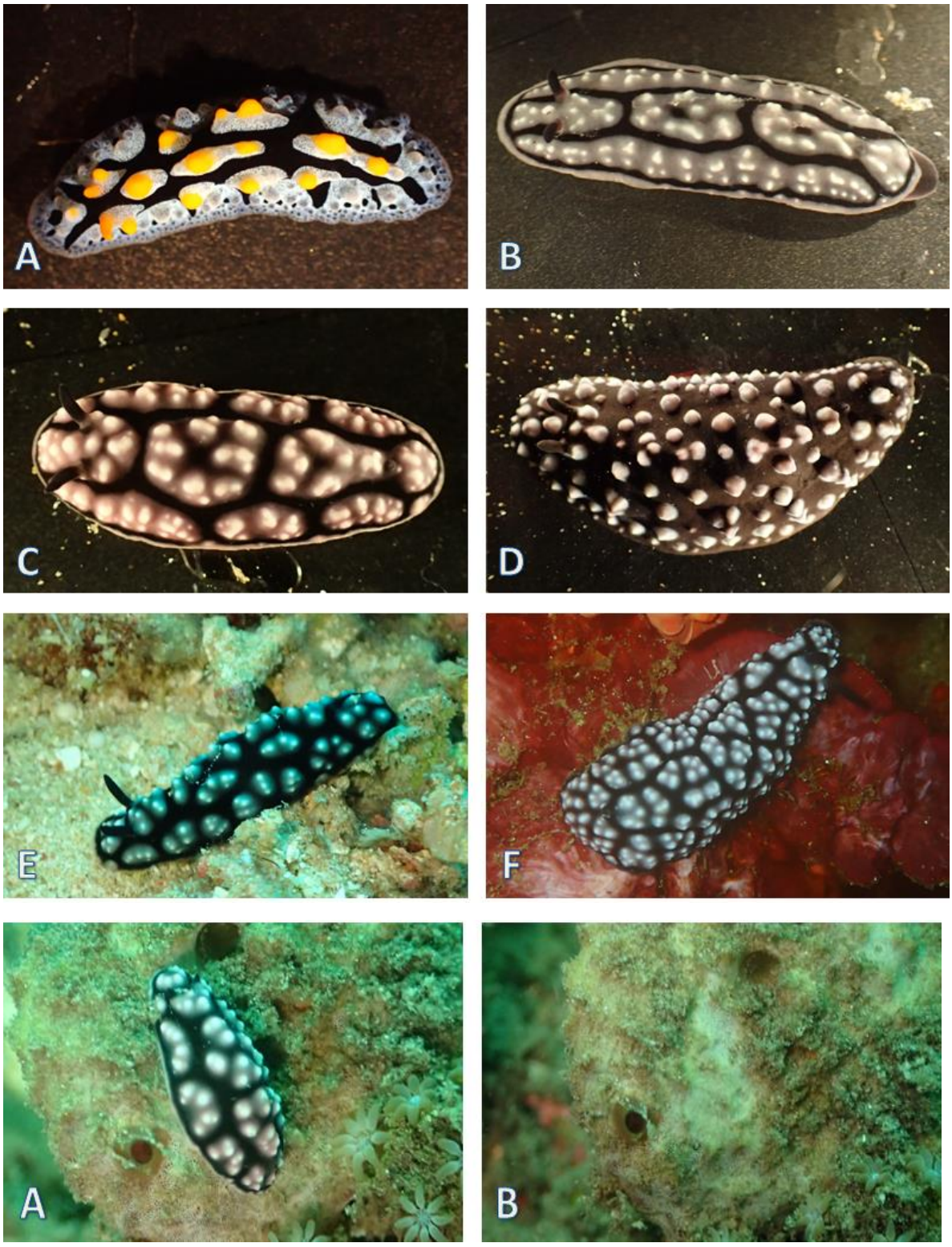


Figure 17. Nudibranchia Doridina: (A) *Phyllidia* sp. a, Phsp18Ba2*;* (B) *Phyllidiella annulata*, Phpu18Ba- 12; (C) *Phyllidiella lizae*, Phli18Ba-1*;* (D) *Phyllidiella nigra*, Phni18Ba-1; (E, F) *Phyllidiella pustulosa*, Phpu18Ba-2, Phpu18Ba-4. (G) Specimen of *Phyllidiella pustulosa* in situ on a sponge; (H) Sponge viewed immediately after *P. pustulosa* was removed.

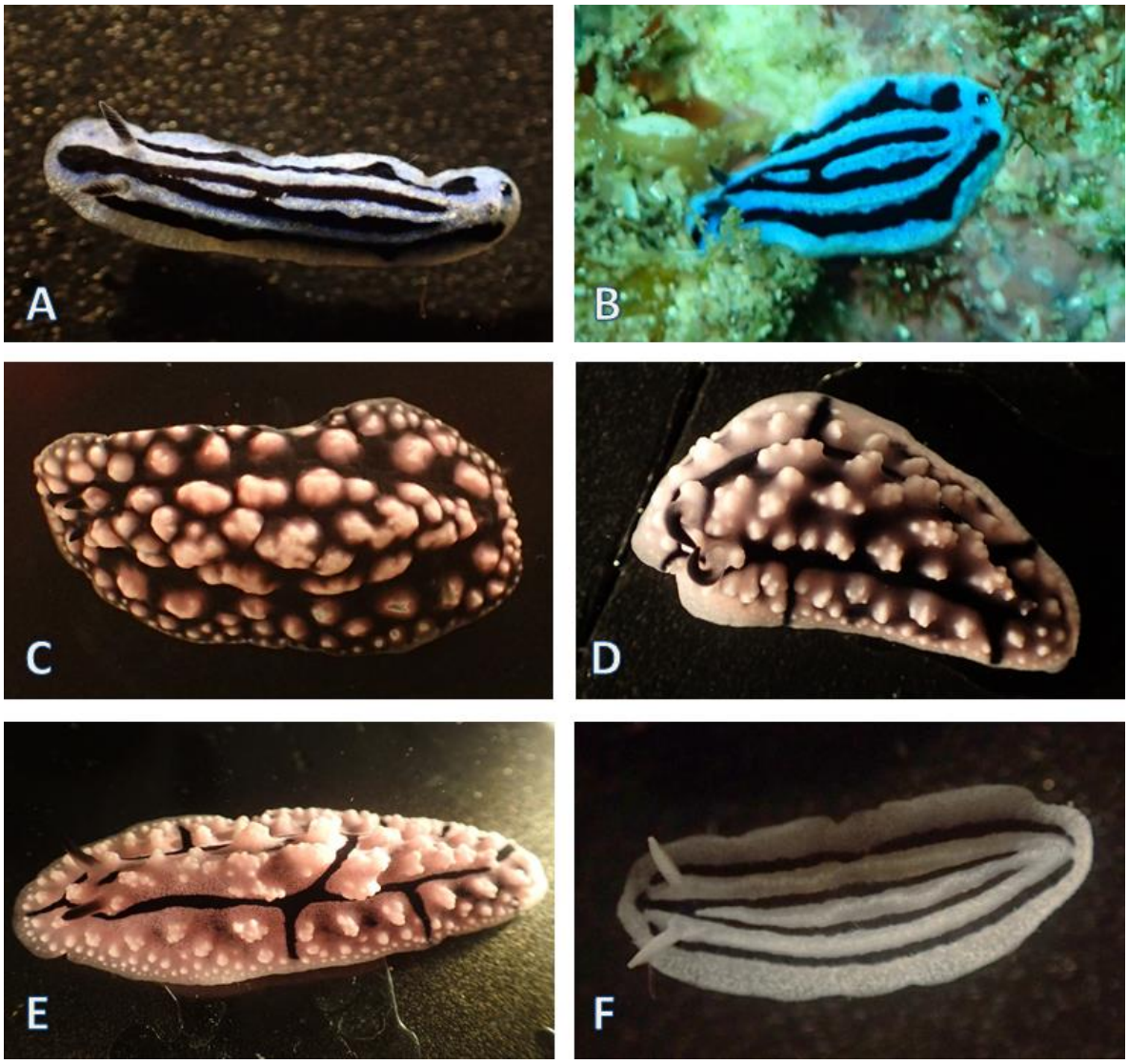


Figure 18. Nudibranchia Doridina: (A, B) *Phyllidiopsis annae,* Phsan18Ba-1 and in situ*;* (C, D) *Phyllidiopsis* cf. *burni,* Phssp18Ba-1*;* (E) *Phyllidiopsis krempfi,* Phskre18Ba-1, Phspi18Ba-1; (F) *Phyllidiopsis xishaensis,* Phsxi18Ba-1.

Chromodorididae: This family is represented in our study by 263 specimens, which could be assigned to 37 species. *Ceratosoma tenue* (Figure 10G) was only identified by photo-documentation. Eight species of the genus *Chromodoris* are present in our collection. *Chromodoris dianae* (Figure 10H), a very common species in BNP, was only represented by two specimens, collected at depths in the range of 13–15 m. Of all chromodorids, *C. annae* (Figure 11A, B) is the species with the highest specimen numbers and distribution (Table 2); rhinophores and gills vary in color from light yellow to a rather unusual orange. *Chromodoris elisabethina* (Figure 11C) is recorded only by photographs from Sempini. *Chromodoris lochi* (Figure 11D, E), the second most common *Chromodoris* species, is represented by two color morphs: rhinophores and gills are either pink or pale yellow, and the mantle is always

translucent white. Molecular barcoding has confirmed both their identities as *C. lochi.* The same two color morphs are also found in BNP (Eisenbarth et al. 2018). One specimen of *C. magnifica* (Figure 11F) with the typical whitish background was found on sand underneath coral rubble. Another specimen was collected that looked in general very similar to *C. magnifica* with a discontinuous dark line in the middle of the notum, but with a distinct bluish body. According to molecular barcoding, both genes (*CO1* and *16S*) show a 99% similarity with sequences from NCBI assigned to *C. quadricolor* (Figure 11G). This would then indicate another record in the Indo-Pacific Ocean of a species that is primarily distributed in the Red Sea. Two specimens of *C. strigata* (Figure 11H) were collected at depths of 7– 14 m, one during a night dive crawling on the green alga *Halimeda.* Only two specimens of *C. willani* (Figure 12A) were found close to each other at 8 m depth, another species much more common in BNP. Four specimens of *Doriprismatica atromarginata* (Figure 12B, C) with sizes of 17–74 mm were collected from different dive sites. In one of these dive sites, we observed a yellow flatworm *Pseudoceros* sp. (Figure 12D), which clearly mimics *D. atromarginata*. Only one specimen of *D. sibogae* (Figure 12E) was found, differing from *D. atromarginata* in gill color. *Glossodoris cincta* is a common species and widespread in the Indo-Pacific, and is also recorded from BNP, Sangihe, and Lembeh Strait. However, there are several species with very similar coloration (Matsuda and Gosliner 2018). We assign two specimens to this species (Figure 12F). One was collected at 6 m depth during a night dive underneath coral rubble, and one at 1 m depth. Both animals had a yellowish to green band followed by a black and then a white margin on the mantle (typical feature of *G. cincta).* *Glossodoris rufomarginata* (Figure 12G) was identified only by photo-documentation. It is described in the literature as having an orange brown spackled pattern on the notum, brown marginal band, and white submarginal band (Gosliner et al. 2015). The genus *Goniobranchus* was represented by six different species. *Goniobranchus coi* (Figure 12H) was only collected in 2017 during a night dive. It was not recorded from BNP (Eisenbarth et al 2018) or Sangihe (Undap et al 2019). *Goniobranchus fidelis* (Figure 13A) is a smaller member of the genus, and our single animal was only 13 mm in length, collected at 17 m depth. *Goniobranchus geometricus* (Figure 13B) is more common in North Sulawesi, and we collected six specimens at 5–15 m depth. *G. kuniei* (Figure 13C) and *G. reticulatus* (Figure 13D) were collected at deeper than 20 m depth. Yonow (2017) outlines the problems of correct identification of this species, and misidentification of *G. inopinata* and even *G. tinctorius* cannot be excluded: *G. tinctorius* usually has a yellow band along the edge of the notum (Winters et al. 2018), which is not present in our specimens.

In contrast to BNP, where only five species have been recently depicted (Eisenbarth et al 2018), 12 species of *Hypselodoris* are recorded in Bangka Archipelago: *H. apolegma* (Figure 13F), *H. bullockii* (Figure 13G), *H. cerisae* (Figure 13H), *H. decorata* (Figure 14A), *H. emma* (Figure 14B), *H. iacula* (Figure 14C), *H. lacuna* (Figure 14D), *H. maculosa* (Figure 14E), *H. maridadilus* (Figure 14F), *H. zephyra* (Figure 14G), *H. tryoni* (Figure 14H) and *Hypselodoris* sp. a (Figure 15A). Many of the species are easily recognized by their distinct coloration and are not discussed in detail below. A recently described new species, *H. iba,* is very similar to *H. bullockii;* however, our animal, which was only photo-documented (Figure 13G), shows the features typical of *H. bullockii* also found in Ambon (Yonow 2001) and BNP (Eisenbarth et al 2018). However, confirmation of the identification of this individual would require specimens and possibly molecular analyses. One member of the newly described species *H. cerisae* (Figure 13H) (Epstein 2019) was collected in front of Coral Eye, and molecular analyses verified the identification based on 99% similarity of our specimen with the sequence of *H. cerisae* CASIZ 178350 deposited in NCBI. Our specimen is pale brown with some purple pigment concentrated at the anterior mantle and foot margin; white dots are aligned on three longitudinal brown lines on the notum; brownish diagonal lines are present on the posterior foot; there are nine gill branches. Externally, *H. decorata* (Figure 14A) is very similar to *H. maculosa* (Figure 14E). According to recent studies (Epstein 2019), they are closely related and form a clade with *H. juniperae*. *H. decorata* can be distinguished from *H. maculosa* by the three reddish rhinophoral bands, versus only two reddish rings. Our specimen of *H. decorata* has these three rhinophoral rings, as well as the other typical color features, e.g., the white dots on the broad brown margin of the mantle and longitudinal lines on the middle part of the mantle with brown dots. Our single specimen of *H. iacula* (Figure 14C) has a light honey-colored body, with a brown band around the mantle margin, violet at the foot margin, and a white net-like pattern on the notum and top of the foot. Eight gill branches arise from a relatively high gill pocket. Two specimens are assigned to *H. lacuna* (Figure 14D), whose name is based on the translucent areas on the notum that resemble holes in the body wall (Epstein 2019). Both specimens have a white mantle, with many translucent patches and two large translucent circles posterior to the rhinophores with two others in front of the gills; further characteristics are blue dots on the lateral margin of the mantle, and white gills and rhinophores with reddish apices. The body of *Hypselodoris* sp. a (Figure 15A) is cream, as well as the rhinophores, which are more translucent at the base and with one orange ring in the cream area. Red dots spread along the margin of the notum and a few along the midline of the notum. Externally, the species is very similar to *Thorunna australis* and

Hypselodoris maculosa. However, *Hypselodoris* sp. a lacks contrasting pigment in the middle of the notum, where *H. maculosa* shows dots all over the notum. Two specimens of *Mexichromis aurora* (Figure 15B) were sitting next to each other on corals at 8.6 m depth, but only one was collected. *M. trilineata* (Figure 15C) was only identified by photo-documentation.

One small animal (Dorid18Ba-1; Figure 15D) was found in Yellow Coco at a depth of 20 m. It is characterized by a pale blue body color with an orange mantle rim, orange gills and rhinophores, as well as orange patches on the dorsal notum and tail. The body shape resembles some goniodorids. However, it could be identified as a juvenile of the species *Miamira magnifica*. One further small member of probably the same genus, *Miamira* sp. a (Figure 15E) (10 mm), was found at a depth of 4 m. It is reddish to brown in color, covered with darker spots and many tiny white dots on the mantle; the edge of the mantle is cream with brown dots. The rhinophores are translucent cream with white lines and an orange hue at the tip, whereas the translucent gills exhibit brown and white patches. *Verconia simplex* (Figure 15F), 6 mm in size, was sitting on black sponges at 5.2 m depth. Both specimens of *Thorunna furtiva* (Figure 15G) were found close to each other on a stone, covered by encrusting red sponge, and were observed to have vibrating gills, a typical character of the genus. *Dendrodoris nigra* (Figure 15H) is the only member of the family Dendrodorididae that was collected. It is a juvenile of only 6 mm length and was found crawling out of the coral rubble sorted in the laboratory.

Phyllidiidae: Members of this family are probably the most common sea slugs in tropical waters. More than 100 specimens were collected in the Bangka Archipelago, assigned to 16 species and three genera, *Phyllidia, Phyllidiella,* and *Phyllidiopsis*. *Phyllidia* cf. *babai* (Figure 16A) was found in Batu Belah and was only identified by photo-documentation. Eight specimens of *Phyllidia coelestis* (Figure 16B) were collected at 1–20 m depth. The other *Phyllidia* species represented in our collection are *P. elegans (*Figure 16C), *P. exquisita* (Figure 16D), *P. ocellata* (Figure 16E), and *P. picta* (Figure 16F). *Phyllidia picta* often co-occurred at the same locations as *P. ocellata*. *Phyllidia varicosa* (Figure 16G, H) was probably the most common *Phyllidia* species with 16 specimens collected at nine sites, ranging between 23 and 85 mm in size and between 2 and 20 m in depth. One specimen originally assigned to *Phyllidia picta* (Phsp18Ba-2; Figure 17A), based on external coloration, clusters with several other specimens from various regions of North Sulawesi in a separate clade, distinct from the *P. picta* clade. It therefore represents a new species with very similar appearance to *P. picta*

(Papu et al., in preparation). Four species of *Phyllidiella* collected in Bangka Archipelago comprise *Phyllidiella* cf. *annulata* (Figure 17B), *P. lizae* (Figure 17C), *P. nigra* (Figure 17D) and *P. pustulosa* (Figure 17E, F). Only one specimen of *Phyllidiella* cf. *annulata* (Figure 17B) was found in Talisei Island, a collection site with strong tidal currents and ocean waves, as well as small compact coral formations. *Phyllidiella nigra* (Figure 17D) was collected from mangrove roots. It is characterized by the dark gray hyponotum and foot, and black notum with single pale pink round tubercles. *Phyllidiella pustulosa* (Figure 17E, F) is the most common species of the genus *Phyllidiella* and also within Phyllidiidae. However, this species was shown to represent a complex of several species (Stoffels et al. 2016), which are very difficult to distinguish by external characteristics alone. Figure 17G and H depict a specimen which we removed from the substrate to show feeding marks on the sponge. *Phyllidiopsis annae* (Figure 18A, B) is a small species, and our four specimens ranged in length from 3 to 12 mm. Further collected *Phyllidiopsis* species are *P.* cf. *burni* (Figure 18C), *P. krempfi* (Figure 18D, E), and *P. xishaensis* (Figure 18F).

b. Nudibranchia, Cladobranchia (42 species in 22 genera belonging to 12 families)

Arminidae: Six different species of *Dermatobranchus* were collected during the two studies: *D. caeruleomaculatus* (Figure 19A), *D. rudmani* (Figure 19B), *D. pustulosus* (Figure 19C), and three undescribed *Dermatobranchus* species (Figure 19D–F). Most of them were collected in 2018 close to Coral Eye under coral rubble.

Proctonotidae: One *Janolus* species (Figure 19G) exists only as a photo-documentation in our collection. It is already depicted in Gosliner et al. as *Janolus* sp. 1.

Bornellidae: Several individuals of *Bornella anguilla* (Figure 19H) (of which only two were collected) were found crawling on encrusting corals and tunicates, and some were mating at approximately 8 m depth. One *Bornella* specimen was found in coral rubble at 1 m depth, which resembles most *B. dotoides* when comparing the shape of the cerata (Figure 20A; Yonow 2017).

Tethydidae*: Melibe bucephala* (Figure 20B) was found together with *B. stellifera* in the collected coral rubble. *Melibe engeli* (Figure 20C; length of 34 mm) is a well-camouflaged species due to its transparent body. The species is recorded to be associated with algae of the genus *Padina* or *Achantophora* (Gosliner et al. 2015); however, our animal was collected from an encrusting sponge.

Dotidae: The brownish-cream to olive-colored species *Doto ussi* (Figure 20D) was always found at the base of the hydrozoan *Aglaophenia cupressina*.

Tritoniidae: Two undescribed species were collected, both at the same site, with many different soft coral species close by. The dorsal appendages of the undescribed *Marionia* species (Figure 20E, F) look like green algae, and the animal is very similar to *M.* sp. 2, depicted in Gosliner et al. (2015). The dorsal appendages of the undescribed *Tritonia* sp. (Figure 20G) resemble polyps of soft corals. The animal is similar to *T.* sp. 3 depicted by Gosliner et al. (2015).

Flabellinidae*: Coryphellina exoptata* (Figure 20H), *C. rubrolineata* (Figure 21A), and two undescribed species (*Flabellina* sp. 2, *Flabellina* sp. 3) are members of the family Flabellinidae. The color of *Flabellina* sp. 2 (Figure 21B) (also depicted in Gosliner et al. (2015)) is similar to *C. rubrolineata*, but our animal has only one ring around each ceras, and no lines in the middle and margin of the body. The specimen of *Flabellina* sp. 3 (Figure 21C, similar to *F.* sp. 3 in Gosliner et al. (2015)) has a rather translucent body with a single red line in the middle of the body. The white cerata carry a few red dots, and the rhinophores are translucent with a tinge of white in the middle part.

Samlidae: *Samla riwo* (Figure 21D) was collected from a non-specific substrate. **Eubranchidae**: *Eubranchus* sp. 22 (Figure 21E) was only found once, with one specimen on a hydrozoan of the family Plumulariidae (Gosliner et al. 2015).

Trinchesiidae: The conspicuous *Trinchesia yamasui* (Hamatani, 1993) (Figure 21F) was only recorded by photo-documentation. It is very similar to the original and subsequent descriptions (Sea Slug Forum homepage; Coleman et al. 2015; Korshunova et al. 2017). The animal is grayish, with a black head followed by a violet band in front of the rhinophores. The cerata are black to gray, with a subapical orange ring, followed by a black apex. Two specimens of an undescribed *Cuthona* species (Figure 21G) depict a uniformly orange body color with the oral tentacles and rhinophores showing a darker orange on the distal parts, followed by a translucent apical part. The specimens resemble *Cuthona* sp. 57 in Gosliner et al. (2015). One unidentified aeolid specimen (Aeol18Ba-1; Figure 21H) was collected from coral rubble and is characterized by a pale white body and cerata with the digestive gland shining through in a darker shade.

Facelinidae: This cladobranch family is represented by the highest number of specimens (99) and species (20) in this study. Several new species were also recorded. One

specimen probably represents an undescribed *Antonietta* species (Figure 22A). It measured only 2 mm alive and has an opaque white body, violet cerata with a dark violet ring on each ceras, and yellow rhinophores. One of the most common facelinid species is *Caloria indica* (Figure 22B), which we collected at depths of 5–14 m. An unidentified specimen probably belonging to the genus *Cratena* (Figure 22C) was collected during a night dive at 4 m depth. It is characterized by the orange color of the jaws, a typical character of the genus. Many members of *Favorinus* collected during this study were observed feeding on other nudibranch egg masses. *F. japonicus* (Figure 22D) was collected from the macroalga *Udotea* while laying eggs. *Favorinus* sp. (Figure 22E) was also found on *Udotea*, feeding on a large nudibranch egg mass. Our animal is similar to *F.* sp. 1 in Gosliner et al. (2015). *F. tsuruganus* (Figure 22F) was collected from coral rubble. Two specimens of an undescribed *Moridilla* species (Figure 22G, H) were collected in 2017 and 2018. Both of these specimens are white, with white and yellow on the oral tentacles. The cerata are shorter than the rhinophores, which carry papillae along the posterior side. Molecular analyses clearly distinguish these specimens as a separate species and group them as sister taxa to a new *Moridilla* species recently described from Bunaken National Park, *M. jobeli* (Schillo et al. 2019). The animals exhibit an interesting behavior of thrusting and wriggling the cerata when disturbed, and swimming with lateral movements. Three undescribed *Noumeaella* species were collected: one looks very similar to *Noumeaella* sp. 2 depicted in Gosliner et al. (2015) (Figure 23A), the second resembles *Noumeaella* sp. 3 (Figure 23B) (Gosliner et al. 2015), and the third is similar to *Noumeaella* sp. 13 (Figure 23C) (Gosliner et al. 2015). The genus *Phyllodesmium* was represented in our collection by eight species. *P. briareum* (Figure 23D) is usually found on the soft coral *Briareum*, where the cerata are very well camouflaged by the tentacles of the octocoral. *P.* cf. *crypticum* (Figure 23E) was found while laying an egg mass at the base of an unidentified xeniid soft coral species. Three specimens of *P. lizardense* (Figure 23F) were collected at various depths, down to 10 m. One large *P. longicirrum* (Figure 23G; 125 mm) was collected on the sand flat at Efrata site at 25 m depth. It usually feeds on the soft coral *Sarcophyton*, which was also quite common on that sandy flat. *P. magnum* (Figure 22H) and *P. parangatum* (Figure 24A) were collected from members of the family Xeniidae, while *P. pecten* (Figure 24B) was extracted from coral rubble. Two specimens of *P. poindimiei* (Figure 24C, D) were collected from an unspecific substrate. *Pteraeolidia semperi* (Figure 24E) is one of the largest facelinid species and was the most common cladobranch species observed, due to an aggregation of more than 100 specimens in shallow water (Sempini), probably for mating.

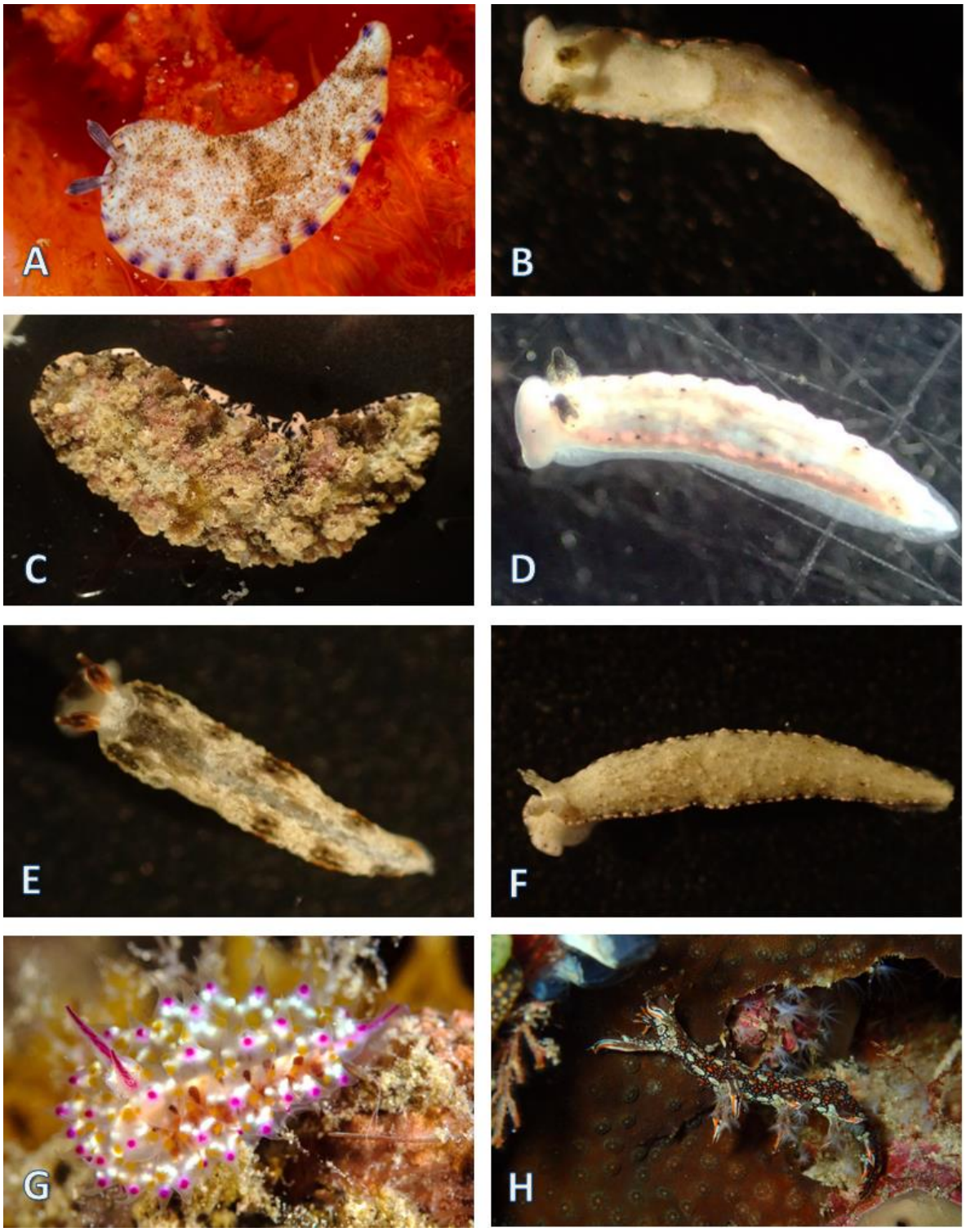


Figure 19. Nudibranchia Cladobranchia: (A) *Dermatobranchus caeruleomaculatus**; (B) *Dermatobranchus rodmani*, Dero18Ba-1; (C) *Dermatobranchus pustulosus*, Depu18Ba-1; (D) *Dermatobranchus* sp. a, Desp.a18Ba-1; (E) *Dermatobranchus* sp. b, Desp18Ba-1; (F) *Dermatobranchus* sp. c, Desp17Ba-1; (G) *Janolus* sp. (*Janolus* sp. 1 in Gosliner et al. (2015): 305)*; (H) *Bornella anguilla*, Boan17Ba-1. *Specimen not collected.

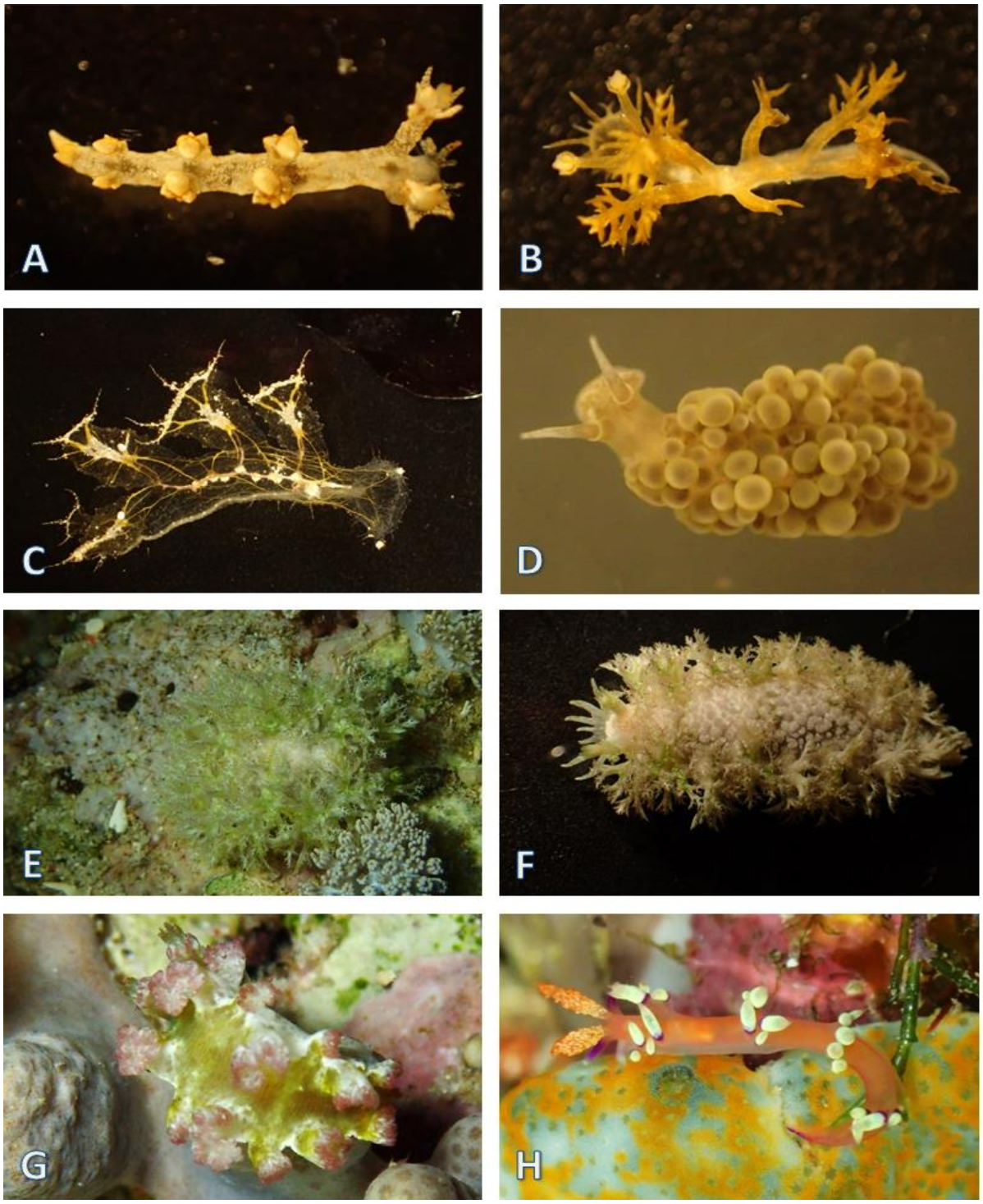


Figure 20. Nudibranchia Cladobranchia: (A) *Bornella dotoides*, Bohe18Ba-1*;* (B) *Melibe bucephala*, Mesp.a18Ba-1*;* (C) *Melibe engeli*, Mebu18Ba-1*;* (D) *Doto ussi*, Dous18Ba-2; (E, F) *Marionia* sp. (*Marionia* sp. 2 in Gosliner et al. (2015): 324), Masp2.18Ba-1*;* (G) *Tritonia* sp. (*Tritonia* sp. 3 in Gosliner et al. (2015): 320), Trsp3_17Ba-1; (H) *Coryphellina exoptata*, Flex17Ba-1.

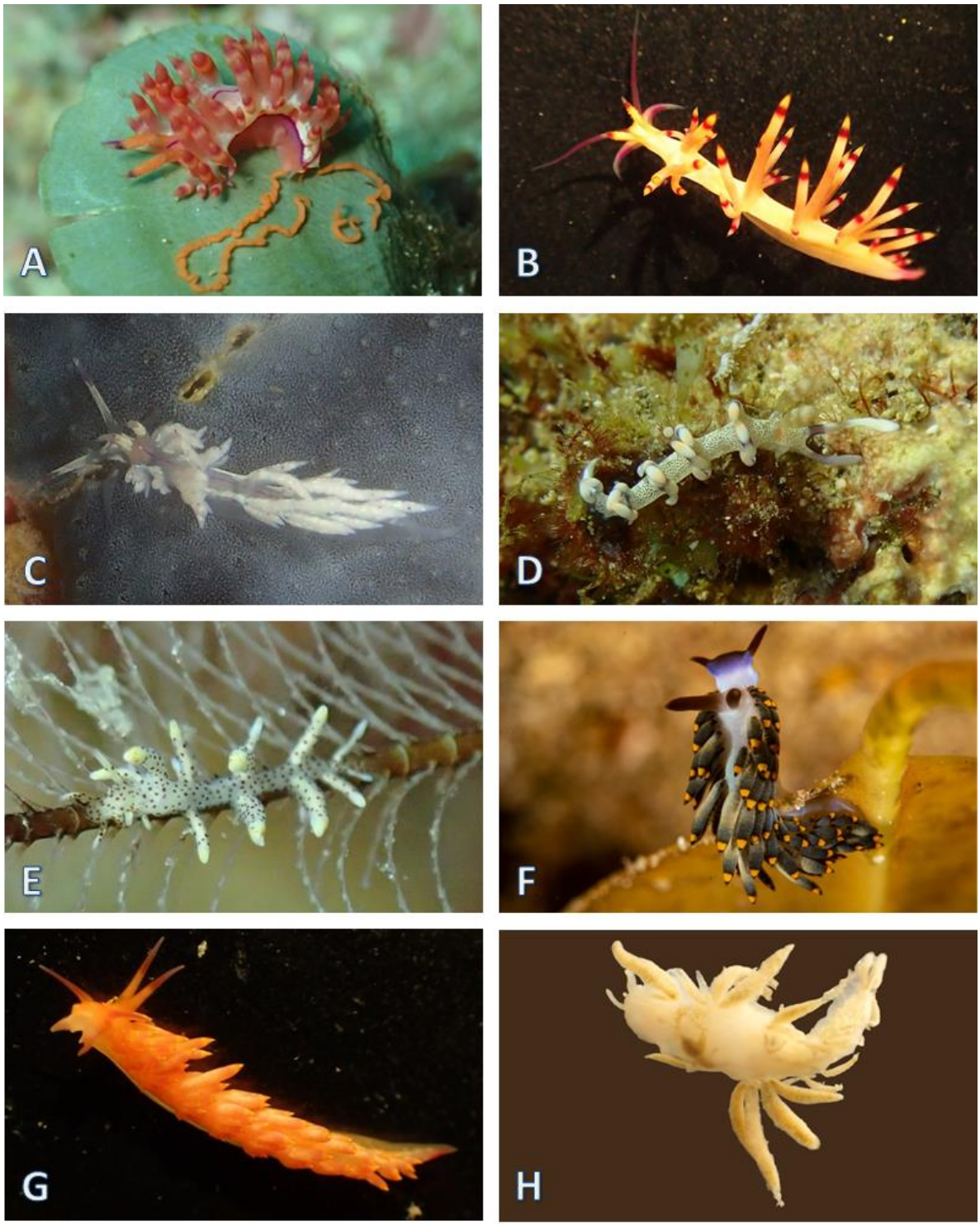


Figure 21. Nudibranchia Cladobranchia: (A) *Coryphellina rubrolineata*, Flru17Ba-1*;* (B) *Flabellina* sp. (*Flabellina* sp. 2 in Gosliner et al. (2015): 333), Flsp2-18Ba-1*;* (C) *Flabellina* sp. (*Flabellina* sp. 3 in Gosliner et al. (2015): 333), Flsp3-17Ba-1*;* (D) *Samla riwo*, Flri17Ba-3; (E) *Eubranchus* sp. *(Eubranchus* sp. 22 in Gosliner et al. (2015): 341), Eusp22-18Ba-1; (F) *Trinchesia yamasui*;* (G) *Cuthona* sp. (*Cuthona* sp. 57 in Gosliner et al. (2015): 353), Cusp57-18Ba-1; (H) *Aeolidia* sp. A, Aeol18Ba-1. *Specimen not collected.

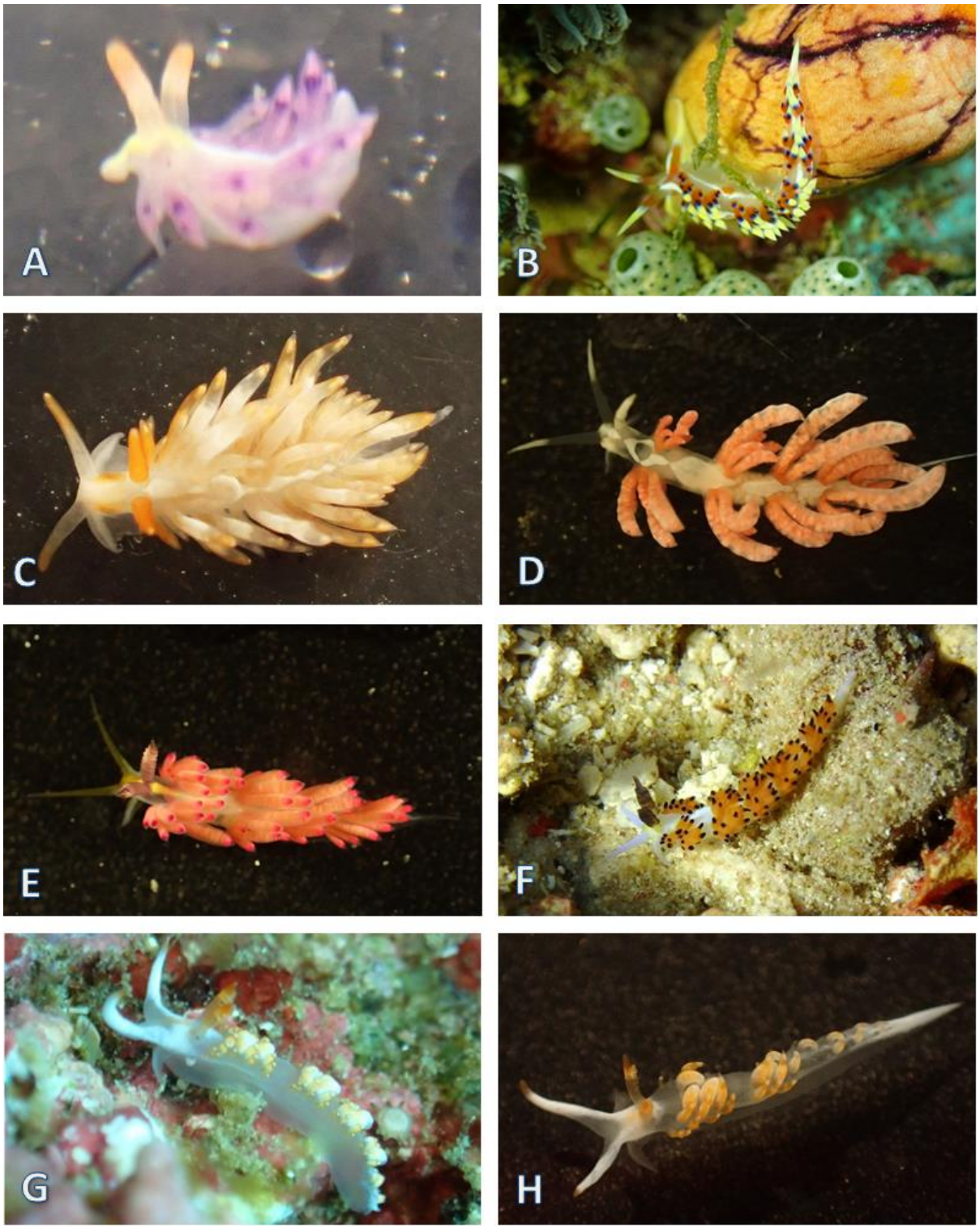


Figure 22. Nudibranchia Cladobranchia: (A) *Antonietta* sp. a, Ansp17Ba-1*;* (B) *Caloria indica*, Cain18Ba- 3*;* (C) *Cratena* sp. a, Crsp17Ba-1*;* (D) *Favorinus japonicas*, Faja18Ba-1; (E) *Favorinus* sp. (*Favorinus* sp. 1 in Gosliner et al. (2015): 363), Fasp1.18Ba-1; (F) *Favorinus tsuruganus*, Fats17Ba-1*;* (G, H) *Moridilla* sp. a, Nosp1.17Ba-1, Mojo18Ba-1.

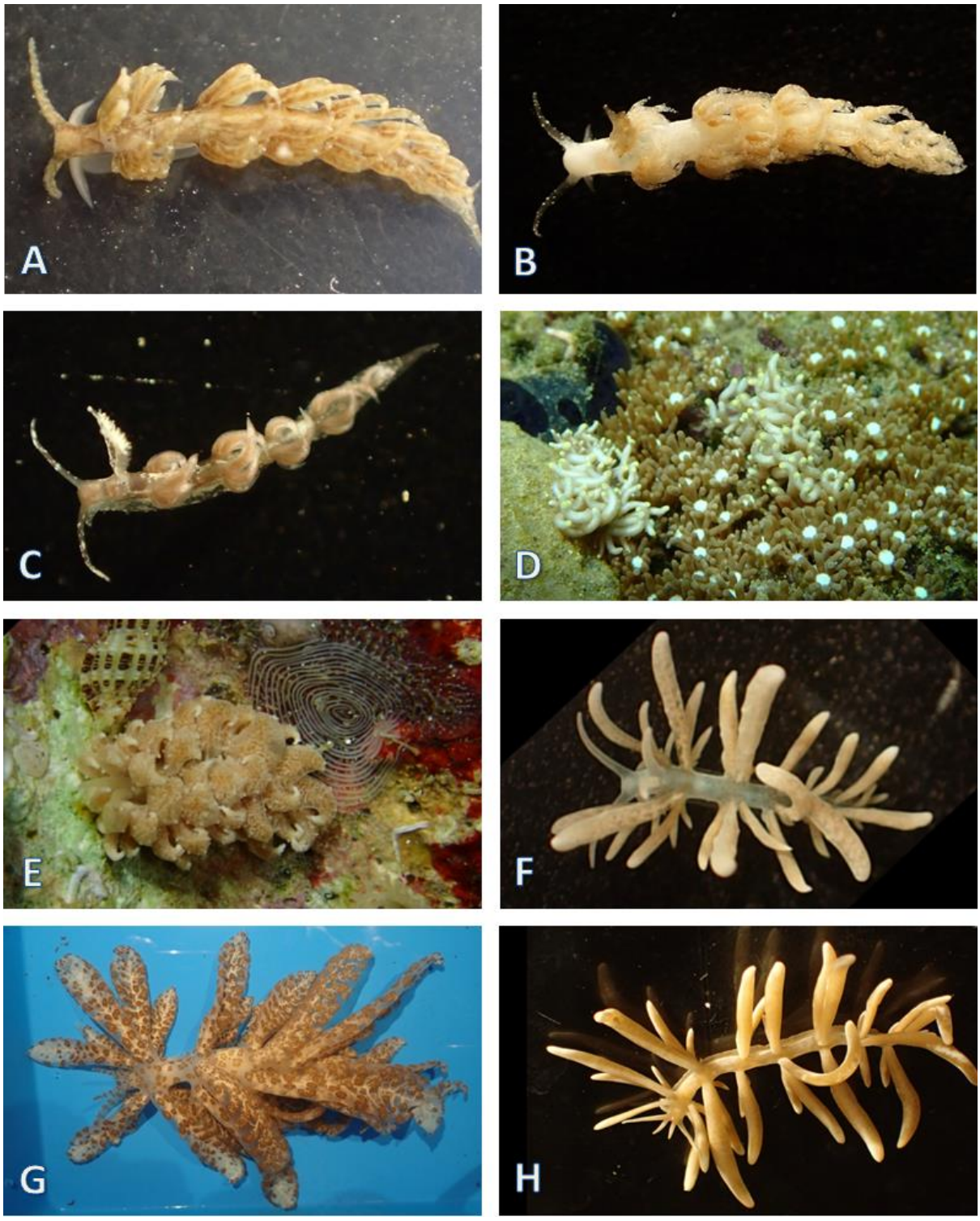


Figure 23. Nudibranchia Cladobranchia: (A) *Noumeaella* sp. (*Noumeaella* sp. 2 in Gosliner et al. (2015): 367), Nosp2_17Ba-1*;* (B) *Noumeaella* sp. (*Noumeaella* sp. 3 in Gosliner et al. (2015): 367), Nosp3Ba-3*;* (C) *Noumeaella* sp. (*Noumeaella* sp. 13 in Gosliner et al. (2015): 369), Nosp13Ba-4*;* (D) *Phyllodesmium briareum*, Phbr18Ba-2-16; (E) *Phyllodesmium* cf. *crypticum*, Phcr17Ba-4; (F) *Phyllodesmium lizardense*, Phliz18Ba-3*;* (G) *Phyllodesmium longicirrum*, Phlo18Ba-1; (H) *Phyllodesmium magnum*, Phma18Ba-1.

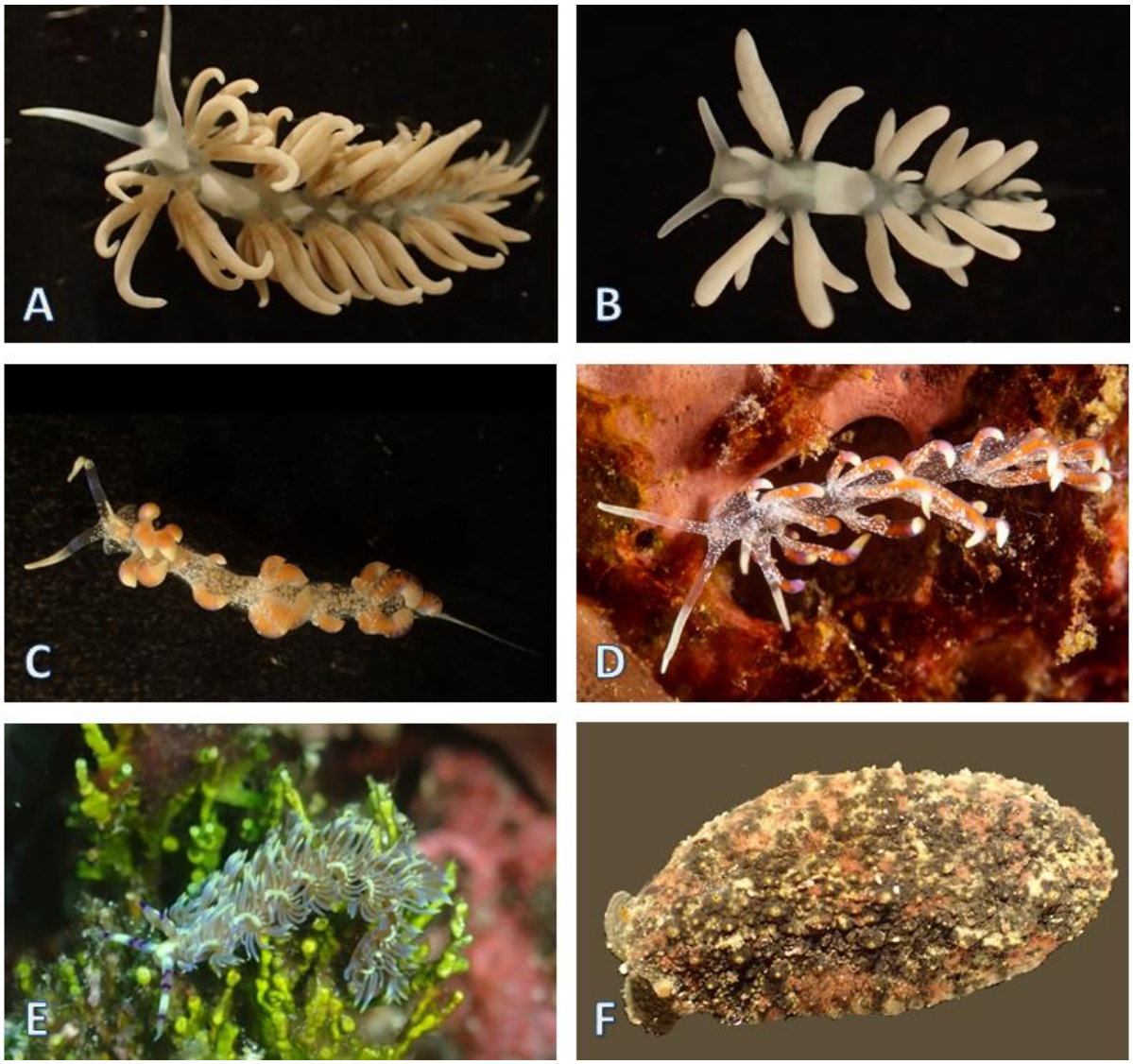


Figure 24. Nudibranchia Cladobranchia and Eupulmonata: (A) *Phyllodesmium parangatum*, Phpa18Ba- 4*;* (B) *Phyllodesmium pecten*, Phpe18Ba-1*;* (C, D*) *Phyllodesmium poindimiei*, Phpo18Ba-1*;* (E) *Pteraeolidia semperi*, Ptse17Ba-2; (F) *Peronia* sp. a, Onsp18Ba-1.

3.1.6. Eupulmonata (One Species in One Genus Belonging to One Family)

Onchidiidae: One member of this family, an undescribed *Peronia* species (confirmed by barcoding) (Figure 24F), was collected in the mangrove. It lives in tidal areas and was nearly 40 mm in size. The notum is a cream background color, with pale green and orange patches. Small cream colored tubercles are spread over the notum.

Figure 25 provides an overview of the species numbers in the various sampling sites, taking into consideration the number of sampling events per site. The high sampling effort at Coral Eye contributed considerably to overall species numbers in this area.

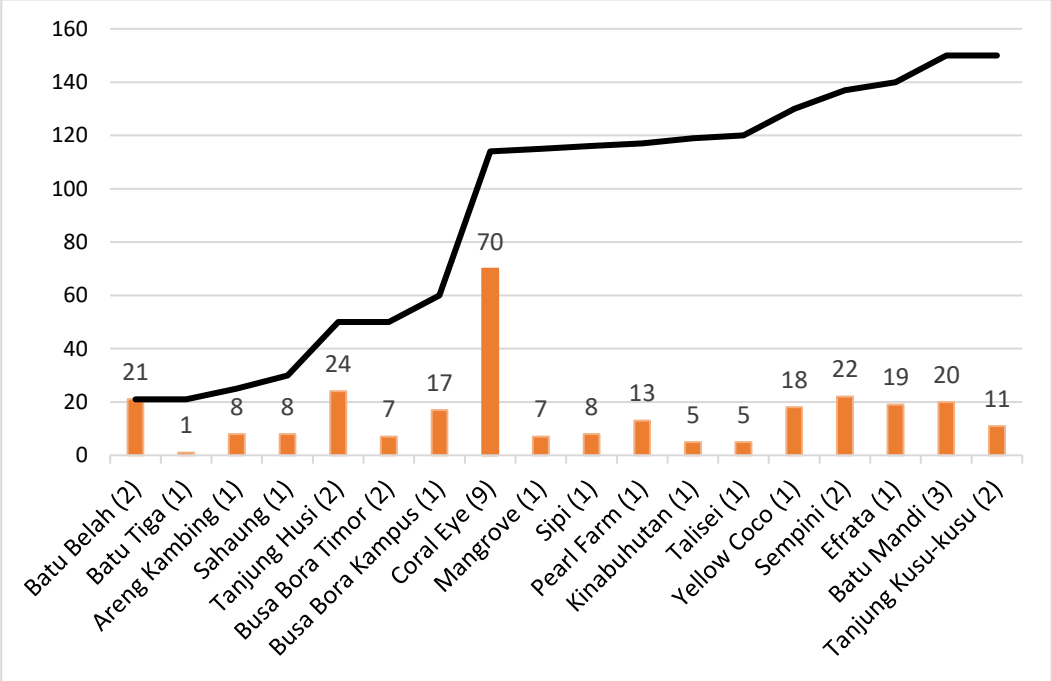


Figure 25. Species accumulation curve (black line) and number of species per locality (red bars). Numbers in brackets after the locality name indicate the number of sampling events at that particular site. Note the high number of sampling events at the locality Coral Eye.

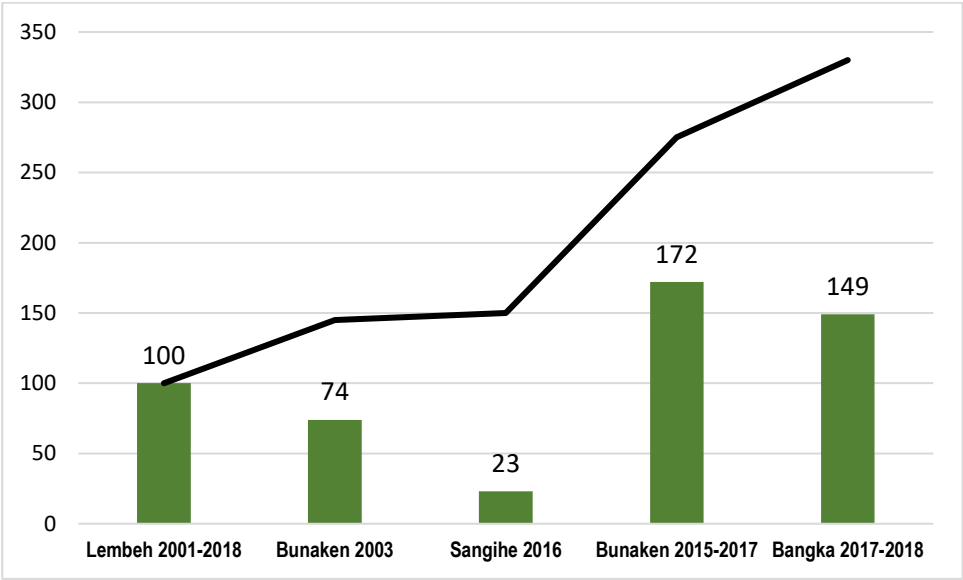


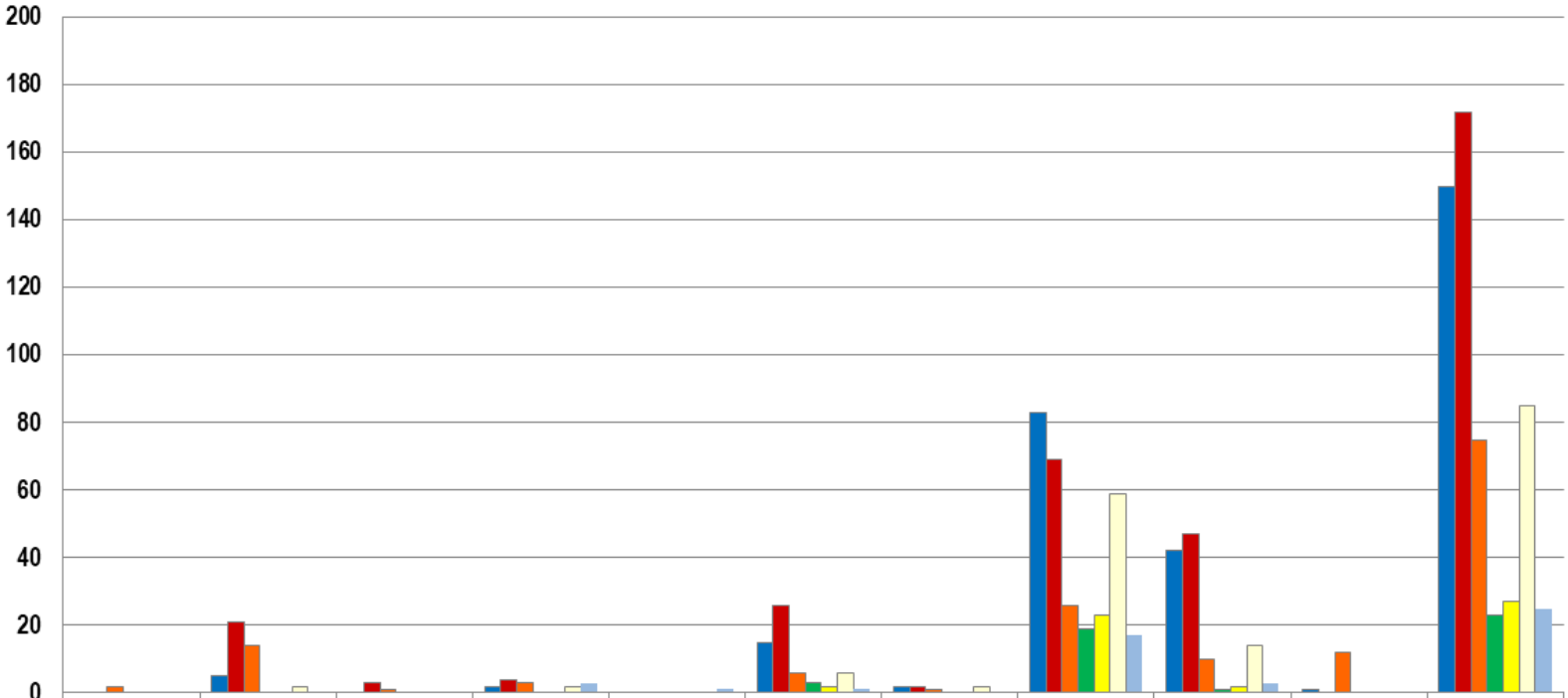
Figure 26. Species accumulation curve and number of species per locality based on the available studies from Lembeh Strait (Tonozuka 2003; Ompi et al. 2019**;** Sea Slug Forum Home page) with the earliest information on diversity from North Sulawesi, Bunaken National Park (BNP) (Burghardt et al. 2006; Kaligis et al. 2018; Eisenbarth et al. 2018)**,** and Sangihe Island (Undap et al. 2019).

4. Discussion

This study is the first of its kind in the Bangka Archipelago (BA). In total, 484 specimens representing 149 species are recorded for the first time from this area, with a continuous increase in species with every collection area (Figure 25). With 33 undescribed species, more than 20% of the total species record is not known to science. In comparison to the recent study of Bunaken National Park (BNP) (Eisenbarth et al. 2018), which recorded 69 undescribed species out of a total of 215 species, the percentage of new species in BA is lower. Summarizing our data from Bangka Archipelago and comparing them with previous studies from North Sulawesi (Figure 26), BA represents the second most diverse area with regard to marine heterobranchs. The species accumulation curve also indicates the increase in species around Bangka Archipelago not recorded before. In total, 333 recorded species are now recorded from North Sulawesi.

When comparing higher order levels in the studied regions of North Sulawesi (Figure 27), the distribution of species within the higher taxa is quite similar, with Doridina and Cladobranchia being the most common taxa, followed by Sacoglossa and Cephalaspidea. However, BA shows the highest number of Doridina, whereas Cladobranchia are more common in BNP.

Distribution of specific taxa is highly variable when comparing the different localities in BA (Table 2), with *Chromodoris annae* (46 individuals) and *Phyllidiella pustulosa* (39 individuals) dominating the overall collection and being encountered in almost all sites. Unpublished results confirm earlier results from Stoffel et al. (Stoffels et al. 2016) and clearly show cryptic speciation in *Phyllidiella pustulosa*, with sympatric occurrences of the various clades in Bangka Archipelago, BNP, and the island of Sangihe. If these clades are considered separate species, the diversity would increase by five to seven species and thus render *P. pustulosa* a much less common species. All available *C. annae* specimens from this study and the prior studies seem to cluster in one clade as one species (unpublished data). Other species were also found in high numbers, but not at many sites. A large population of *Pteraeolidia semperi* was found in one place (Sempini) with more than 100 specimens recorded, probably coming together for mating. More than 50 specimens of *Phyllodesmium briareum* were found on a single large soft coral colony of *Briareum*, which extended nearly 2 m2. However, these larger aggregations are unusual. Many species (62) were found with only one individual.



	Pteropoda	Cephalaspidea	Runcinacea	Aplysiida	Umbraculiida	Sacoglossa	Pleurobranchida	Nudibranchia Doridina	Nudibranchia Cladobranchia	Eupulmonata	Total
Exp Bangka 2017-2018	0	5	0	2	0	15	2	83	42	1	150
Exp Bunaken 2015-2017	0	21	3	4	0	26	2	69	47	0	172
Exp Bunaken 2003	2	14	1	3	0	6	1	26	10	12	75
Exp Sangihe 2017	0	0	0	0	0	3	0	19	1	0	23
Exp Lembeh 2018	0	0	0	0	0	2	0	23	2	0	27
Lembeh Sea slug forum 2001-2010	0	2	0	2	0	6	2	59	14	0	85
Lembeh Tonozuka 2003	0	0	0	3	1	1	0	17	3	0	25

Figure 27. Higher taxa diversity of marine Heterobranchia in Bangka Archipelago compared with other areas in North Sulawesi. Considered are the expeditions to Bunaken National Park (Burghardt et al. 2006; Kaligis et al. 2018; Eisenbarth et al. 2018), to Sangihe Island (Undap et al. 2019), and to Lembeh Strait (Tonozuka 2003; Ompi et al. 2019). Additional information is accumulated for Lembeh Strait from Sea Slug Forum Home Page. Numbers above columns indicate species numbers.

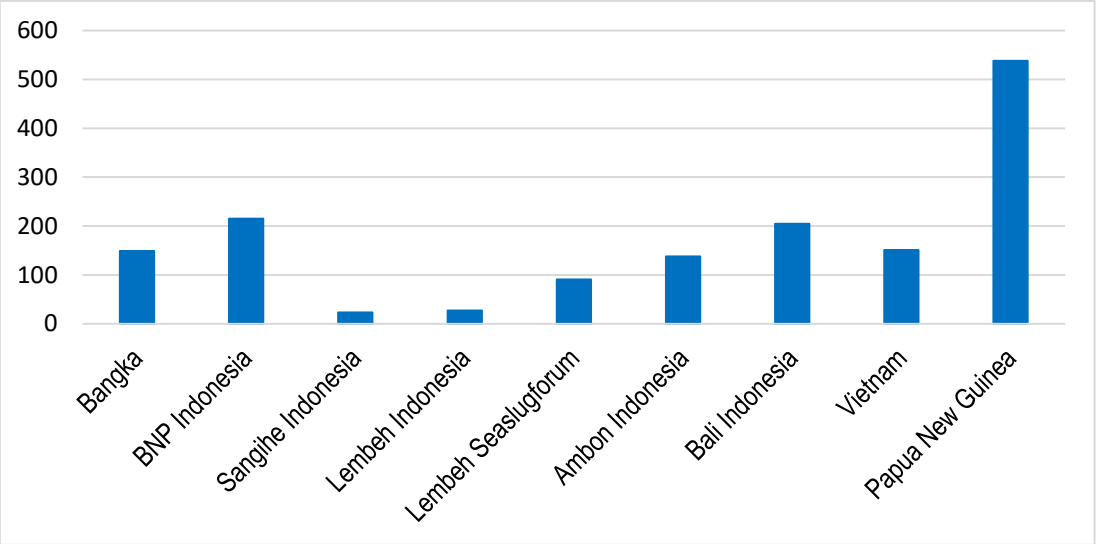


Figure 28. Species diversity from Bangka Archipelago compared with other studies in Indonesia and areas nearby. Data taken from the following literature: BNP, Indonesia (Burghardt et al. 2006; Eisenbarth et al. 2018); Sangihe, Indonesia (Undap et al. 2019); Lembeh Island, Indonesia (Ompi et al. 2019; Sea Slug Forum Home Page); Ambon, Indonesia (Yonow 2001; 2011; 2017; 2018) Bali, Indonesia (Tonozuka 2003); Vietnam (Martinov & Korshunova 2012); and Papua New Guinea (Gosliner 1992).

With regard to animals encountered during diving, the family Phyllidiidae seems to be the most dominant one, with members found at almost all dive sites. The animals feed on sponges, on which they sometimes leave a scar (Figure 17G, H). This was already noted before (Yasman 2003; Alqudah et al. 2016) where, e.g., Yasman (2003) interpreted the scars as an effect of extra-oral digestion.

A few genera are represented by a higher number of species than in other areas of North Sulawesi. Eight species of *Phyllodesmium* are now recorded from BA; this number is thus much higher than that recorded in BNP, with only four species. Members of *Phyllodesmium* feed on octocorals, which are very common in our study area.

Recording diversity can be biased by collection efforts (Figure 25). The highest number of species was collected in front of Coral Eye, due to the proximity of this site to the marine laboratory station; this enabled several night dives and also a more thorough search in the coral rubble, which was collected and examined in the laboratory. Coral rubble is an important habitat for smaller heterobranchs, providing a wide array of food items. Specific searches in this habitat yielded many species not found while diving, e.g., the rather cryptic *Melibe bucephala*, several *Gymnodoris* spp., *Dermatobranchus rodmani*, and *Dermatobranchus* species.

The lowest number of species and specimens occurred in mangrove areas (close to Sipi and Kinabuhutan). This is certainly because of lower food availability. However, a species typical of this habitat, *Jorunna funebris*, was recorded from the locality in high numbers (but not all were collected). The record of only one species from Batu Tiga is certainly due to the difficult diving situation on that particular day with strong currents. The locality is dominated by a coral sand flat at a depth of ca. 20 m with a few coral pinnacles. Snorkeling in front of the destroyed area at the mining site close to Sipi did not result in any records of nudibranchs, due to the still rather fresh stone blocks dumped in front of the site, where only a few corals and sponges were observed to have begun to colonize them. We did not include the mining site in our list of localities, because we did not perform any scuba diving at this particular locality.

Table 3 and Figure 28 list all recorded marine Heterobranchia species from North Sulawesi including our data from Bangka Archipelago (BA) and those published from the three other study areas: Bunaken National Park (BNP) (Burghardt et al. 2015; Eisenbarth et al. 2018; Kaligis et al. 2018), Sangihe Island (SA) (Undap et al. 2019, and Lembeh Strait (LS) (Ompi et al. 2019). Lembeh Strait is particularly famous for its richness in marine heterobranchs, although detailed studies are still lacking and most of the data are only available on the internet. For better comparison, we extracted additional information from Tonozuka (2003) and especially from seaslugforum.net. Photographic records provided by many scientists and citizen scientists on this website were evaluated by Bill Rudman and the scientific community in the past. Using information from citizen scientists has become more important lately in documenting changes in species composition (Pasternak & Galil 2012; Nimbs et al. 2016; Padula et al. 2016; Smith & Davis 2019). This is a state-of-the-art species list and enables future monitoring to assess earlier data from this locality. In the following section, we highlight some results from this comparison.

Only 10 species (*Thuridilla gracilis, Chromodoris annae, C. strigata, Glossodoris cincta, Goniobranchus geometricus, G. reticulatus, Hypselodoris tryoni, Phyllidia ocellata, P. varicosa*, and *Phyllidiella pustulosa*) are recorded from all areas. Of these, *C. annae, P. varicosa, and P. pustulosa* show the broadest distribution with records from nearly all dive sites of the four study areas, and can probably be considered as the most dominant species in North Sulawesi. However, there are also distinct differences in

species composition at each of the various study areas. A few of them we would like to address here, with an emphasis on Bunaken National Park (BNP) and Lembeh Strait (LS). A much higher number of cephalaspideans and sacoglossans are now recorded from BNP than from BA or LS. However, the sacoglossan *Thuridilla gracilis* is very common in BA and LS, but very rare in BNP. Future studies will show which of the recently identified 14 *T. gracilis* lineages (Martín-Hervás et al. 2019) are present. In contrast, its congener T. lineolata is a common species in BNP, especially in the lagoons behind the fringing reefs. This habitat structure is less common in BA and LS and is probably the reason why *T. lineolata* was not found in BA (but recorded in LS).

Six species of *Nembrotha* were collected in BA, but only two of these species, the highly conspicuous *Nembrotha kubaryana* and *N. cristata*, are reported from BNP, which are also very common in BA. Members of the genus feed on ascidians, which usually need higher nutrition in the water column (Pola et al. 2008). No bryozoan-feeding members of the genus *Polycera* are recorded from BA, although several species are recorded from BNP (Eisenbarth et al. 2018). However, the bryozoan-feeding genus *Tambja* is represented by two species in BA, but only one in BNP.

Halgerda batangas was represented in BA with 10 specimens from five different localities, a much higher number than the records in BNP (Eisenbarth et al. 2018). Localities in the two different study areas are similar in as much as all sites with *Halgerda* present were noted to have a highly diverse sponge community. Why diversity and also number of specimens of *Halgerda* species seems to be higher in BA and LS is therefore not clear, but can probably be explained by the distribution and environmental needs of the food sponges (Gosliner et al. 2015; Tibiriçá et al. 2017).

Within Chromodorididae, the genus *Hypselodoris* is quite common in BA and LS, with 11 and 10 species, respectively. In BNP, five species are recorded: the three species *H. apolegma*, *H. maculosa*, and *H. tryoni* are also distributed in BA and LS, but two undescribed species are only recorded from BNP.

Some taxa are small and very inconspicuous, and therefore seldom reported by citizen scientists. The genus *Dermatobranchus* has many small forms with cryptic coloration and is therefore easily overlooked. Our study revealed six different species in BA; Eisenbarth et al. (2018) also recorded six species from BNP; however, the overlap

of species includes only one undescribed species of *Dermatobranchus*. None of these *Dermatobranchus* species are recorded from LS (Sea Slug Forum Home page, Tonozuka 2003).

Dotidae are represented by *Doto* and *Kabeiro* in North Sulawesi. Interestingly, the genus *Kabeiro* was quite common in BNP, but was not found in BA. Members of this genus exclusively feed on hydroids of the family Plumulariidae, which are less common in BA.

Many biotic and abiotic factors influence both the occurrence and distribution of sea slugs. The main island of Bunaken National Park, Bunaken Island, is formed by an old reef and is characterized by fringing reefs with wall-like structures, exposed to hydrodynamics varying in strength (e.g., high waves and strong currents). Lagoons behind the fringing reefs are formed by white sand partly covered by seagrass or mangroves and are influenced by the tides. Lembeh Strait, located in the south-east of the Bangka Archipelago, is of volcanic origin and dominated by slopes with coral patches, volcanic sand slopes, and beds. Fringing reefs, typical for Bunaken National Park, are uncommon in LS and BA. Bangka Island lies between these two areas and supports both habitats: it is partly fringed by reefs, and areas are partly dominated by volcanic sands. However, habitat structure and substrate are not the only factors that influence species composition; temperature and water currents also have a strong effect on the connectivity of populations within these three localities, affecting the distribution of both heterobranch predator and prey larvae (Brodie & Brodie 1995; Fetzer 2004; García & Bertsch 2009). Understanding how these factors contribute to the differences would require much more information about the lifestyle of those particular species that are more common or rare in the respective geographic areas.

Table 3. Compilation of the species recorded in this study and those recently published from Bunaken National Park (Eisenbarth et al. 2018), a previous study from Bunaken National Park (Kaligis et al. 2018), from Sangihe Island (Undap et al. 2019), and three sources on Lembeh Strait: Ompi et al. (2019), species records from Lembeh Strait available from the seaslugforum.net in the time period of 2001–2010, and data extracted from Tonozuka (2003). (*) indicate species that were identified only by photo-documentation.

Expedition in Bangka Archipelago and Islands Adjacent									
Higher Taxon Affiliation	Identifier	Species Name	Exp Bangka 2017-2018	Exp Bunaken 2015-2017	Exp Bunaken 2003	Exp Sangihe 2016	Exp Lembeh 2018	Lembeh Sea slug forum 2001-2010	Tonozuka 2003
Pteropoda									
Limacinidae Gray, 1840		*Limacina* cf. *Helicina* (Phipps, 1774)			X				
Cavoliniidae Gray, 1850 (1815)		*Cavolinia globulosa* Gray, 1850			X				
Cephalaspidea									
Bullidae Lamarck, 1801		*Bulla* cf *ampulla* Linnaeus, 1758			X				
Haminoeidae Pilsbry, 1895		*Atys* cf. *Semistriata* Pease, 1860			X				
		Haminoea curta (Adams, 1850)			X				
	Hasp15Bu-1, Hasp16Bu-1	*Haminoea* sp. (*Haminoea* sp. 2 in Gosliner et al. (2015): 30)		X	X				
		Haminoeid sp. (*Haminoeid* sp. 2 in Gosliner et al. (2015): 34))		X					
	Hasp18Ba-1	*Haminoea* sp. (*Haminoea* sp. 3 (in Gosliner et al. (2015): 30)	X						
	Hasp2_15Bu-1	*Haminoea* sp.		X					
	Hasp2_16Bu-1	*Haminoea* sp.		X					
		Limulatys cf. *ooformis* Habe, 1952			X				
		Phanerophthalmus cf. *Albocollaris* Heller and Thompson, 1983		X	X				
		Phanerophthalmus olivaceus (Ehrenberg, 1828) as *P. smaragdinus*			X				
		Phanerophthalmus sp. (*Phanerophthalmus* sp. 3 in Gosliner et al. (2015): 33)		X					
Philinidae Gray, 1850 (1815)	Ilsp17Bu-1	*Philine* sp.		X	X				
Philinoglossidae Hertling, 1932		*Philinoglossa marcusi* Challis, 1969			X				
		Philinoglossa sp. (in Gosliner et.al (2015): 103)			X				
Colpodaspididae Oskars, Bouchet & Malaquias, 2015		*Colpodaspis thompsoni* G. H. Brown, 1979	X	X	X				
Aglajidae Pilsbry, 1895 (1847)	Agsp15Bu-1	*Aglajidae* sp.		X					
		Chelidonura amoena Bergh, 1905	X	X	X			X cf amoena	
		Chelidonura hirundinina (Quoy and Gaimard, 1833)		X	X				
	Odsp.a18Ba-1	*Odontoglaja* cf. *guamensis* Rudman, 1978	X	X					
		Philinopsis speciosa Pease, 1860						X	
	Phisp16Bu-1	*Tubulophilinopsis* sp.		X					
Gastropteridae Swainson, 1840	Gasp5_16Bu-1	*Gastropteron* sp. (*Gastropteron* sp. 5 in Gosliner et al. (2015) 56))		X					
		Sagaminopteron psychedelicum Carlson and Hoff, 1974	X	X					

	Sasp17Bu-1	*Sagaminopteron* sp.		X				
		Siphopteron Brunneomarginatum (Carlson & Hoff, 1974)		X				
		Siphopteron ladrones (Carlson and Hoff, 1974)		X				
		*Siphopteron nigromarginatu*m Gosliner, 1989		X				
		Siphopteron tigrinum Gosliner, 1989		X	X			
	Sini15Bu-19+20	*Siphopteron* sp.		X				
Runcinacea								
Runcinidae H. Adams and A. Adams, 1854	Rusp15Bu-1	*Runcina* sp.		X	X			
	Rusp16Bu-1	*Runcina* sp.		X				
	Rusp2_16Bu-1 Rusp3_16Bu-1	*Runcina* sp.		X				
Aplysiida								
Aplysiidae Lamarck, 1809		*Aplysia oculifera* A. Adams & Reeve, 1850						X
		Bursatella leachii Blainville, 1817					X	
		Dolabella auricularia (Lightfoot, 1786)						X
		Petalifera ramosa Baba,1959						X
		Aplysia parvula Mörch, 1863	X	X				
		Stylocheilus striatus (Quoy & Gaimard, 1832)	X	X	X			
		Dolabella auricularia (Lightfoot, 1786)		X	X			
		Dolabrifera dolabrifera (Rang, 1828)		X				
		Phylaplysia sp.			X			
		Syphonota geographica (A. Adams & Reeve, 1850)					X	
		Umbraculiida						
Umbraculidae Dall, 1889		*Umbraculum umbraculum* (Lightfoot, 1786)						X
		Sacoglossa						
Cylindrobullidae Thiele, 1931	Assp1_17Bu-1-4	*Cylindrobulla* sp.		X				
Oxynoidae Stoliczka, 1868 (1847)		*Lobiger nevilli* Pilsbry, 1896		X				
	Losp1-18Ba1; Losp1-18Ba2	*Lobiger* sp. (*Lobiger* sp. 1 (in Gosliner et al. (2015): 70)	X	X				
Hermaeidae H. Adams & A. Adams, 1854		*Cyerce bourbonica* Yonow, 2012	X	X	X *C.* sp. 1			
		Cyerce elegans Bergh, 1870			X			
		Cyerce nigra Bergh, 1871	X					
	Cysp2_15Bu-5	*Cyerce* sp.		X				
		Hermaea sp. (*Hermae* sp. 2 in Gosliner et al. (2015): 81) as *Aplysiopsis* sp.1 (Sea slug forum)					X	
		Sohgenia palauensis Hamatani, 1991		X				
Costasiellidae Clarke, 1984	Cosp17Bu-1	*Costasiella kuroshimae* Ichikawa, 1993		X			X *C.* sp. 3	
	Cosp1_17Bu-1-2	*Costasiella* sp. (*Costasiella* sp. 1 in Gosliner et al. (2015): 79)		X				
	Cosp8_17Bu-1	*Costasiella* sp. (*Costasiella* sp. 8 in Gosliner et al. (2015): 81)		X				
	Cosp3_16Bu-1-5	*Costasiella* sp.		X				
Plakobranchidae Gray, 1840		*Elysia asbecki* Wägele, Stemmer, Burghardt and Händeler, 2010	X	X				
		Elysia grandifolia Kelaart, 1858					X	
		Elysia marginata (Pease, 1871)	X	X				
		Elysia mercieri (Pruvot-Fol, 1930)		X				

		Elysia cf *nigropunctata* (Pease, 1871)	X							
		Elysia ornata (Swainson, 1840)							X	
		Elysia pusilla (Bergh, 1871)	X	X	X	X				
	Elsp.a18Ba-1	*Elysia* sp. (*Elysia* sp. a)	X	X						
	Elsp.b18Ba-1	*Elysia* sp. (*Elysia* sp. b)	X	X						
	Elsp1_16Bu-1	*Elysia* sp.		X						
	Elsp16Bu-1	*Elysia* sp.		X						
	Elsp4_16Bu-1	*Elysia* sp.		X						
	Elsp24-18Ba-1	*Elysia* sp. 24 (in Gosliner et al. (2015): 89)	X							
	Elsp27-18Ba-1	*Elysia* sp. 27 (in Gosliner et al. (2015): 89)	X							
		Plakobranchus ocellatus van Hasselt, 1824		X						
		Plakobranchus cf. *papua*				X				
		Thuridilla albopustulosa Gosliner, 1995		X					X	
		Thuridilla carlsoni Gosliner, 1995	X							
		Thuridilla flavomaculata Gosliner, 1995	X	X						
		Thuridilla vataae (Risbec, 1928)	X	X						
		Thuridilla gracilis (Risbec, 1928)	X	X	X *T. bayeri*	X			X	
		Thuridilla cf. *hoffae* Gosliner, 1995			X					
		Thuridilla lineolate (Bergh, 1905)		X	X					X
		Thuridilla livida (Baba, 1955)		X						
		Thuridilla undula Gosliner, 1995		X						
		Pleurobranchida								
Pleurobranchidae Gray, 1827		*Berthellina citrina* (Pease, 1861)		X						
		Berthella martensi (Pilsbry, 1896)							X	
		Pleurobranchus forskalii Rüppell and Leuckart, 1828	X	X						
		Pleurobranchus peronii Cuvier, 1804	X		X				X	
		Nudibranchia, Doridina								
Hexabranchidae Bergh, 1891	Glsp1_17Ba-1	*Hexabranchus sanguineus* (Rüppell & Leuckart, 1830)	X	X	X					
Polyceridae Alder and Hancock, 1845		*Kaloplocamus dokte* Vallès and Gosliner, 2006		X						
	Kalsp8_16Bu-1	*Kaloplocamus* sp. (*Kaloplocamus* sp. 8 in Gosliner et al. (2015): 116)		X						
	Kalsp9_16Bu-1	*Kaloplocamus* sp. (*Kaloplocamus* sp. 9 in Gosliner et al. (2015): 116)		X						
		Nembrotha chamberlaini Gosliner & Behrens, 1997	X							
		Nembrotha cristata Bergh, 1877	X	X						
		Nembrotha kubaryana Bergh, 1877	X	X				X	X	
		Nembrotha lineolata Bergh, 1905	X							
		Nembrotha megalocera Yonow, 1990								X
		Nembrotha milleri Gosliner & Behrens, 1997	X							
		Nembrotha mullineri Gosliner & Behrens, 1997	X							
		Nembrotha purpureolineata O'Donoghue, 1924 as *N. rutilans*							X	
	Nesp1_17Ba-1	*Nembrotha* sp. 1 (in Gosliner et al. (2015): 122)	X							

		Polycera japonica Baba, 1949		X					
		Polycera risbeci Odhner, 1941		X					
	Posp516Bu-1	*Polycera* sp. (*Polycera* sp. 5 in Gosliner et al. (2015): 113)		X					
		Roboastra gracilis (Bergh, 1877)		X	X				
		Tambja gabrielae Pola, Cervera & Gosliner, 2005	X						X
		Tambja morosa (Bergh, 1877)	X		X				
		Thecacera picta Baba, 1972							X
		Gymnodoris aurita (Gould, 1852)*	X						
		Gymnodoris citrina (Bergh, 1877)			X				
	Gysp2_16Bu-1	*Gymnodoris* sp. (*Gymnodoris* sp. 2 in Gosliner et al. (2015): 152)		X					
	Gysp16Bu-1	*Gymnodoris* sp. (*Gymnodoris* cf. sp. 35 in Gosliner et al. (2015): 159)		X					
	Gysp1_17Bu-1 Gysp22_17Bu-1	*Gymnodoris* sp. (*Gymnodoris* cf. sp. 46 in Gosliner et al. (2015): 161)		X					
	Gysp1_15Bu-2	*Gymnodoris* sp.		X					
		Gymnodoris tuberculosa Knutson & Gosliner, 2014	X	X					
		Thecacera sp.						X	
	Gysp20-18Ba-1	*Gymnodoris* sp. 20 (in Gosliner et al. (2015): 156)	X						
	Gysp25-18Ba-1-2	*Gymnodoris* sp. 25 (in Gosliner et al. (2015): 157)	X						
Goniodorididae H. Adams and A. Adams, 1854	Gosp7-18Ba-1	*Goniodoris* sp. 7 (in Gosliner et al. (2015): 153)	X						
		Okenia kendi Gosliner, 2004						X	
		Okenia pellucida Burn, 1967						X	
		Trapania armilla Gosliner & Fahey, 2008	X					X	
		Trapania euryeia Gosliner and Fahey, 2008		X					
		Trapania safracornia Fahey, 2004	X						
Aegiridae P. Fischer, 1883		*Aegires citrinus* Pruvot-Fol, 1930		X					
	Aesp7-18Ba-1	*Aegires* sp. 7 (in Gosliner et al. (2015): 149)	X						
		Notodoris minor Eliot, 1904*	X						X
		Notodoris serenae		X		X			
Doridoidea Rafinesque, 1815	Dosp17Bu-3	*Doridoidea* sp.		X					
	Scsp1_17Bu-1	*Doridoidea* sp.		X					
Cadlinidae Bergh, 1891		*Aldisa albatrossae* Elwood, Valdés & Gosliner, 2000						X	
Discodorididae Bergh, 1891		*Asteronotus cespitosus* (van Hasselt, 1824)*	X	X					X
		Asteronotus mimeticus Gosliner & Valdés, 2002	X	X					
		Atagema intecta (Kelaart, 1859)	X						X
	Disp1_Bu-1	*Diaulula* sp. (*Diaulula* sp. 1 in Gosliner et al. (2015): 197)		X					
		Carminodoris estrelyado (Gosliner & Behrens, 1998)						X	X
		Discodoris cebuensis Bergh, 1877	X						
		Halgerda batangas Carlson & Hoff, 2000	X	X	X		X	X	
		Halgerda carlsoni Rudman, 1978	X	X					
		Halgerda okinawa Carlson & Hoff, 2000						X	

		Halgerda tessellata (Bergh, 1880)		X					
		Jorunna funebris Kelaart, 1859	X				X	X	
		Paradoris liturata (Bergh, 1905)	X					X	
		Platydoris ellioti (Alder & Hancock, 1864)						X	
		Platydoris formosa (Alder & Hancock, 1864)							X
		Platydoris inframaculata (Abraham, 1877)						X	
		Platydoris sanguinea Bergh, 1905	X	X					
		Sclerodoris tuberculata Eliot, 1904						X	
	Scsp2_16Bu-1	*Sclerodoris* sp. (*Sclerodoris* sp. 2 in Gosliner et al. (2015):195)		X					
		Taringa halgerda Gosliner and Behrens, 1998		X					
	Dosp17Bu-1	*Discodorididae* sp.		X					
	Dosp17Bu-2	*Discodorididae* sp.		X					
		Ardeadoris averni (Rudman, 1985)							X
		Ardeadoris cruenta (Rudman, 1986)						X	
		Ceratosoma bicolor Baba, 1949						X	
		Ceratosoma tenue Abraham, 1876*	X					X	X
		Ceratosoma trilobatum (J.E. Gray, 1827)						X	X
	Cesp2_15Bu-3 Cesp1_17Bu-1	*Ceratosoma* sp. (*Ceratosoma* sp. 1 in Gosliner et al. (2015): 266)		X					
		Chromodoris annae Bergh, 1877	X	X	X	X	X	X	
		Chromodoris cf. *boucheti* Rudman, 1982		X					
		Chromodoris colemani Rudman, 1982						X	
		Chromodoris dianae Gosliner & Behrens, 1998	X	X	X	X			
		Chromodoris elisabethina Bergh, 1877*	X						
		Chromodoris lochi Rudman, 1982	X	X	X		X	X	X
		Chromodoris magnifica (Quoy & Gaimard, 1832)	X					X	
Chromodorididae Bergh, 1891		*Chromodoris* cf. *michaeli* Gosliner and Behrens, 1998		X					
		Chromodoris quadricolor (Rüppell & Leuckart, 1830)	X						
		Chromodoris strigata Rudman,1982	X	X	X	X		X	
		Chromodoris willani Rudman, 1982	X	X	X			X	
		Doriprismatica atromarginata (Cuvier, 1804)	X						
		Doriprismatica sibogae Berg, 1905	X						
		Doriprismatica stellata (Rudman, 1986)		X					
		Glossodoris cincta (Bergh, 1888)	X	X		X	X	X	
		Glossodoris hikuerensis (Pruvot-Fol, 1954)		X					
		Glossodoris rufomarginata (Bergh, 1890)*	X					X	
		Goniobranchus aureopurpureus (Collingwood, 1881)						X	
		Goniobranchus coi (Risbec, 1956)	X						
		Goniobranchus decorus (Pease, 1860)			X				
		Goniobranchus fidelis (Kelaart, 1858)	X	X			X	X	
		Goniobranchus geometricus (Risbec, 1928)	X	X	X	X	X	X *C. geometrica*	

		Goniobranchus hintuanensis (Gosliner & Behrens, 1998) as C. hintuanensis						X	
		Goniobranchus kuniei (Pruvot-Fol, 1930)	X					X *C. kuniei*	
		Goniobranchus preciosus (Kelaart, 1858) as *C. preciosa*						X	
		Goniobranchus reticulatus (Quoy & Gaimard, 1832)	X	X		X	X	X *C.reticulata*	
		Goniobranchus roboi (Gosliner & Behrens, 1998)						X	
		Goniobranchus tinctorius (Rüppell & Leuckart, 1830) as *C. tinctoria*			X				
		Goniobranchus verrieri (Crosse, 1875) as *C. verrieri*	X					X	
	Gosp40_16Bu-1	*Goniobranchus* sp. (*Goniobranchus* sp. 40 in Gosliner et al. (2015): 230)		X					
		Hypselodoris apolegma (Yonow, 2001)	X	X	X			X	
		Hypselodoris bullockii (Collingwood, 1881) *	X						
	Hysp1_17Ba-1	*Hypselodoris cerisae* Gosliner & Johnson, 2018	X					X	
		Hypselodoris decorata Gosliner & Johnson, 2018	X						
		Hypselodoris emma Rudman, 1977*	X						
	Chromos18Ba-1	*Hypselodoris iacula* Gosliner & R. F. Johnson, 1999	X						
		Hypselodoris iba Gosliner & R. F. Johnson, 2018							X
		Hypselodoris infucata (Rüppell & Leuckart, 1830)							X
		Hypselodoris kanga Rudman, 1977						X	
		Hypselodoris cf krakatoa Gosliner & R. F. Johnson, 1999						X	
	Hysp19_17Ba-1-2	*Hypselodoris lacuna* Gosliner & Johnson, 2018	X						
		Hypselodoris maculosa (Pease, 1871)	X	X				X	X
		Hypselodoris zephyra (Eliot, 1904)	X						
		Hypselodoris nigrostriata (Eliot, 1904)	X					X	
		Hypselodoris purpureomaculosa Hamatani, 1995						X	X
		Hypselodoris tryoni (Garret 1873)	X	X	X *Risbecia tryoni*	X	X	X	
	Thsp1-18Ba-1	*Hypselodoris* sp. a	X	X				X	
	Hysp16Bu-1	*Hypselodoris* sp.		X					
	Hysp2_16Bu-1	*Hypselodoris* sp.		X					
		Mexichromis aurora (R. F. Johnson & Gosliner, 1998)	X						
		Mexichromis multituberculata (Baba, 1953)						X	
		Mexichromis trilineata (A. Adams & Reeve, 1850)	X					X	
		Miamira sinuata (van Hasselt, 1824)		X	X				
	Dorid18Ba-1	*Miamira magnifica*	X						
	Misp17Ba_1	*Miamira* sp. a	X						
		Noumea simplex Pease, 1871	X						
		Thorunna florens (Baba, 1949)						X	
		Thorunna furtiva Bergh, 1878	X	X					
		Verconia varians (Pease, 1871)		X	X				
	Dosp17Bu-4	*Verconia* sp.		X					
		Dendrodoris albobrunnea Allan, 1933		X					

Dendrodoridae O'Donoghue, 1924 (1864)		*Dendrodoris carbunculosa* (Kelaart, 1858)						X	
		Dendrodoris elongata Baba, 1936			X				
		Dendrodoris guttata (Odhner, 1917)						X	
		Dendrodoris nigra (Stimpson, 1855)	X	X					
	Rosp17Bu-1	*Dendrodoris* sp.		X					
Phyllidiidae Rafinesque, 1814		*Phyllidia* cf *babai* Brunckhorst, 1993*	X					X	X
		Phyllidia coelestis Bergh, 1905	X	X	X	X			
		Phyllidia elegans Bergh, 1869	X	X	X		X	X	
		Phyllidia exquisita Brunckhorst, 1993	X					X	
		Phyllidia madangensis				X			
		Phyllidia ocellata Cuvier, 1804	X	X	X	X		X	
		Phyllidia picta Pruvot-Fol, 1957	X			X			
		Phyllidia polkadotsa Brunckhorst, 1993						X	
	Phsp18Ba-2-3	*Phyllidia* sp. a	X	X		X			
		Phyllidia varicosa Lamarck, 1801	X	X	X	X	X	X	
		Phyllidiella annulata (Gray, 1853)	X	X					
		Phyllidiella lizae Brunckhorst, 1993	X		X	X			
		Phyllidiella nigra (van Hasselt 1824)	X		X	X			
		Phyllidiella pustulosa (Cuvier, 1804)	X	X	X	X	X	X	
		Phyllidiopsis annae Brunckhorst, 1993	X					X	
	Phssp.aBa-1	*Phyllidiopsis* cf *burni*	X						
		Phyllidiopsis krempfi Pruvot-Fol, 1957	X			X			
		Phyllidiopsis pipeki Brunckhorst, 1993		X					
		Phyllidiopsis shireenae				X		X	
		Phyllidiopsis sphingis Brunckhorst, 1993		X					
		Phyllidiopsis xishaensis (Lin,1983)	X	X	X				
Nudibranchia, Cladobranchia									
Arminidae Iredale and O'Donoghue, 1923		*Dermatobranchus caeruleomaculatus* Gosliner & Fahey, 2011*	X						
		Dermatobranchus diagonalis Gosliner and Fahey, 2011		X					
		Dermatobranchus fasciatus Gosliner and Fahey, 2011		X					
		Dermatobranchus cf. *kokonas* Gosliner and Fahey, 2011		X					
		Dermatobranchus cf. *piperoides* Gosliner and Fahey, 2011		X					
		Dermatobranchus rodmani Gosliner & Fahey, 2011	X						
		Dermatobranchus pustulosus van Hasselt, 1824	X						
	Desp8_17Bu-1	*Dermatobranchus* sp. (*Dermatobranchus* sp. 8 in Gosliner et al. (2015): 302)		X					
	Desp17Ba-1	*Dermatobranchus* sp.	X						
	Desp.a18Ba-1-2	*Dermatobranchus* sp. a	X						
	Desp1_16Bu-1 Desp18Ba-1	*Dermatobranchus* sp.	X	X					
Proctonotidae Gray, 1853		*Janolus* cf. *mirabilis* Baba and Abe, 1970		X	X				
		Janolus savinkini Martynov & Korshunova, 2012 as *J.* sp 4						X	
		Janolus sp. 1 (in Gosliner et al. (2015): 305)*	X	X					

Scyllaeidae Alder and Hancock, 1855	Cross16Bu-1-8	*Crosslandia daedali* Poorman and Mulliner, 1981		X				
Bornellidae Bergh, 1874		*Bornella anguilla* S. Johnson, 1984	X					
		Bornella dotoides Pola, Rudman, Gosliner 2009	X					
		Bornella stellifera (A. Adams & Reeve [in A. Adams], 1848)			X			
Pseudovermidae Thiele, 1931		*Pseudovermis* cf. *mortoni* Challis, 1969			X			
Tethydidae Rafinesque, 1815	Mesp.a18Ba-1	*Melibe bucephala* Bergh, 1902	X					
		Melibe engeli Riscec, 1937	X					
		Melibe viridis (Kelaart, 1858)						X
Dotidae Gray, 1853		*Doto ussi* Ortea, 1982	X	X				
		Kabeiro rubroreticulata Shipman and Gosliner, 2015		X				
	Dotosp15Bu-1	*Kabeiro* sp.		X				
	Kasp16Bu-1-15+17 Kasp17Bu-1	*Kabeiro* sp.		X				
	Kasp16Bu-16	*Kabeiro* sp.		X				
Tritoniidae Lamarck, 1809		*Marianina rosea* (Pruvot-Fol, 1930)		X				
	Masp2.18Ba-1	*Marionia* sp. 2 (in Gosliner et al. (2015): 324)	X					
	Trsp3_17Ba-1	*Tritonia* sp. 3 (in Gosliner et al. (2015): 320)	X					
	Trsp8_16Bu-1	*Tritonia* sp. (Tritonia sp. 9 in Gosliner et al. (2015): 321)		X				
	Trisp10_16Bu-1	*Tritonia* sp. (Tritonia sp. 10 in Gosliner et al. (2015): 321)		X				
	Trsp16Bu-1	*Tritonia* sp.		X				
Embletoniidae		*Embletonia gracilis* Risbec, 1928			X			
Flabellinidae Bergh, 1889		*Coryphellina delicate* (Gosliner and Willan, 1991)		X				
		Coryphellina exoptata (Gosliner and Willan, 1991)	X	X	X			X
		Coryphellina rubrolineata O'Donoghue, 1929	X	X			X	X
	Flsp2-18Ba-1	*Flabellina* sp. 2 (in Gosliner et al. (2015): 333)	X					
	Flsp3_17Ba-1	*Flabellina* sp. 3 (in Gosliner et al. (2015): 333)	X					
		Flabellina sp. 8 (Sea Slug Forum)						X
Samlidae Korshunova, Martynov, Bakken, Evertsen, Fletcher, Mudianta, Saito, Lundin, Schrödl and Picton, 2017		*Samla bicolor* (Kelaart, 1858)		X				
		Samla bicolor (Kelaart, 1858)						X
		Samla riwo (Gosliner and Willan, 1991)	X	X				X
Trinchesiidae F. Nordsieck, 1972		*Phestilla lugubris* (Bergh, 1870)		X				
		Phestilla minor Rudman, 1981		X				
		Trinchesia yamasui (Hamatani, 1993)	X					
Eubranchidae Odhner, 1934		*Eubranchus* sp. (*Eubranchus* sp. 22 in Gosliner et al. (2015): 341)	X	X				
Cuthonidae Odhner, 1934	Cusp4_16Bu-1	*Cuthona* sp. (*Cuthona* sp. 4 in Gosliner et al. (2015): 343)		X				
	Cusp54_16Bu-1	*Cuthona* sp. (*Cuthona* sp. 54 in Gosliner et al. (2015): 353)		X				
		Cuthona sp. 57 (in Gosliner et al. (2015): 353)	X					
	Cusp65_16Bu-1	*Cuthona* sp. (*Cuthona* cf. sp. 65 in Gosliner et al. (2015): 343)		X				
Aeolidiidae Gray, 1827	Aeol18Ba-1	*Aeolidia* sp.	X		X	X		
		Bulbaeolidia alba (Risbec, 1928)		X				X
		Baeolidia australis (Rudman, 1982) as *Spurilla australis*						X

		Cerberilla ambonensis Bergh, 1905							X
Facelinidae Bergh, 1889	Ansp17Ba-1	*Antonietta* sp.	X						
		Caloria indica (Bergh, 1896)	X	X					
		Caloria sp. (*Caloria* sp. 1 in Gosliner et al. (2015): 362)		X					
		Cratena sp. (*Cratena* sp. 5 in Gosliner et al. (2015): 383)		X					
	Crsp17Ba-1	*Cratena* sp.	X						
		*Facelina rhodopos*Yonow, 2000		X					
	Fasp3_16Bu-1+3-5	*Facelina* sp. (*Facelina* sp. 3 in Gosliner et al. (2015): 359)		X					
	Fasp3_16Bu-2	*Facelina* sp. (*Facelina* sp. 4 in Gosliner et al. (2015): 359)		X					
	Fasp8_16Bu-1	*Facelina* sp. (*Facelina* sp. 8 in Gosliner et al. (2015): 360)		X					
		Favorinus japonicus Baba, 1949	X	X					
		Favorinus mirabilis Baba, 1955		X					
		Favorinus tsuruganus Baba and Abe, 1964	X						
	Fasp1-18Ba-1-2	*Favorinus* sp. 1 (in Gosliner et al. (2015): 363)	X						
	Mojo18Ba-1	*Moridilla* sp. a	X						
		Noumeaella sp. 2 (in Gosliner et al. (2015): 367)	X						
		Noumeaella sp. 3 (in Gosliner et al. (2015): 367)	X	X					
	Nosp6_17Bu-1-2	*Noumeaella* sp. (*Noumeaella* cf. sp. 6 in Gosliner et al. (2015): 368)		X					
	Nosp12_16Bu-1	*Noumeaella* sp. (*Noumeaella* sp. 12 in Gosliner et al.(2015):367)		X					
		Noumeaella sp. 13 (in Gosliner et al. (2015): 369)	X						
	Nosp2_15Bu 1 Nosp2_16Bu-1-6	*Noumeaella* sp.		X					
		Phyllodesmium briareum (Bergh, 1896)	X	X	X			X	
		Phyllodesmium cf *crypticum* Rudman, 1981	X						
		Phyllodesmium jakobsenae Burghardt & Wägele, 2004			X				
		Phyllodesmium lizardense Burghardt, Schrödl & Wägele, 2008	X						
		Phyllodesmium longicirrum (Bergh, 1905)	X						
		Phyllodesmium magnum Rudman, 1991	X					X	
		Phyllodesmium parangatum Ortiz & Gosliner, 2003	X						
		Phyllodesmium pecten Rudman, 1981	X						
		Phyllodesmium poindimiei (Risbec, 1928)	X	X					
		Phyllodesmium rudmani Burghardt & Gosliner 2006			X as sp 1			X as sp. 11	
		Phyllodesmium undulatum Moore & Gosliner, 2014							X
		Phyllodesmium sp. 9 (Sea Slug Forum)						X	
		Pteraeolidia semperi (Bergh, 1870) as *P. ianthina (*Burghardt and Sea Slug Forum)	X	X	X			X	X
	Fasp17Bu-1	*Facelinidae* sp.		X					
Eupulmonata									
Onchidiidae Rafinesque, 1815		*Peronia* sp.	X		X				
Total		**326**	**149**	**172**	**75**	**23**	**27**	**85**	

5. Conclusions

When comparing our study in BA with other available studies from Indonesia, e.g., Ambon, Bali, and Papua New Guinea (Naturalis Biodiversity Center; Gosliner 1992; Alqudah et al. 2016; Eisenbarth et al. 2018), we can consider the number of 149 species as reasonable for this region after only a few collection events. Only the study in Papua New Guinea exhibits a much higher number of species (Figure 28), probably due to the exceptionally high collection effort in this area (Gosliner 1992). We also cannot exclude the influence of seasons on the success of collection. Larkin et al. (2018) showed a considerable difference in the abundance of marine Heterobranchia seen during the day and night, a factor that we took into consideration to a certain extent. However, we collected only during the late dry season, another factor that may influence overall diversity numbers (Smith & Nimbs 2017; Larkin et al. 2018).

The comparison between the various regions in North Sulawesi (Figure 27) and other Indonesian regions (Figure 28) allows the conclusion that the true number of species is actually certainly much higher than those recorded in these studies, not only in Bangka Archipelago but also in the other Indonesian areas sampled to date. A continuous increase in species records in the Indo-Pacific can already be seen when comparing the identification book of Gosliner et al. (2015) with his follow-up study published in 2018 (Gosliner et al. 2018), with an increase of more than 100 species, from less than 2000 documented ones in the Indo-Pacific to more than 2100. Although we have mainly used the version from 2015, we subsequently checked our findings with the latest version, which did not reveal new information or species relevant for our study. Thus, our results contribute to the overall species numbers for the Indo-Pacific, and they form a baseline for future monitoring in the region, which still appears to be unaffected by environmental stress factors. According to the planktonic community index based on recent planktonic studies, the marine environment of BA is considered to be in a good condition (Usman et al. 2013). However, Ponti et al. (2016) consider the health of the corals in the area as critical, despite the low impact of disease, and refer to the general problems in BA of increased human activities, including mining, which irredeemably compromises reef health (Ponti et al. 2016). Snorkeling in front of the destroyed area at the mining site did not result in any records of nudibranchs, due to the recent stone blocks dumped in front of the site, where only a few corals and sponges were observed to colonize them. Probably due to land erosion and the lack of mangroves to filter nutrients and sediments, the water was extremely murky, and the high concentration of tiny jellyfish might

be an additional indicator of eutrophication (Qu et al. 2014; Smith & Nimbs 2017). However, the nearby investigated areas, such as Sipi, revealed a pristine environment, and 13 heterobranch species were recorded from this locality (Table 2) when diving for approximately one hour with five divers involved. Since the mining project stopped recently, the highly disturbed habitat in front of the mines will provide a good study area for future studies of recolonization and recovery, and the results of this study with detailed information from each locality are a good baseline for comparison.

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Chapter 4

Metabolome of the *Phyllidiella pustulosa* Species Complex (Nudibranchia, Heterobranchia, Gastropoda) Reveals Rare Dichloroimidic Sesquiterpene Derivatives from a Phylogenetically Distinct and Undescribed Clade

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Abstract: Phyllidiid nudibranchs are brightly colored gastropod mollusks, frequently encountered in coral reefs of the tropical Indo-Pacific. The lack of a protective shell is suggested to be compensated by toxic secondary metabolites that are sequestered from specific prey sponges. Our ongoing reconstruction of phyllidiid phylogeny using molecular data of more than 700 specimens, based on published data and newly collected specimens in various seasons and localities around North Sulawesi (Indonesia), demonstrates that *Phyllidiella pustulosa* is a species complex with at least seven well-supported clades. A

metabolomics analysis of 52 specimens from all seven clades of *P. pustulosa* was performed. Secondary metabolite profiles were found to correlate with the phylogenetic study and not the prevailing food sponges as expected. GNPS molecular networking revealed a unique chemotype in clade 6. Detailed chemical analysis of a specimen from this chemically and genetically distinct *P. pustulosa* clade led to the identification of seven new sesquiterpenoids with a rare dichloroimidic moiety (**1** and **4**) and derivatives thereof (**2, 3, 5−7**). Our findings suggest that *P. pustulosa* clades should be raised to the species level.

Introduction

Indonesia is a famous hotspot of biodiversity and the focus of a project aiming to monitor the diversity of one group, the marine Heterobranchia (Gastropoda, Mollusca). One of the most prominent features of mollusks is their shell, which mainly serves as a protection against predators. However, reduction or complete loss of the shell is common in several molluscan groups and well known for the gastropod taxon Heterobranchia. Within this group, the marine forms, formerly known as Opisthobranchia, are well investigated with regard to alternative defense systems. These comprise a variety of strategies, including the presence of calcareous spicules, incorporation of cnidocystes from their prey, and the uptake or de novo biosynthesis of chemicals (Wägele 2004; 2005; Cimino & Gavagnin 2006; Wägele et al 2006; Putz et al. 2010; Avila et al. 2018; Goodheart et al. 2018). Chemical defense is considered a driving force for speciation in heterobranchs (Cimino & Ghiselin 1999; 2009) and may explain the extraordinarily high species numbers in certain groups, such as the nudibranch family Chromodorididae (Nudibranchia, Anthobranchia) with probably more than 500 species.

Chromodorididae are sponge feeders, as are members of the anthobranch family Phyllidiidae, of which only ca. 75 species are known. Recent analyses by molecular means have shown that this latter group probably has a much higher diversity than previously assumed. Stoffels and co-workers (2016) indicated that *Phyllidiella pustulosa*, one of the most common nudibranchs in tropical to temperate waters of the Indo-Pacific (Undap et al. 2019), actually comprises several species.

Many investigations focused on secondary metabolites across the phyllidiid taxa, e.g., *Phyllidia coelestis* (Jasaimut et al. 2013; Carbone et al. 2019), *P. ocellata* (Fusetani et al. 1992; White et al. 2015), *P. (Fryeria) picta* (Sim et al. 2018), *P. varicose* (Burreson et al. 1975; Yasman et al. 2003), and *Phyllidiopsis krempfi* (Okino et al. 1996). *P. pustulosa* has been studied to the largest extent (Kassühlke et al. 1991; Hirota et al. 1998; Wright 2003;

Manzo et al. 2004; Lyakhova et al. 2010; Jomori et al. 2015; White et al. 2017). As a result of these studies, a large number of various sesqui- and diterpene-type secondary metabolites, mostly substituted with isocyanate and isothiocyanate functionalities, have been described. The reviews by Garson and Simpson (2004) and the more recent one by Emsermann et al. (2016) provide comprehensive overviews on the structural diversity of this large compound family. Chemical variability was already noted in *P. pustulosa* by Okino and co-workers (1996), who compared four populations from different sites and found different terpenoid compounds in each population.

In this study we provide the first results evolved from the overall phylogenetic reconstruction of more than 700 phyllidiid specimens using molecular 16S and CO1 markers and morphological characters (Papu et al., in preparation). In addition to more evidence for cryptic speciation in the various phyllidiid species and genera, this analysis confirms cryptic species within the *P. pustulosa* species complex, involving seven clades. In order to find chemical differences between the clades supporting the molecular results, we chemically analyzed 52 specimens of these seven distinct clades, as well as the closely related species *P. nigra, Phyllidiopsis burni*, and *P. krempfi.* These analyses provided results that, interestingly, highlighted one clade specifically, from which we report the isolation and structure elucidation of seven new dichloroimidic sesquiterpenoids and their derivates (**1-7**) and a structure assignment of two unstable dichloroimidic sesquiterpenoids (**8, 9**).

RESULTS AND DISCUSSION

During several expeditions to Bunaken National Park, Bangka Archipelago, and Sangihe Island (North Sulawesi, Indonesia) between 2015 and 2018, more than 500 phyllidiid specimens were collected (Undap et al. 2019; Kaligis et al. 2018; Eisenbarth et al. 2018; Papu et al. 2020). Based on morphological inspection in the field, 269 specimens of these were tentatively identified as *Phyllidiella pustulosa*. A tree of the Phyllidiidae, including these specimens, was retrieved from two mitochondrial genes (16S and CO1 partial gene sequences), including sequences from NCBI (Papu et al., in preparation). The phylogenetic analysis revealed the species *P. pustulosa* as a species complex and thus confirms previous studies (Stoffels et al. 2016). *P. nigra* and *P. rudmani* cluster within *P. pustulosa* clades, additionally rendering *P. pustulosa* a paraphyletic group (Figure 1). Specimens assigned to *P. lizae* (Table 1) also cluster with *P. pustulosa* specimens from clade 1 and clade 5, as well as *P. rudmani*. These results clearly indicate the necessity of a revision of systematics within the

genus Phyllidiella. Figure 1 highlights (in colored boxes) all *P. pustulosa* clades including specimens from the closely related genus Phyllidiopsis, of which selected specimens were also chemically analyzed during this project. Based on this phylogenetic reconstruction, 52 specimens representing all seven clades of *P. pustulosa* (including specimens with a typical color variations, thus probably misidentified in the field) and closely related *P. nigra* and *Phyllidiopsis* spp. were selected (see identifiers in Figure 1) and subjected to metabolomic analysis.

Table 1. List of sequences (own and published data) used in this study for tree reconstruction (Valdés 2003; Stoffel et al. 2016; Hallas et al. 2017; Undap et al. 2019). NCBI accession numbers for CO1 and 16S partial genes are provided, as well as individual sequence voucher/IDs. Localities of the specimens according to published data are additionally provided. Specimens analyzed chemically in this study are highlighted in bold.

Species	CO1 gene	16S gene	Voucher/ID	Locality
Phyllidiella pustulosa	KX235966	-	336586	Southeast Gam, Desa Besir
Phyllidiella pustulosa	KX235969	-	336585	Southeast Gam, Desa Besir
Phyllidiella pustulosa	KX235962	-	336587	South Gam, Eastern entrance Besir Bay, Cape Besir
Phyllidiella pustulosa	KX235961	-	336584	West Pulau Yeben Kecil
Phyllidiella pustulosa	KX235964	-	336583	South Gam, Eastern entrance Besir Bay, Cape Besir
Phyllidiella pustulosa	KX235957	-	336474	Tanjung Tabam
Phyllidiella pustulosa	KX235956	-	336470	Northwest side of Maitara, North Moluccas
Phyllidiella pustulosa	KX235954	-	336460	Desa Tahua
Phyllidiella pustulosa	KX235955	-	336461	Desa Tahua
Phyllidiella pustulosa	KX235959	-	336508	Dufadufa/ Benteng Toloko
Phyllidiella pustulosa	KX235970	-	336588	West Pulau Yeben Kecil
Phyllidiella pustulosa	KX235963	-	336581	South Gam, Besir Bay
Phyllidiella pustulosa	KX235960	-	336510	Dufadufa / Benteng Toloko
Phyllidiella pustulosa	-	KP873167	KP873167	Bidong Island (Terengganu)
Phyllidiella pustulosa	KX235967	-	336580	Southwest Pulau Kri
Phyllidiella pustulosa	KX235971	-	336579	South Gam, Besir Bay
Phyllidiella pustulosa	KX235968	-	336582	Southwest Pulau Kri
Phyllidiella pustulosa	KX235953	-	336436	Off Danau Laguna
Phyllidiella pustulosa	KX235965	-	336578	South Gam, Southeast Besir Bay
Phyllidiella pustulosa	KX235958	-	336495	Tanjung Ratemu (South of river)
Phyllidiella pustulosa	MN248608	MN243996	Phpu16Sa-5	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478669	MT483964	Phpu15Bu-14	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	KJ001310	-	KJ001310	Lizard Island, Queensland, Australia
Phyllidiella pustulosa	-	AF430366	AF430366	Baie du Santal, Lifou, New Caledonia
Phyllidiella pustulosa	-	AF249232	AF249232	Baie du Santal, Lifou, New Caledonia
Phyllidiella pustulosa	MN248610	-	Phpu16Sa-68	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248601	MN243991	Phpu16Sa-3	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248602	MN244006	Phpu16Sa-6	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478670	MT483965	Phpu15Bu-4	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MT478671	-	Phpu16Sa-4	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478672	MT483966	Phpu15Bu-9	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MN248627	MN243980	Phpu16Sa-9	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248621	MN244002	Phpu16Sa-52	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248615	MN243989	Phpu16Sa-76	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248619	MN243988	Phpu16Sa-79	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248630	-	Phpu16Sa-90	Sangihe Island, North Sulawesi

Phyllidiella nigra	MT478673	MT483967	Phpu15Bu-19	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478674	MT483968	Phpu15Bu-10	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478675	MT483969	Phpu15Bu-18	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478676	MT483970	Phpu15Bu-7	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478677	MT483971	Phpu15Bu-6	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478678	MT483972	Phpu15Bu-43	Bunaken Island, North Sulawesi
Phyllidiella nigra	MT478679	MT483973	Phpu16Sa-64	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248625	MN243978	Phpu16Sa-24	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248626	MN243979	Phpu16Sa-26	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248634	MN244014	Phpu16Sa-60	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248633	MN244013	Phli16Sa-7	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478680	MT483974	Phpu15Bu-3	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MT478681	MT483975	Phpu15Bu-25	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MT478682	MT483976	Phpu15Bu-11	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MN248636	MN244015	Phpu16Sa-2	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248638	MN244018	Phpu16Sa-70	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248598	MN244010	Phpu16Sa-39	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248599	MN244012	Phpu16Sa-84	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248596	MN244009	Phpu16Sa-27	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248594	MN244007	Phpu16Sa-7	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248595	MN244008	Phpu16Sa-25	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478683	-	Phpu15Bu-27	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MN248580	-	Phpu16Sa-14	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248592	MN243967	Phpu16Sa-92	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478684	MT483977	Phpu15Bu-35	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MT478685	MT483978	Phpu16Sa-37	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MT478686	MT483979	Phpu16Bu-8	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	MT478687	MT483980	Phpu15Bu-21	Bunaken Island, North Sulawesi
Phyllidiella pustulosa	-	MT483981	Phpu17Ba-4	Bangka Island, North Sulawesi
Phyllidiella pustulosa	-	MT483982	Phpu16Sa-32	Sangihe Island, North Sulawesi
Phyllidiella pustulosa	MN248641	MN243958	Phpu16Sa-50	Sangihe Island, North Sulawesi
Phyllidiella nigra	KX235948	-	336472	Maitara Northwest, North Moluccas
Phyllidiella nigra	KX235949	-	336501	Sulamadaha I, North Moluccas
Phyllidiella nigra	KX235946	-	336434	Off Danau Laguna, North Moluccas
Phyllidiella nigra	KX235947	-	336471	Maitara Northwest
Phyllidiella nigra	KX235952	-	336576	South Gam, Eastern entrance Besir Bay, Pulau Bun
Phyllidiella nigra	KX235951	-	336577	South Gam, Southeast Besir Bay
Phyllidiella nigra	KX235950	-	336505	Sulamadaha II
Phyllidiella nigra	-	MF958280	CASIZ 186196A	Maricaban Strait, Batangas Prov. Luzon,Philippines
Phyllidiella lizae	KJ001309	-	KJ001309	Lizard Island, Queensland, Australia
Phyllidiella lizae	-	AF430365	AF430365	Baie du Santal, Lifou, New Caledonia
Phyllidiella lizae	-	KJ018918	KJ018918	Lizard Island, Queensland, Australia
Phyllidiella lizae	MN248576	MN243972	Phli16Sa-5	Sangihe Island, North Sulawesi
Phyllidiella rudmani	KX235945	-	336589	Southeast Gam, Friwen Wonda
Phyllidiopsis fissuratus	KX235944	-	336590	Yenweres Bay
Phyllidiopsis krempfi	MN248650	MN244074	Phpu16Sa-58	Sangihe Island, North Sulawesi
Phyllidiopsis krempfi	MN248647	MN244073	Phpu16Sa-66	Sangihe Island, North Sulawesi
Phyllidiopsis krempfi	KX235976	-	336512	Dufadufa / Benteng Toloko.
Phyllidiopsis krempfi	KX235974	-	336466	Tanjung Ebamadu
Phyllidiopsis krempfi	KX235972	-	336453	Kampung Cina / Tapak 2
Phyllidiopsis krempfi	KX235984	-	336595	Southwest Pulau Kri
Phyllidiopsis krempfi	KX235979	-	336594	Southwest Pulau Kri, Kuburan

Phyllidiopsis krempfi	KX235983	-	336596	Northwest Pulau Mansuar, Lalosi reef
Phyllidiopsis krempfi	KX235973	-	336462	Tanjung Ebamadu
Phyllidiopsis krempfi	KX235975	-	336469	West Maitara, North Moluccas
Phyllidiopsis krempfi	KP873168	-	KP873168	Bidong Island (Terengganu)
Phyllidiopsis krempfi	KX235982	-	336599	East Kri, Sorido Wall
Phyllidiopsis krempfi	KX235981	-	336600	Northeast Mansuar
Phyllidiopsis krempfi	KX235977	-	336650	Teluk Dodinga; West Karang Ngeli.
Phyllidiopsis krempfi	KX235980	-	336598	North Batanta, North Pulau Yarifi
Phyllidiopsis krempfi	KX235978	-	336597	Southwest Pulau Kri, Kuburan
Phyllidiopsis krempfi	MT478688	MT483983	Phpu15Bu-37	Bunaken Island, North Sulawesi
Phyllidiopsis burni	MN248655	MN244078	Phpu16Sa-93	Sangihe Island, North Sulawesi
Phyllidiopsis burni	MN248652	-	Phpu16Sa-19	Sangihe Island, North Sulawesi
Phyllidiopsis burni	-	MN244081	Phpu16Sa-55	Sangihe Island, North Sulawesi
)endrodoris atromaculat	MF958434	MF958307	CASIZ 181231	Janao Bay, Luzon, Philippines

Extracts of individually stored nudibranchs were first analyzed by ^{1}H NMR spectroscopy and HR-LCMS. The occurrence of several chemotypes became evident after the preliminary ^{1}H NMR analysis and was finally confirmed by HR-LCMS. Obtained MS data also allow molecular network analyses using the Web-based platform Global Natural Products Social Molecular Networking (GNPS), which provides a method to rapidly assess the diversity and chemical relatedness of secondary metabolites in complex extracts, based on similarities in MS/MS parent ion fragmentation patterns (Wang et al. 2016). Interestingly, a locality-dependent distribution of the secondary metabolite profiles, e.g., dependent upon the prevailing food sponges, as originally hypothesized by us, was not found. Instead, the chemical profiles correlated with the results of the phylogenetic study. The metabolomic analysis using the GNPS platform clearly indicated that the specimens Phpu15Bu-21, Phpu16Sa-32, and Phpu17Ba-4 possess unique chemical features (Figure 2, yellow nodes). All three specimens were collected in localities lying 40 to 200 km apart (Bunaken Island, Bangka Archipelago, and Sangihe Island) and during expeditions that took place in 2015, 2016, and 2017, respectively. Nevertheless, the metabolomic profiles of the specimens Phpu15Bu-21 and Phpu16Sa-32 were found to be identical (Figure 3), whereas the extract of the specimen Phpu17Ba-4 contained mostly derivatives/degradation products of the major compounds detected in the two above mentioned phyllidiids. The most intriguing finding, however, was that these three specimens form the separate clade 6 (Figure 1, yellow), providing strong evidence of specific chemical profiles characteristic for the genetically distinct *P. pustulosa* clades or even separate as yet undescribed species.

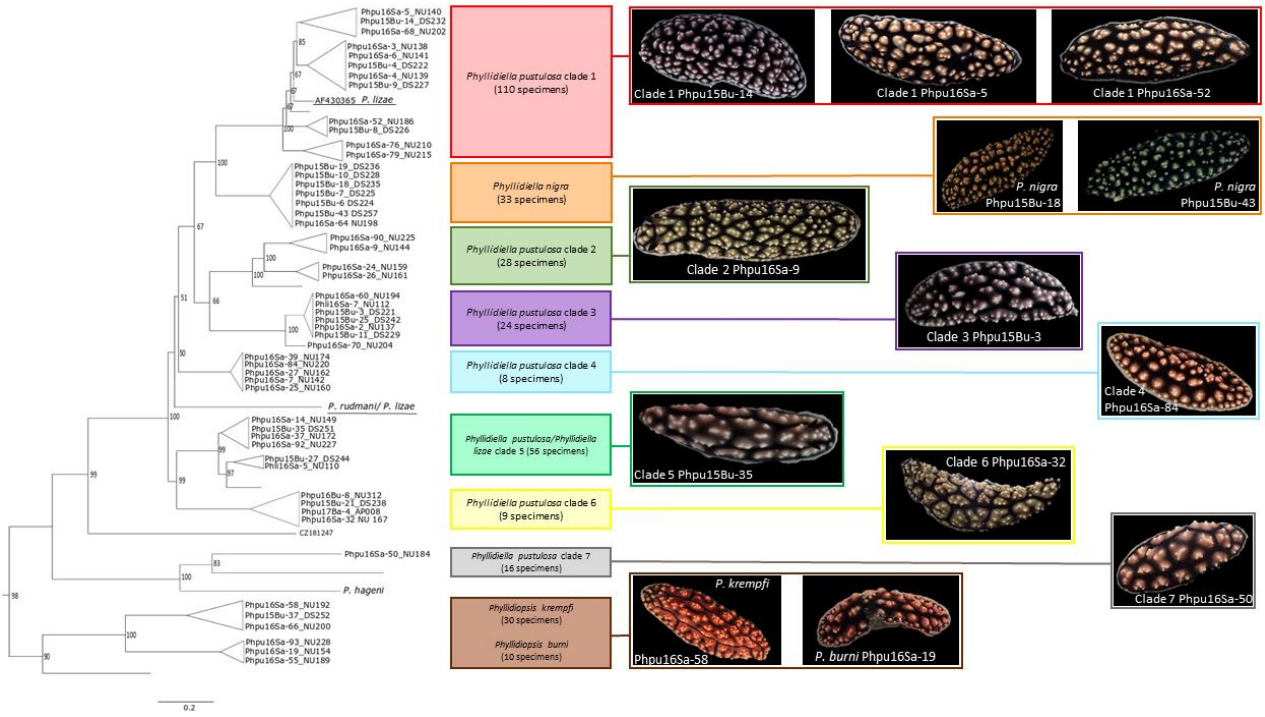


Figure 1. Condensed phylogenetic tree including the chemically investigated 52 phyllidiid specimens. The depicted tree reconstruction, based on 16S and CO1 partial gene sequences, include 340 *Phyllidiella* and *Phyllidiopsis* specimens (own and from NCBI). Clades are partly collapsed to increase clarity. Numbers within the colored clades indicate the overall specimens used for tree reconstruction. The distinct *P. pustulosa* clade 6 (yellow frame) possesses a unique chemotype and the specimen Phpu15Bu-21 was chemically analyzed in detail for its secondary metabolites. Bu in identifier signifies Bunaken National Park as locality, Ba Bangka Island and Sa Sangihe Island.

Structure Elucidation of New Compounds 1-9. As outlined above, GNPS molecular networking of the extracts of 52 phyllidiid specimens indicated a unique secondary metabolome of the phylogenetically well separated clade 6 within the *P. pustulosa* species complex. Out of this clade one specimen (internal code Phpu15Bu-21) was chosen for detailed chemical analysis. Chromatographic separations using SPE and HPLC followed by 1D and 2D NMR methods led to the isolation and structure elucidation of two new dichloroimidic sesquiterpenes (**1** and **4**) and five new derivatives (**2**, **3**, **5**-**7**) thereof. Molecular structures of two additional dichloroimidic sesquiterpenes (**8** and **9**) could only be established by the interpretation of the HR-LCMS data due to their chemical instability.

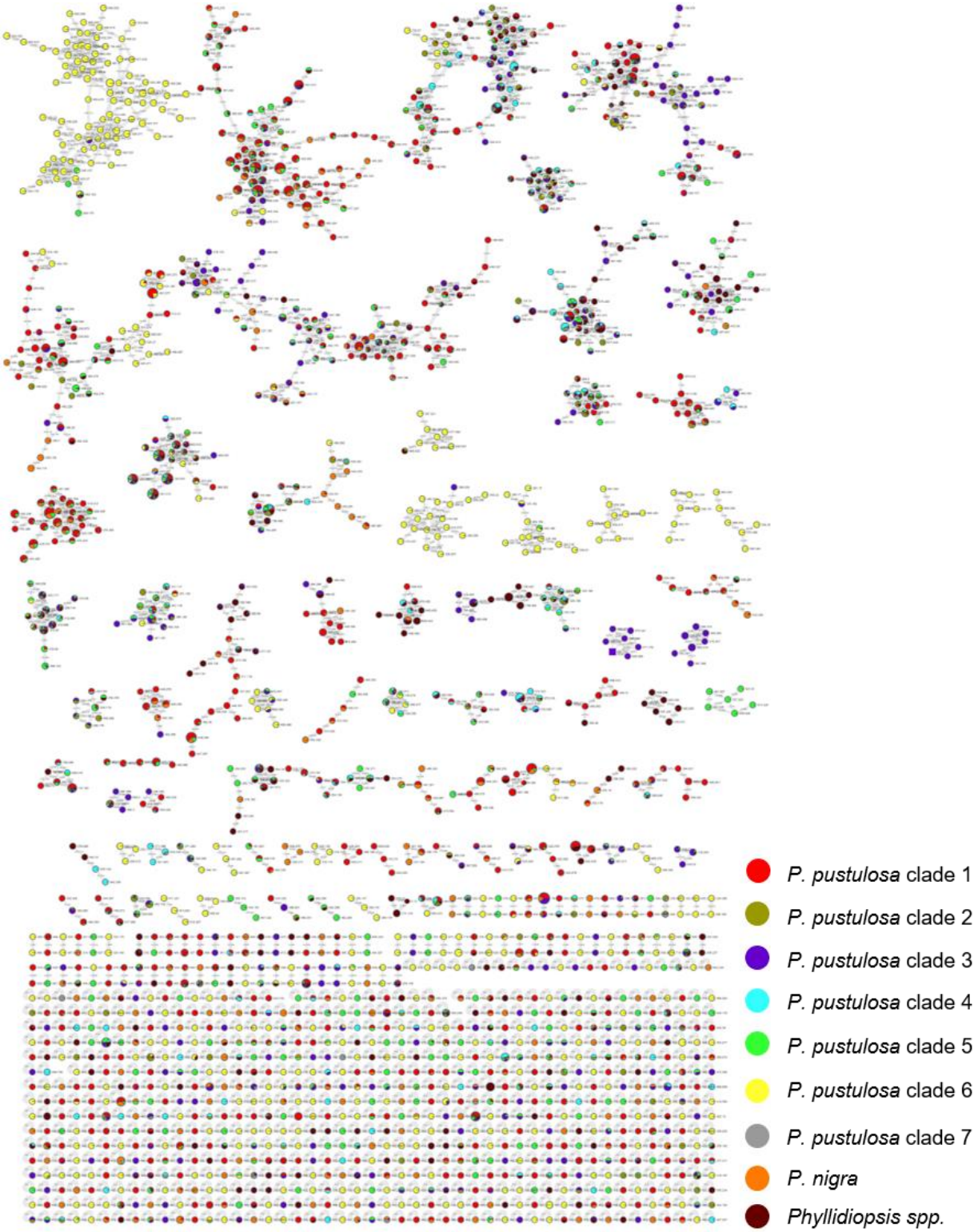


Figure 2. GNPS analysis of HR-LCMS data obtained from crude extracts of 52 phyllidiid specimens. Unique secondary metabolites detected in the *Phyllidiella pustulosa* clade 6 form distinct clusters (yellow nodes).

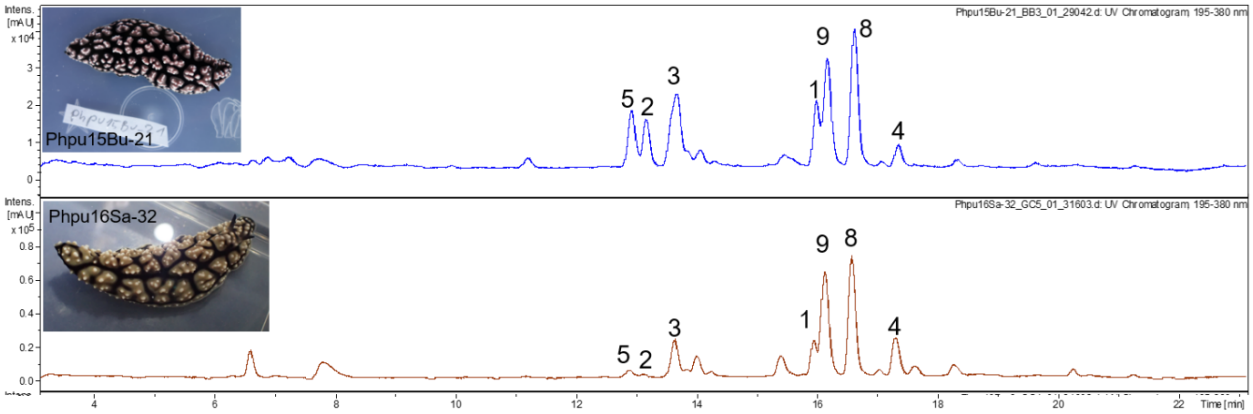


Figure 3. LC-DAD chromatograms (UV trace 195–380 nm) of two specimens phylogenetically assigned to *Phyllidiella pustulosa* clade 6. Phpu15Bu-21 and Phpu16Sa-32 were collected at different localities (Johnson diving site at Bunaken Island and Talengen Bay at Sangihe Island) about 200 km apart, and in different years (2015 and 2016, respectively). Structures **1-9** are shown in chart 1.

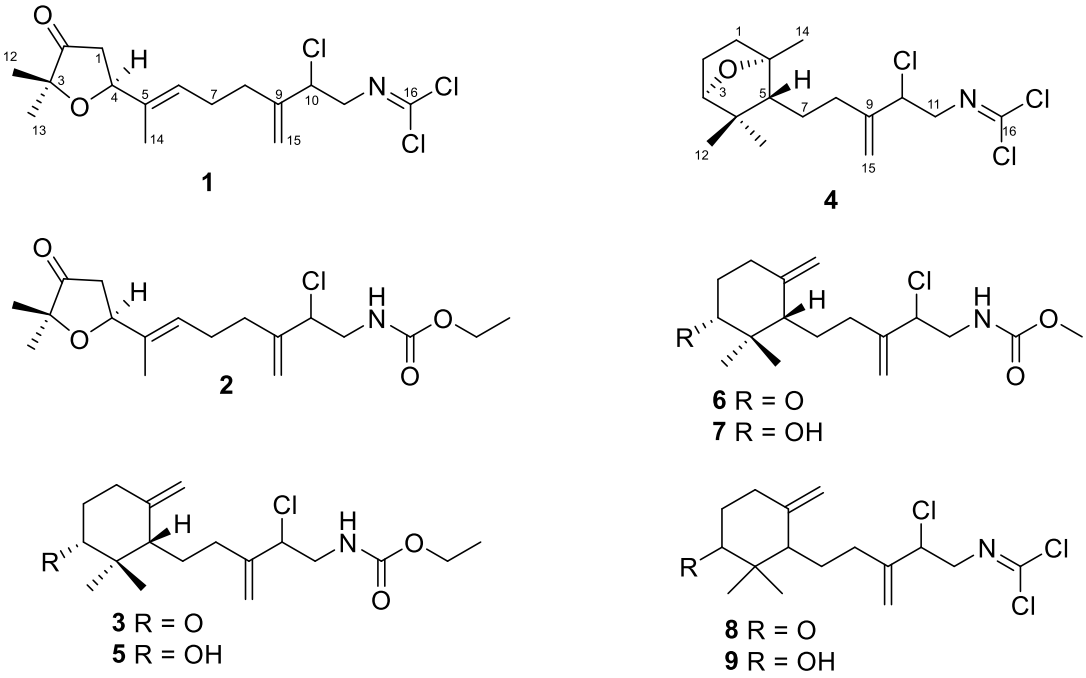


Chart 1. Structures of the new metabolites from Phyllidiella pustulosa clade 6.

Compound **1** was isolated as a colorless oil. The analysis of the mass spectrum obtained via an HR-ESIMS experiment revealed an isotope pattern typical for a polychlorinated compound. The molecular formula $C_{16}H_{22}Cl_3NO_2$ was established from *m/z* 366.064 $[M + H]^+$ and an ion resulting from loss of chlorine 330.099 $[M - Cl]^+$. The molecule showed five degrees of unsaturation. The presence of the carbonimidic dichloride was evident from a strong IR absorption at 1654 cm^{-1} and a weak nonprotonated ^{13}CNMR signal at δ 127.3. The ^{13}C NMR spectrum consisted of 16 carbon resonances, which were assigned to five nonprotonated carbons, four CH, five CH_2, and three CH_3 groups using DEPT and HSQC experiments (Table 2). The presence of four double bonds (2 C=C; 1 C=O; 1 C=N) indicated

the molecule to be monocyclic. The ^{1}H NMR spectrum contained three singlet methyl signals (H3-13, -12, -14; δ_H 1.25, 1.30, and 1.73), two broad singlet resonances at δ_H 5.29 and 5.14 that were assigned to an exomethylene group (H_2-15, δ_C 115.2), and a triplet at δ_H 5.66 (H-6, J = 6.0 Hz) attributable to an sp^2 proton. Two further deshielded resonances at δ_H 4.67 (H-4, doublet of doublets, J = 6.5, 9.5 Hz) and δ_H 4.76 (H-10, triplet J = 6.8 Hz) indicated that they are connected to a heteroatom, i.e., CH-4 to oxygen and CH- 10 to chlorine. Three spin systems (**A** including H_2-1 and H-4; **B** from CH_3-14 to H_2-8, and **C** ranging from H-10 to H_2-11) were established by the interpretation of a COSY experiment (Figure 4). These ^{1}H-^{1}H spin systems were connected with each other using HMBC long-range correlations. Thus H_3-14 has a heteronuclear coupling to C-4 connecting spin systems **A** and **B**, and HMBC cross-peaks observed from the exomethylene H_2-15 to C-8 and C-10 connected B with **C**. The ^{13}C NMR chemical shift (δ_C 11.5) of the resonance for the methyl C-14 demonstrated the E configuration of the Δ^5 double bond. Long-range CH correlations of both methyl groups CH_3-12 and CH_3-13 to each other, to C-3, and to carbonyl C-2 indicated their position on nonprotonated carbon C-3. The ^{13}C NMR resonance of C-3 at δ_C 81.9 is characteristic for the presence of an attached oxygen atom. A weak long-range correlation of H-4 to C-3 gave evidence for the oxygen bridge, and thus a dihydrofuranone ring system was established, providing the last unsaturation requirement. The presence of a chlorine atom at C-10 was delineated from a characteristic ^{13}C NMR shift of δ_C 63.6. Finally, an HMBC correlation of H2-11 to the nonprotonated carbon C-16 indicated the position of the dichloroimidic moiety at the terminal C-11 of the C_{15} sesquiterpene skeleton and finally led to the planar structure of **1**.

Compound **2** was isolated as a colorless oil. The molecular formula $C_{18}H_{28}ClNO_4$ was established by HR-ESIMS and has five degrees of unsaturation. The dichloroimidic band at 1654 cm^{-1} was missing in the IR spectrum. Instead an additional carbonyl band appeared at 1707 cm^{-1}. ^{1}H and ^{13}C NMR spectra resembled those of **1** (Table 2). The major differences in the ^{1}H NMR spectrum were the additional signal at δ_H 4.11 (H_2-17, quartet *J* = 7.0 Hz) and an additional triplet signal of a methyl group at δ_H 1.26 (H_3-18, *J* = 7.0 Hz), indicating the presence of an ethoxy functionality. The ^{13}C NMR resonance of the dichloroimidic carbon could not be observed. Instead, an additional carbonyl signal at δ_C 159.0 for C-16 was detected. While the remaining ^{1}H and ^{13}C NMR resonances corresponded with those of the dihydrofuranone **1**, the differences mentioned above led us to conclude that we had an EtOH degradation product of **1**, because the dichloroimidic moiety is highly reactive. An HMBC experiment revealed a long-range correlation of H_2-11 and ethoxy H_2-17 to the carbonyl

carbon C-16, and thus compound **2** is the ethyl carbamate of dihydrofuranone dichloroimide **1**.

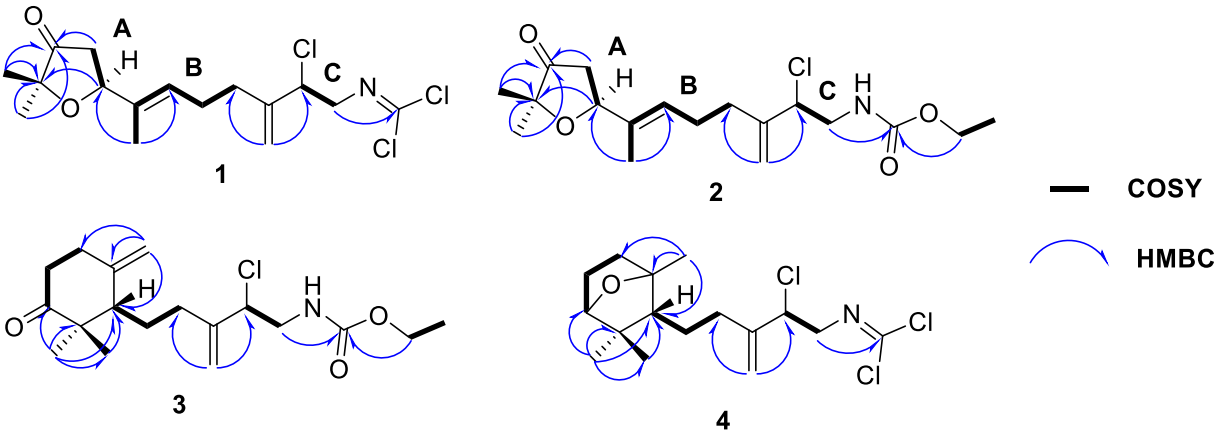


Chart 2. Key COSY and HMBC correlations in compounds 1–4.

C-4 Absolute Configurations of Dihydrofuranones 1 and 2. Whereas the eastern part of the compound **1** and its ethyl carbamate derivative **2** is typical for the rare dichloroimidic sesquiterpenes from marine sponges (Musman et al. 2001; Garson & Simpson 2004) and the phyllidiid nudibranch *Reticulidia fungia* (Tanaka 1999), the western part is structurally identical to that of the fungal metabolite ascofuranone (Figure 5) (Sasaki et al. 1973; Araki et al. 2019).

The absolute configuration of ascofuranone was established in 1975 using X-ray crystallography (Ando et al. 1975). For the assignment of the configuration at carbon C-4, first NOESY data analysis (Figure 6) and comparison of NMR spectroscopic data of **1** and **2** with those of ascofuranone was undertaken. The S configuration at C-4 in ascofuranone causes a negative Cotton effect at 300 nm as predicted by the ketone octant rule. Due to the instability of the dichloroimide **1**, circular dichroism (ECD) measurement was performed on the dihydrofuranone carbamate **2** and demonstrated a significant negative Cotton effect at 300 nm. Considering the same biosynthetic origins of both compounds **1** and **2**, the S configuration was assigned at C-4, as found in ascofuranone. The 13C NMR data of the dihydrofuranone moiety of compounds **1** and **2** are identical and match perfectly those published for ascofuranone isolated from the sponge-derived fungus Acremonium sp (Zhang et al. 2009). The configuration at the chlorine bearing C-10 remains to be solved in all herein described compounds.

Compound **3** was isolated as a colorless oil with a specific rotation of $[\alpha]^{25}_{D}$ +4 (*c* 0.09, acetone). A group of isotopic signals with a characteristic pattern for a monochlorinated organic molecule with the most intense one at *m/z* 342.184 $[M + H]^+$ was observed in the HR-ESIMS spectrum. The molecular formula was established as $C_{18}H_{28}ClNO_3$ with five degrees of unsaturation. A characteristic dichloroimidic band at 1654 cm^{-1} was missing in the IR spectrum, instead of a strong carbonyl band at 1707 cm^{-1}, indicated, as in the case of 2, a degradation of the dichloroimidic moiety. The planar structure of 3 could be established by the analysis and comparison of 1D and 2D NMR data. ^1H and ^{13}C NMR spectra of compound **3** shared many similarities with those of sesquiterpene **2**, and it was found that the molecules are identical from C-18 to C-15. For the remaining part of the structure ^1H–^1H NMR spin systems from H-5 to H_2-7 to H_2-8 and from H_2-1 to H_2-2 and H_2-14 were detected. These fragments were then connected via HMBC correlations from the exomethylene groups H_2-15 to C-8 and H_2-14 to C-5. The two geminal methyls CH_3- 2 and CH_3-13 had long-range couplings with the quaternary C-4, tertiary C-5, and the carbonyl C-3 (δ_C 217.5). The HMBC correlation of H_2-2 with the carbons C-3 and C-4 led to the formation of the cyclohexanone moiety and finally the whole planar structure of 3 (Figure 4).

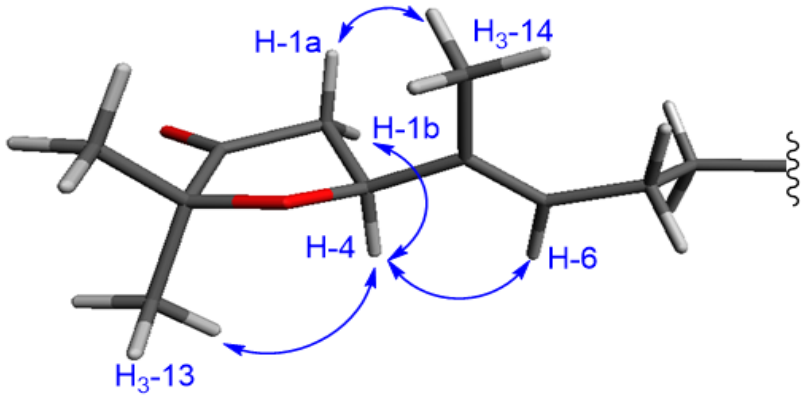
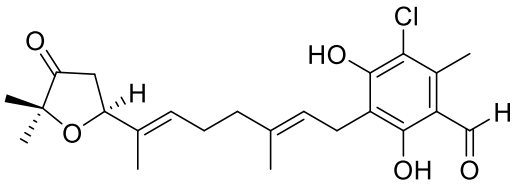


Figure 4. Relative configuration of the dihydrofuranone moiety in compounds **1** and **2**. Blue arrows indicate key NOESY correlations. 3D model constructed with Avogadro 1.20.

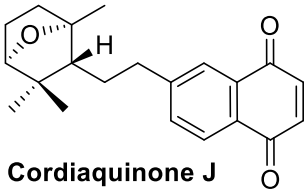
Compound **4** showed a specific rotation of $[\alpha]^{25}_{D}$ +24 (*c*0.08, acetone). An HR-ESIMS measurement in the positive mode revealed a protonated molecule having an *m/z* 352.096 $[M + H]^+$, which was part of a group of low-intensity peaks with a diagnostic isotope pattern of a molecule bearing three chlorine atoms. MS signals with higher intensity were observed with *m/z* 334.088 $[M – H2O]^+$ and *m/z* 316.119 $[M – Cl]^+$. The molecular formula was calculated to be $C_{16}H_{24}Cl_3NO$ with four degrees of unsaturation. The IR spectrum showed a characteristic band at 1654 cm^{-1} resulting from a dichloroimidic function. The NMR spectroscopic data of compound **4** partially resembled those of **1**, indicating that the eastern

parts of the molecules, i.e., from the C 15 methylene function to C- 16, were identical. Compared to compound **3**, the lack of a carbonyl group next to the geminal methyls CH_3-12 and CH3- 13 was most obvious. Instead, these methyl groups exhibited HMBC correlations to tertiary carbon C-3 with a ^{13}C NMR resonance at δ_C 87.7 that clearly indicated its connection with an oxygen atom. HMBC correlations were also detected between CH_3-12 and CH_3-13 to the tertiary carbon C-5 and the quaternary carbon C-4. Methyl group CH3-14 was located at the oxygen-bearing nonprotonated carbon (C-6, δ_C 88.6) and exhibited HMBC long range couplings to both C-5 and C- 1. A COSY spin system ranging from H_2-1 via H_2 2 to H-3 enabled together with the above-mentioned HMBC correlations the deduction of a cyclohexane ring. The oxygen bridge between C-3 and C-6 was evident due to a long-range HMBC coupling of H-3 with C-6. An additional ^1H-^1H spin system from H 5 via the methylene groups H_2-7 to H_2-8 and a clear HMBC correlation of the methylene protons H_2-15 to carbon C-8 connected the ring with the eastern part and completed the structure.

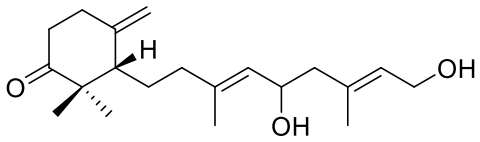
Compound **5** was only detected as part of a mixture together with compound **3**. LC-HRMS (Supporting Information Figure S26) analysis of this mixture showed the presence of compound **3**, as well as signals for a compound with an *m/z* 344.198 $[M + H]^+$. This MS result was indicative for the presence of two additional hydrogens in **5**. The formula was thus established as $C_{18}H_{30}ClNO_3$ with four degrees of unsaturation. The planar structure of **5** was elucidated unambiguously basing on 1D and 2D NMR experiments after the subtraction of the signals that were already assigned to compound **3**. The only major differences between **3** and **5** were found in the cyclohexane ring. NMR signals for the carbonyl group C-3 were missing in **5**, and instead an additional signal appeared in the ^1H NMR spectrum (δ_H 3.40, m) that exhibited an HSQC to an oxygen bearing carbon (δ_C 77.8) indicating the reduction of the ketone to a secondary alcohol. Compound 5 seems to convert to **3** under chromatographic separation conditions; thus, repeated attempts to isolate **5** as a pure compound were unsuccessful.



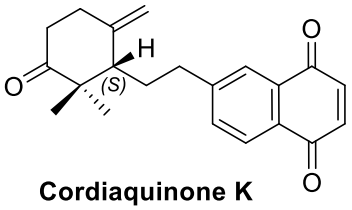
Ascofuranone



Cordiaquinone J



11,12-Dihydroxy-9,10-seco-ent-labda-8*E*,13*E*,(20)-triene-3-one



Cordiaquinone K

Chart 3. Natural products sharing structural similarities the new sesquiterpenes 1–4.

Compounds **6** and **7** were also isolated in a mixture after repeated HPLC separations. Careful inspection of the NMR and MS data indicated that they are the methyl carbamate derivatives of compounds **3** and **5**. Most indicative were HMBC correlations of the C-17 methoxy group resonances at δ_H 3.66 and 3.67, respectively, to the carbonyl carbon at δ_C 159.4 (C-16). Remaining ^{1}H and ^{13}C NMR shifts corresponded with those of **3** and **5**.

Configurations of Compounds 3-7. The configuration of the cyclic part of the molecules was established by the interpretation of NOESY data, circular dichroism, molecular modeling, and comparison of spectroscopic data with those of similar compounds from the literature (Figure 5), i.e., secolabdanes (Zdero et al 1990) and cordiaquinones J and K (Ioset et al. 2000). The cyclohexanone moieties of compounds **3** and **6** possess one stereogenic carbon, *i.e.*, the tertiary C-5. To depict the conformation of the cyclohexanone structure and its relative configuration, minimal energy conformer searches using the Avogadro software version 1.20 (Hanwell et al. 2012) combined with the analysis of the NOESY spectra were performed. NOE correlations between proton H-14b and H-5 and between H-5 and both the methyl group H_3-12 and the methylene group H_2-8 can only be explained with the pseudoaxial orientation of the side chain (Figure 7). Additionally, an ECD measurement of compound **3** revealed a positive Cotton effect at 300 nm, indicating the S configuration at C-5, in accordance with the observations for the secolabdane by Zdero and co-workers (Zdero et al. 1990).

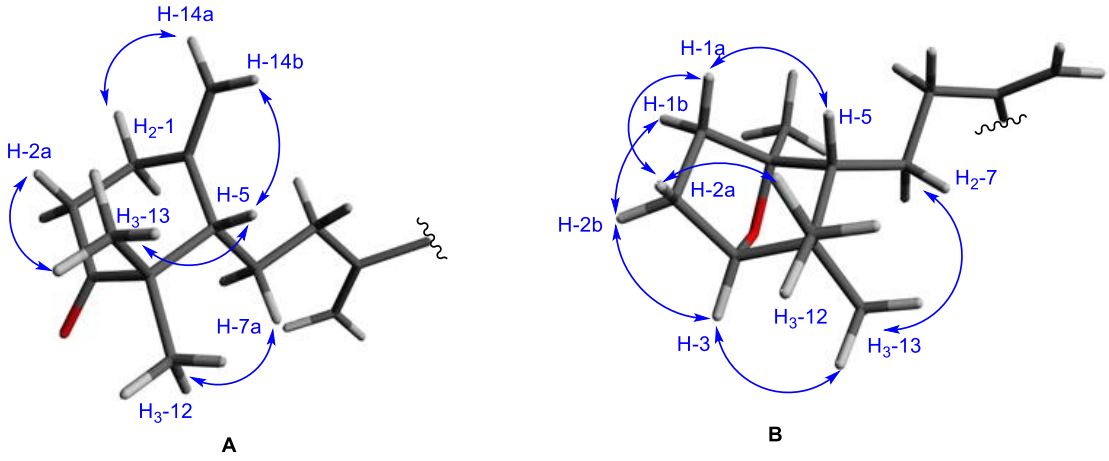


Figure 5. Configuration of the cyclic moieties. **A**: as found in compound **3**. **B**: as found in compound **4**. Blue arrows indicate key NOESY correlations. 3D model constructed with Avogadro 1.20.

As described in the literature the cyclohexane moiety in the western part of compound **4** has a preferred boat conformation due to the oxygen bridge (Hanwell et al. 2012). The assignment of the relative configuration of the oxabicyclo ring system using NOESY is not straightforward. NOE correlations between H-5 and H-1a, between H-1a and H-2a, and between H-2a and H_3-12 indicated the same pseudoaxial orientation of protons H-1a, H-2a, H-5, and H_3-12. The bulky side chain at C-5 thus has an energetically favored pseudoequatorial orientation, which is further confirmed by a clear NOE correlation of H3-13 with H_2-7. The ^{13}C NMR shifts of the whole oxabicyclo moiety of compound **4** are identical with the reported values for cordiaquinone J (Ioset et al 2000), demonstrating the same relative configuration of both natural products. Yajima and co-workers synthesized different enantiomers of cordiaquinone J and provided NMR data of them confirming the relative configuration of the natural cordiaquinone J (Yajima et al. 2005). Considering the same biosynthetic origin of compounds **3** and **4**, the same spatial orientation of the substituents at C-5 is very likely. We thus suggest the absolute configuration of the oxabicyclic moiety in compound **4** to be 3*R*, 5*R*, 6*S*.

Because the cyclohexanones (**3** and **6**) and their cyclohexanol counterparts co-occur and moreover seemingly interconvert, the same configuration at C-5 is very likely. Considering the R configuration of C-5, the configuration of the hydroxy group bearing carbon C-3 could be established by an NOE correlation between H-3 and H-5 observed for compound **7**, demonstrating the spatial orientation to the same face. Thus, the configuration of cyclohexanols **5** and **7** is 3*S*, 5*R*.

Assignment of the Structures 8 and 9. The analysis of the HR LCMS data of the extract of the herein investigated *P. pustulosa* specimen pointed toward the presence of two additional

major metabolites. However, they could not be isolated by chromatographic means. ^{1}H and ^{13}C NMR analysis of the initially collected fraction SPE3 HPLC2 in the course of the preparative HPLC showed that the fraction contained several substances. Repeated HPLC was performed and resulted in the isolation of an inseparable mixture of the artifacts **6** and **7** that were formed during chromatography or the NMR measurement in deuterated MeOH (as seen in the LC HRMS analysis). The original natural products are highly reactive and quickly degrade during the workup procedure.

Combining the knowledge obtained during the identification of the structures of **1**-**7** and a detailed analysis of MS data obtained in the course of our initial LC HRMS measurements of the Phyllidiid extracts enabled the structure assignment for metabolites **8** and **9**. Prominent HPLC peaks with retention times of 16.1 and 16.6 min (Figure 2, Figures S34-S36) revealed the characteristic isotopic patterns of polychlorinated compounds, indicating the presence of the dichloroimidic moiety. The *m/z* values of 350.081 $[M + H]^+$ and 314.103 $[M - HCl]^+$ found for the HPLC peak at t_R 16.6 min were attributable to a compound with a molecular formula of $C_{16}H_{24}Cl_3NO$. The HPLC peak at 16.1 min led to *m/z* values of 352.095 $[M + H]^+$, 334.086 $[M + H - H2O]^+$, and 316.122 $[M - HCl]^+$ that was deduced as a molecular formula of $C_{16}H_{26}C_{l3}NO$. These formulas are attributable to the sesquiterpenoids with the same core structure as found for compounds **3**, **5**, **6**, and **7**, but in the case of **8** and **9** with an intact dichloroimidic moiety instead of carbamates. Thus, compound **8** is a cyclohexanone (t_R 16.6 min) and **9** is its corresponding alcohol, eluting earlier from the RP stationary phase (at 16.1 min).

This study led to the identification of the structurally intriguing dichloroimidic sesquiterpenoids **1, 4, 8**, and **9** along with the solvent artifacts **2, 3**, and **5**-**7** thereof (Chart 1) in a distinct phylogenetic clade of the *P. pustulosa* species complex. The presence of chlorine and the dichloroimidic moiety in these molecules allows their specific detection by MS, via the characteristic isotopic as well as fragmentation patterns, directly from extracts of the nudibranchs. However, no further dichloroimides could be detected in any other *P. pustulosa* clade nor in any other phyllidiid species chemically analysed using HR-LCMS in the course of this study (partially unpublished data*: Phyllidia varicosa, P. elegans, P. ocellata, Phyllidiella lizae, P. nigra, Phyllidiopsis burni, P. krempfi,* and *P. shireenae*). This observation leads to the conclusion, that dichloroimidic sesquiterpenoids and their derivatives formed under the influence of solvents during the workup (**1**-**9**) represent a unique chemotaxonomic feature of *Phyllidiella pustulosa* clade 6.

Natural products containing a dichloroimidic moiety are extremely rare, although the first ones were isolated in the late 1970s from the Indo-Pacific sponge *Pseudaxinyssa pitys* (accepted now as *Axinyssa mertoni*) (Van Soest et al. 1954) by Faulkner and coworkers (Wratten et al. 1977; 1978). Despite their early discovery at the very beginning of research on marine natural products, there are only 16 metabolites bearing this functionality known to date, all derived from marine sponges and their nudibranch predators (Garson & Simpson 2004). Biogenetically, the dichloroimidic functionality originates from the corresponding isocyanates and isothiocyanates, as demonstrated by incubation experiments with ^{14}C labeled precursors in the tropical marine sponge, *Stylotella aurantium* (Brust & Garson 2003; Simpson et al. 2004).

The presence of dichloroimidic compounds **1, 4, 8**, and **9** in extracts of animals of only one clade, *i.e.*, clade 6 (Figure 1), indicates that speciation processes may be based on food priority. Members of this clade were found at all three collection sites (Bunaken National Park, Bangka Archipelago, and Sangihe Island). Phylogenetic analyses provided evidence for further cryptic species within the *P. pustulosa* complex, with clades usually found in all three locations, but with one exception, clade 2. The specimens within this latter clade were all only collected around Sangihe Island. Although sampling is still limited with regard to specimens in the various populations, the confinement of clade 2 to only Sangihe Island could indicate a restricted gene flow based on different larval development (with low, if at all, dispersal by free swimming larvae). However, it can also not be excluded that specific food availability (here probably an unknown sponge) restricts distribution of this clade.

Within this study we show that results of chemical and molecular phylogenetic analyses support each other, demonstrating that subclades within the *P. pustulosa* complex are in part also characterized by different chemical constituents. *P. pustulosa* was already recognized as a species complex (Stoffels et al. 2016), and our studies even go further, by not only showing this species as a complex of several distinct clades but also the clustering of known and described species (*P. nigra, P. rudmani, P. lizae*) within the *P. pustulosa* complex.

The absence of clear morphologically characterizing features renders this very common nudibranch taxon, characterized by the presence of unusual natural products, an extremely difficult group with regard to correct taxonomic identification. A revision of the genus is warranted and needs to include more species than those involved in our study. Phyllidiid nudibranchs sequester the secondary metabolites from sponges; however not much is known about the preferences toward a certain favored prey. The presence of different metabolomes

in sympatrically living “species” indicates that at least some of the Phyllidiidae like *P. pustulosa* clade 6 appear to be highly specialized feeders. The ongoing speciation process is thus mainly driven by food that provides the specific compounds. Providing information on clade-specific metabolomic markers, chemotaxonomic analyses might help in solving the P. pustulosa conundrum and strengthen the hypotheses of different species being present in one visually nearly identical group as we have shown here for clade 6.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Jasco P-2000 polarimeter. UV and IR spectra were obtained using PerkinElmer Lambda 40 and PerkinElmer Spectrum BX instruments, respectively. ECD spectra were taken on a Jasco J-810 CD spectropolarimeter. All NMR spectra were recorded in MeOH-d_4 using Bruker Avance 300 DPX or Bruker Ascend 600 spectrometers. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0. Mass spectra were recorded on a micrOTOF-QIII mass spectrometer (Bruker) with an ESI-source coupled with an HPLC Dionex Ultimate 3000 (Thermo Scientific) using an EC10/2 Nucleoshell C_{18} 2.7 μm column (Macherey-Nagel). The column temperature was 25 °C. MS data were acquired over a range from 100 to 3000 *m/z* in positive mode. Auto MS/MS fragmentation was achieved with rising collision energy (35-50 keV over a gradient from 500 to 2000 *m/z*) with a frequency of 4 Hz for all ions over a threshold of 100. HPLC begins with 90% H_2O containing 0.1% acetic acid. The gradient starts after 1 min to 100% CH_3CN (0.1% acetic acid) in 20 min. A 5 μL amount of a 1 mg/mL sample solution (MeOH) was injected at a flow of 0.3 mL/min. All solvents were LCMS grade. Preparative HPLC was performed on a Merck Hitachi HPLC system equipped with an L-6200A pump, an L- 4500A PDA detector, a D-6000A interface with D-7000A HSM software, and a Rheodyne 7725i injection system. A Nucleodur C_{18} 5 μm Pyramid 250 mm × 10 mm (Macherey-Nagel) and a Kinetex PFP 5 µm C_{18} 100 Å 250 mm × 4.6 mm (Phenomenex) column were used.

Samples. The animals for the chemical analyses were collected in 2015 to 2017 in the course of the biodiversity survey around North Sulawesi by Nani Undap, Adelfia Papu, and collaborators (Eisenbarth et al. 2018, Undap et al. 2019, Papu et al. 2020). The collections are registered at UNSRAT collection under the numbers SRU2015/01, SRU2016/01, SRU2016/02, and SRU2017/01. The specimens were

fixed individually in EtOH (96%) immediately after the collection and preliminary identification. After the transfer to University of Bonn samples were stored at -20 °C until extraction and further processing.

Extraction and Isolation. The ethanolic storage solution was decanted and evaporated under reduced pressure. The bodies were cut into small pieces and repeatedly extracted with MeOH (3 × 30 mL) and CH_2Cl_2 (1 × 30 mL). The fractions were combined to give the crude extract. HR-LCMS analysis was performed using a 1 mg/ mL LCMS-grade MeOH solution of the crude extracts.

The extract of the specimen Phpu15Bu-21 was submitted for further detailed chemical analysis. The extract (300 mg) was resuspended in 30 mL of H_2O and extracted three times with 30 mL of EtOAc in a 100 mL separation funnel. The obtained lipophilic part (56.6 mg) underwent fractionation on a reversed-phase SPE Bakerbond 2000 mg column using a stepwise gradient (50:50, 70:30, 90:10, 100:0 MeOH/H_2O v/v, 20 mL each) to obtain four fractions. The fifth fraction was eluted with 20 mL of acetone. ^{1}H NMR experiments demonstrated that SPE fractions 2 (4 mg) and 3 (25 mg) were identical. They were combined and submitted to HPLC separation using the Nucleodur C_{18} 5 µm Pyramid 250 mm × 10 mm column and 85:15 MeOH/H_2O mobile phase at 2.5 mL/min flow to yield four fractions, two of which were already pure compounds (compound **1**, 1.3 mg; compound **4**, 1.1 mg). Repeated HPLC of the remaining fractions using the same column with a 75:25 MeOH/H_2O mobile phase and the analytical column Kinetex PFP 5 µm C_{18} 100 Å 250 mm × 4.6 mm with a 68:32 MeOH/H2O mobile phase at 0.7 mL/min flow resulted in the isolation of pure compounds **2** (0.8 mg) and **3** (1.0 mg) as well as poorly separable mixtures containing compounds **3** and **5** (1.5 mg) and **6** and **7** (2.0 mg).

*Compound **1***: colorless oil, $[\alpha]^{25}_D$ -32 (*c* 0.1, acetone); IR (neat) ν_{max} 2928, 1755, 1654, 1172, 1112, 883 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Table 2; HR-ESIMS *m/z* 330.099 $[M - Cl]^+$ (calcd for $C_{16}H_{22}Cl_2NO_2$, 330.102).

*Compound **2***: colorless oil, $[\alpha]^{25}_D$ -15 (*c* 0.1, $CHCl_3$); ECD (2.8 mM, CH_3CN) λ ($\Delta\varepsilon$) 210 (+0.27), 310 (-0.11); IR (neat) ν_{max} 2926, 2854, 1754, 1707, 1526, 1457, 1376, 1252, 1172, 1112, 1030 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, Table 2; HR-ESIMS *m/z* 358.178 $[M + H]^+$ (calcd for $C_{18}H_{29}ClNO_4$, 358.178).

*Compound **3***: colorless oil; $[\alpha]^{25}_D$ +4 (*c* 0.09, acetone); ECD (2.9 mM, CH_3CN) λ ($\Delta\varepsilon$) 310 (+0.02); IR (neat) ν_{max} 2926, 2854, 1754, 1707, 1526, 1457, 1376, 1252, 1172, 1112,

1030 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table S3; HR-ESIMS *m/z* 342.184 $[M + H]^+$ (calcd for $C_{18}H_{29}ClNO_3$, 342.183).

Compound ***4***: colorless oil; $[\alpha]^{25}_D$ +24 (*c* 0.08, acetone); IR (neat) ν_{max} 2928, 1733, 1654, 1456, 875 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table S4; HR-ESIMS *m/z* 352.096 $[M + H]^+$ (calcd for $C_{16}H_{25}Cl_3NO$, 352.099).

Compound ***5***: isolated in a mixture with **3**; colorless oil; ^{1}H NMR and ^{13}C NMR data, see Table S5; HR-ESIMS m/z 344.198 $[M + H]^+$ (calcd for C18H31ClNO3, 344.199).

Compounds ***6*** and ***7***: isolated in a mixture; colorless oil; ^{1}H NMR and ^{13}C NMR data, see the SI.

MS Data Analysis. The obtained raw .d LCMS/MS folders were converted using the DataAnalysis 4.2 software (Bruker Daltonic) to .mzXML format and uploaded to the MassIVE server (https:// massive.ucsd.edu) via FileZilla (https://filezilla-project.org) FTP client. A molecular network was created using the online workflow (https://ccms-ucsd.github.io/GNPSDocumentation/) on the GNPS Website (http://gnps.ucsd.edu). The data were filtered by removing all MS/MS fragment ions within ±17 Da of the precursor m/z. MS/MS spectra were window filtered by choosing only the top 6 fragment ions in the ±50 Da window throughout the spectrum. The precursor ion mass tolerance was set to 0.05 Da and a MS/MS fragment ion tolerance of 0.02 Da. A network was then created where edges were filtered to have a cosine score above 0.6 and more than 3 matched peaks. Further, edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS's spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 3 matched peaks. Networks were visualized using Cytoscape v3.7.2 https://cytoscape.org/ (Shannon 2003). Nodes attributable to the MS/MS spectra from blank samples (LCMS grade MeOH) were subtracted together with the whole clusters from the network in order to eliminate unspecific instrument noise.

Molecular Modeling. Minimal energy conformations and 3D representations of the molecules were generated using the Avogadro software version 1.20 (Hanwee et al. 2012).

DNA Extraction, Amplification, Sequencing, and Alignment. DNA was extracted from pieces (foot or notum) of the specimens or whole body, based on the size of animals. DNA isolation has been carried out by means of QIAgen DNeasy blood and tissue kit, following the manufacturer's instructions. Partial sequences of mitochondrial CO1 (ca. 680bp) and ribosomal 16S (ca. 650bp) were amplified by polymerase chain reaction (PCR) using the primers LCO1490-JJ (5′-CHACWAAYCATAAAGATATYGG-3′) and HCO2198-JJ (5′ AWACTTCVGGRTGVCCAAARAATCA-3′) for CO1 (Astrin et al. 2005) and 16Sar-L (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT-3′) for 16S (Palumbi et al. 2002). Amplification of CO1 was performed by an initial step (95 °C for 15 min) followed by 40 touch-down cycles of denaturation (94 °C for 35 s), annealing (55 °C for 90 s), and extension (72 °C for 90 s) with a final extension step 72 °C for 10 min. For 16S rRNA, the PCR started with an initial step (95 °C for 15 min), denaturation (94 °C for 45 s) followed by 34 touch-down cycles, annealing (56 °C for 45 s), extension (72 °C for 90 s), and final extension step at 72 °C for 10 min. PCR products were sequenced by Macrogen Europe Laboratory. GENEIOUS Pro 7.1.9 was used to extract the consensus sequence between the primer regions and checking the quality of sequences. Consensus sequences were blasted against the NCBI database for evaluation of identification (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Specimen sequences together with sequences retrieved from NCBI were aligned in MAFFT Alignment, algorithm FFT-NS-2, scoring matrix 200PAM/k = 2. CO1 and 16S gene sequences were concatenated with GENEIOUS Pro 7.1.9 and used for subsequent tree analyses.

Phylogenetic Analyses. Phylogenetic reconstruction was carried out with maximum likelihood algorithms implemented in the IQ tree Web server. The following settings were applied: Ultrafast Bootstrap analyses, 1000 number of bootstraps, 1000 max iterations, 0.99 min correlation coefficient (Trifinopoulos et al. 2016). Collapsing of clades was processed using Dendroscope 3.5.10 (Huson et al. 2007) and FigTree v1.4.4 (Rambaut 2018). For the analysis within this project, only part of the tree is depicted. In total, 52 sequences of Phyllidiella and Phyllidiopsis specimens used for chemical taxonomic analyses are shown in this tree using Dendroscope version 3.5.10 to indicate the relationship of the targeted groups. The full tree will be published elsewhere.

■ ASSOCIATED CONTENT

Supporting Information: The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00783. Additional information (PDF) or in appendix.

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Chapter 5

Phyllidiidae (Nudibranchia, Heterobranchia, Gastropoda) – a taxonomic and chemical analysis, with surprising results

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Abstract:

Members of the widely distributed and common nudibranch family Phyllidiidae are very often easily spotted in the marine environment because of their conspicuous colours and larger body size. They are interesting with regard to their defensive chemical compounds that may lead to new drug discoveries. Despite their abundance, the family is also well known for its taxonomic problems and the difficulties in species identification due to very similarly coloured species. In this study phyllidiid species were analysed by integrative methods. For species delimitation we used molecular analysis of mitochondrial genes (16S and CO1), applying phylogenetic analyses, species delimitation tests, and haplotype network analyses. Additionally, for the first time, external morphological characters were analysed, museum material was re-analysed, and chemical profiles applied for characterizing species. As well as using published sequences we included sequences of 598 specimens collected in Indonesia. Our study comprises 11 species of *Phyllidia*, seven species of *Phyllidiopsis*, and 11 species of *Phyllidiella;* 11 species in the three genera are new to science. *Phyllidiella albonigra* is resurrected. We could show that some of the external coloration previously used for species identification is not valid, but we provide alternative characters for most species' identification. Chemical analyses lead to species characterization in a few examples, indicating that these species use particular sponge species as food; however, many species show a broader array of compounds and are therefore characterised more by their composition/profile than distinct or unique compounds.

Keywords: phylogeny, taxonomy, molecular, chemotype, metabolomics, chemotaxonomy.

Introduction

Nudibranchia is one of the largest taxa within the marine Heterobranchia. It comprises two lineages, the Cladobranchia and the Doridina. Within doridina, Phyllidiidae is one of the oldest recognised families, described in 1814 by Rafinesque. Phyllidiidae and Dendrodorididae were united under the name Porostomata Bergh, 1878, based on the pore-like mouth opening and the complete loss of radula and jaws in both families. Subsequently, a third family with similar characteristics was described, Mandeliidae Valdés & Gosliner

(1999), and these three families today being the absence of the radula and jaws (Valdes & Gosliner 1999, Valdes 2002).

Specimens of Phyllidiidae are very commonly encountered in Indo-Pacific reefs. Because of their abundance, their large size, and usually striking colours of bright yellow/orange in combination with white and black, they have been investigated with regard to their chemical compounds used for defence (Hagadone et al. 1979; Manzo et al. 2004; Coleman 2015; White et al. 2015; Böhringer et al. 2017; Fisch et al. 2017; Bogdanov et al. 2020). In addition to the compounds that are taken up from the sponge food items, phyllidiids also use a dense layer of calcareous spicules arranged in the mantle and foot that also provide structural support (Kasamesiri et al. 2011; Wallraff 2018) and make them difficult to eat. This combination of defence systems probably allows them to be conspicuously exposed on the substrate during daytime.

Despite their commonness and conspicuous appearance, many members within the Phyllidiidae are known to be difficult to identify, and cryptic speciation and species complexes have been described recently (Stoffels et al. 2016; Bogdanov et al. 2020). Species of Phyllidiidae were first documented from the tropical Indo-Pacific, with *Phyllidia varicosa* Lamarck, 1801 and *Phyllidia ocellata* Cuvier, 1804; type localities of both species were Rėunion Island, western Indian Ocean, and 'Mer des Indes', respectively. Few species are documented in the Atlantic Ocean, Caribbean Sea, and Mediterranean Sea (Brunckhorst 1993; Phyllidiidae in GBIF Secretariat 2019e). A major distribution area lies in the Coral Triangle, including Indonesia. Sulawesi is one of the largest islands of Indonesia and is unique in its position in between the Wallace and the Webber lines, as well as close to the Lydekker line (Cockey 2013). Sulawesi is therefore characterised by a species-overlap zone with members from the Asian continent as well as from the Australian continent. The high number of phyllidiid species and specimens in these areas were recently documented in several studies (Eisenbarth et al. 2018; Kaligis et al. 2018; Ompi et al. 2019; Undap et al. 2019; Papu et al. 2020).

Only few studies analyse phylogenetic relationship between the five recognised genera, containing approximately 81 species (WoRMS Editorial Board 2021). Brunckhorst (1993) provided the first extensive morphological analysis and review on the Phyllidiidae, distinguishing the six genera. Subsequently, Valdés and Gosliner (1999) analysed relationships using morphological data. They included 11 species covering five genera but

the relationships of *Phyllidiella* and *Phyllidia* could not be resolved. In a preliminary molecular study, Valdés (2003) published a phylogenetic tree including 12 specimens from four genera (without *Ceratophyllidia*). In that work, *Phyllidiella* grouped with members of the genus *Phyllidiopsis*, and *Phyllidiopsis* therefore appeared paraphyletic. Subsequent molecular studies using only the mitochondrial gene CO1 were performed by Stoffels et al. (2016), including 18 specimens belonging to four genera, also omitting *Ceratophyllidia*. That analysis recovered the monophyly of *Phyllidia* and *Phyllidiella*; however, *Phyllidiopsis* again appeared paraphyletic. Interestingly, that study presented evidence of cryptic variation in *Phyllidiella 'pustulosa'*, which was most recently confirmed by the more extensive molecular and chemical composition study of Bogdanov et al. (2020): at least seven subclades were identified within the *Phyllidiella pustulosa* complex that were not assigned to species. Certain compounds were specific to certain clades and one metabolite was identified that is unique for one specific clade, confirming the distinctiveness of these cryptic varieties within *Phyllidiella*.

In contrast to the scarce phylogenetic analyses, the chemistry of phyllidiids has evoked interest for many decades. Following an observation of a *Phyllidia varicosa* specimen secreting "a light-grey mucus" that caused deadly poisoning of a lobster in an aquarium setting (Johannes 1963), *P. varicosa* was among the first phyllidiid nudibranchs drawing the attention of natural product chemists who isolated a sesquiterpene isonitrile (Burreson et al. 1975). Several studies focusing on phyllidiid chemistry followed (see review in Fish et al. 2017) and the published results already hinted at the variety of compounds isolated from one and the same species, e.g. *Phyllidiella pustulosa*, implying either a very high degree of metabolomic variation or that species identification might not be correct in all cases.

In the present study we address the phylogenetic relationships of the family Phyllidiidae by using the new and available GenBank sequences from partial mitochondrial genes (CO1 and 16S) and perform species delimitation tests to identify putative cryptic variations/species within the genera. We also attempt to assign morphological characters to the identified species/clades. Finally, we perform metabolomic analyses of some specimens used in the molecular analyses as an additional chemotaxonomic evidence of species delimitation and identification. With 716 sequences, our study is the most comprehensive phylogenetic analysis of the Phyllidiidae to date and this is the first time that metabolome analysis provides unprecedented insights into clade-specific chemical composition that largely supports many of the recognised and new clades in our phyllidiid study.

Materials and methods

Sample collection

Phyllidiidae specimens were collected during several expeditions from 2015 to 2018 in the area of north Sulawesi by SCUBA diving and snorkelling in depths between 0 and 30 m. The localities include Bunaken National Park, Bangka Archipelago and Sangihe Island. Species lists of these expeditions have been published in Kaligis et al. (2018), Eisenbarth et al. (2018), Undap et al. (2019), Ompi et al. (2019), and Papu et al. (2020). In addition, phyllidiids were collected in south and south-east Sulawesi in 2018 (Fig. 1), totalling approximately 610 specimens in total. Each collected specimen was (usually) documented underwater and additionally in the laboratory with a Sony and/or Olympus TG4 camera, then provided with a unique identifier (abbreviation of name, year, location, and number of specimen). Collected specimens were preserved in 96% EtOH and/or in formaldehyde/seawater. For those specimens preserved in formaldehyde, a tiny piece of the foot was cut off first and preserved in EtOH 96% for barcoding. The collections are registered at UNSRAT collection under the numbers SRU2015/1, SRU2016/1, SRU2016/2, SRU2017/1, SRU2017/2, SRU2018/1, and SRU2018/2. Metadata, including images of all animals, will be published in the Diversity Workbench (https://www.gfbio.org/) (Diepenbroek et al. 2014).

Morphological identification

Around 700 specimens were preliminarily identified with the help of available literature (Fahrner and Beck 2000; Domínguez et al. 2007; Yonow 2011, 2012; Gosliner et al. 2018). Additional websites such as the Sea Slug Forum Home Page were used to check the external variability and also to obtain distribution data. Subsequently, original literature was used for final identifications (see details for each species in Results). The validity of names was checked against the World Register of Marine Species (WoRMS Editorial Board 2021). Several type and non-type materials from Australian Museum Sydney, Muséum national d'Histoire naturelle Paris, Natural History Museum of Denmark, Copenhagen, Naturalis Biodiversity Center Leiden, and the British Museum of Natural History, London, were used for morphological confirmation. Three species of *Phyllidiella* and three of *Phyllidiopsis* (Figs. 2, 3) are very relevant for this study, and the following types were examined (see Table 3 Appendix for registration numbers): *Phyllidiella pustulosa* (*Phyllidia nobilis* syntype,

paralectotype, non-type materials, *Phyllidia pustulosa* lectotype; *Phyllidia annulata* nontype; *Phyllidiella lizae* holotype, paratypes; *Phyllidiella annulata*; *Phyllidiopsis pipeki* holotype; *Phyllidiopsis burni* holotype; and *Phyllidiopsis krempfi* non-type.

DNA extraction, amplification, sequencing, and alignment

DNA from 598 of Phyllidiidae specimens were extracted successfully from the foot, notum, or whole body, depending upon the size of animals (Table 1). DNA-isolation was carried out by means of QIAgen® DNeasy Blood and Tissue-Kit, following manufacturer's instructions. Partial sequences of mitochondrial CO1 (ca. 680bp) and ribosomal 16S (ca. 650bp) were amplified by polymerase chain reaction (PCR) using the primers LCO1490-JJ (5' –CHACWAAYCATAAAGATATYGG-3') and HCO2198-JJ (5'-AWACTTCVGGRTGVCCAAARAATCA-3') (Astrin and Stüben 2008) for CO1, and 16Sar-L (5′ -CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5′-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 2002) for 16S. Amplification of CO1 was performed by an initial step (95° C for 15 min) followed by 40 touch-down cycles of denaturation (94° C for 35 sec), annealing (55° C for 90 sec) and extension (72° C for 90 sec), with a final extension step 72° C for 10 min. For 16S rRNA, the PCR started with an initial step (95° C for 15 min), denaturation (94° C for 45 sec), followed by 34 touch-down cycles, annealing (56° C for 45 sec), extension (72° C for 90 sec) and final extension step at 72° C for 10 min. PCR products were sequenced by Macrogen Europe Laboratory (Amsterdam, Netherlands). GENEIOUS Pro 7.1.9 was used to extract the consensus sequence between the primer regions, and for editing and checking the quality of sequences. Consensus sequences were blasted against the NCBI database for evaluation of identification (NCBI Resource Coordinators 2018).

Additionally, 116 phyllidiid sequences available in GenBank were extracted to increase coverage of species and genera (Table 2). Three species of the sister taxon of the Phyllidiidae, the Dendrodoridídae (*Doriopsilla albopunctata*, *Doriopsilla bertschi*, and *Dendrodoris atromaculata)*, were taken from GenBank and used as outgroups. Sequences were aligned using the web program MAFFT Alignment, by applying the algorithm FFT-NS-2, scoring matrix 200 PAM/k = 2. The single gene data sets (635 bp of CO1, 538 bp of 16S) were analysed separately, but both genes were also concatenated using GENEIOUS Pro 7.1.9, resulting in a total alignment length of 1173 bp.

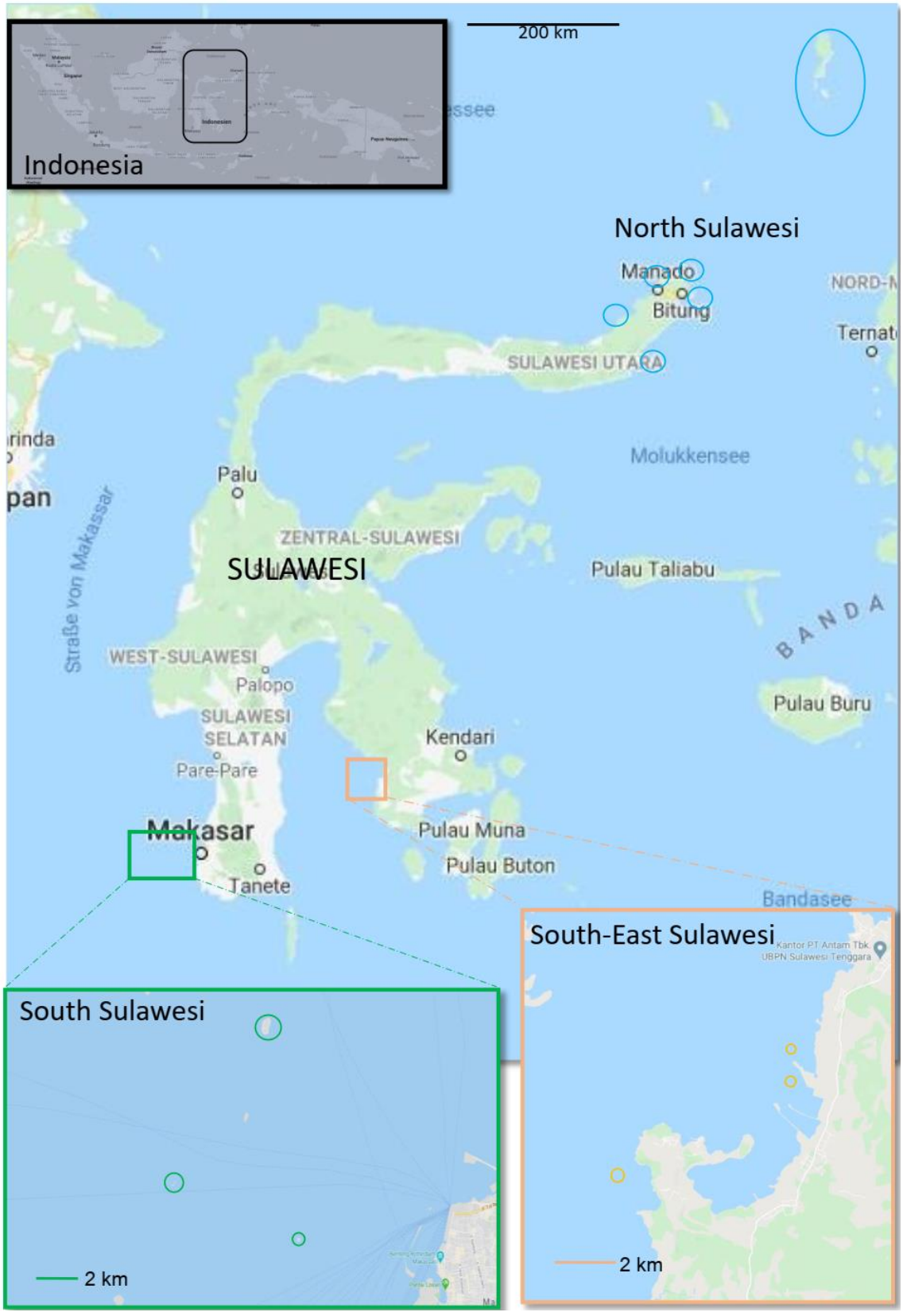

Figure **1**. Sampling locations in Sulawesi, Indonesia.

Fig. 2 Type material of *Phyllidiella*: **a** *Phyllidia nobilis* Syntype NHMD 91773; **b** *Phyllidia nobilis* non type NHMD-633656; **c** *Phyllidia nobilis* non type NHMD-633657; **d** *Phyllidia nobilis* paralectotype NHMD-633658; **e** *Phyllidia pustulosa* lectotype MNHN-IM-2000-35147; **f** *Phyllidia melanocera* holotype NHMUK 1985206/1; **g** *Phyllidiella lizae* holotype C159493; **h** *Phyllidiella lizae* paratype C162784; **i** *Phyllidiella lizae* paratype C159492.

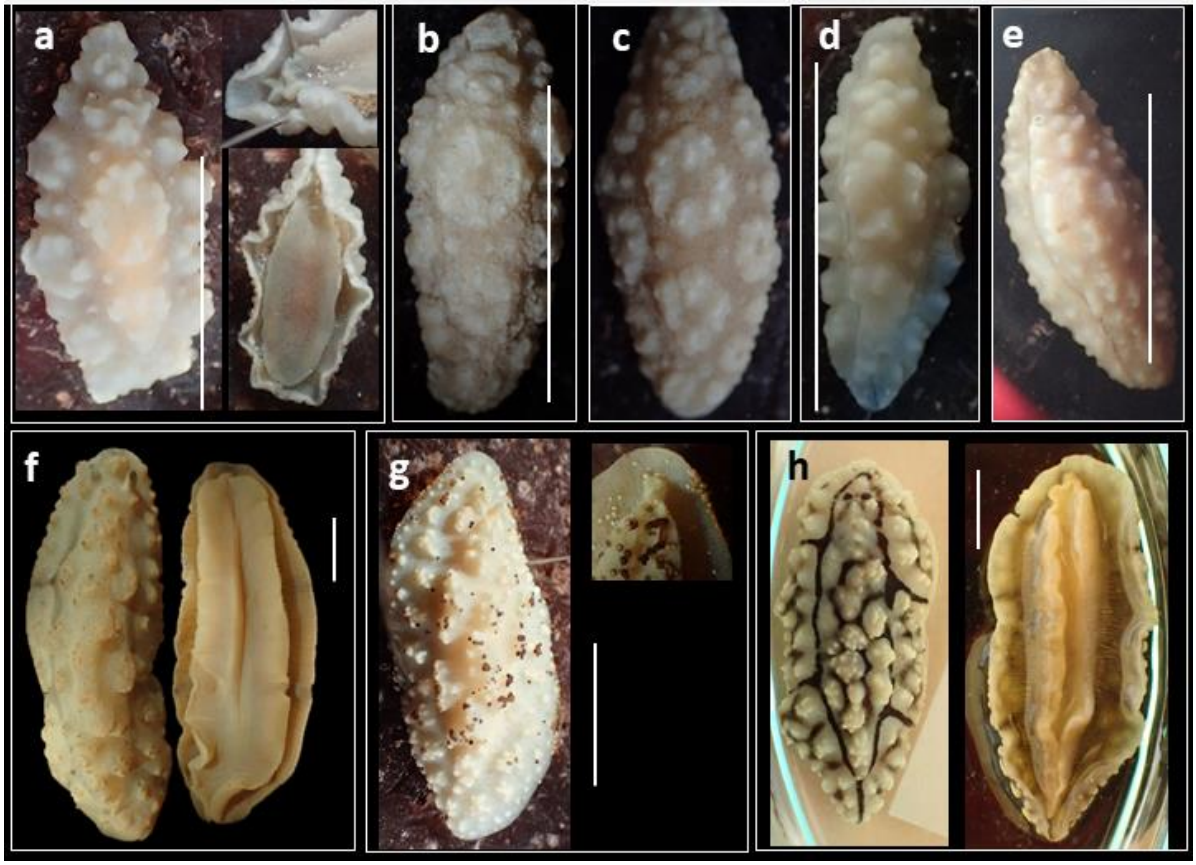

Fig. 3 Type and old material of *Phyllidiella* and *Phyllidiopsis*: **a** *Phyllidiella annulata* non-type C159519; **b** *Phyllidiella annulata* non-type C159520; **c** *Phyllidiella annulata* non-type C159520b; **d** *Phyllidiella annulata* non-type C159522; **e** *Phyllidiella annulata* non-type C159526; **f** *Phyllidiopsis pipeki* holotype C162772; **g** *Phyllidiopsis burni* holotype C159452; **h** *Phyllidiopsis krempfi* non-type RMNH.Mol.336469.

Phylogenetic analyses and species delimitation tests

The single gene datasets as well as the concatenated data set was processed in the online version of IQ-TREE in http://iqtree.cibiv.univie.ac.at/ using default settings for determination of the best-fit substitution model. For our tree reconstruction, TVM+F+I+G4 was selected according to the Bayesian Information Criterion (BIC) (Burnham and Anderson 2009; Nguyen et al. 2015; Hoang et al. 2017; Kalyaanamoorthy et al. 2017). Phylogenetic reconstruction was carried out using the Maximum Likelihood algorithms implemented in the IQ tree web server. Following settings were applied: Ultrafast Bootstrap analyses, 1000 number of bootstrap, 1000max iterations, 0,99 min correlation coefficient (Minh et al. 2017). For visualisation of the trees Dendroscope Vers. 3.5.10 was used (Huson et al. 2012). Collapsing of clades and tree editing was subsequently processed using FigTree v1.4.4 (Rambaut 2009) and edited in inkscape (Draw Freely | Inkscape).

To provide further evidence of species hypotheses, datasets of CO1 and 16S were analysed separately with the help of the ABGD (Automatic Barcode Gap Discovery)

methodology (Puillandre et al. 2012), using the web server https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. This method helps to characterise barcode gaps between hypothetical species, especially when these are difficult to identify based solely on morphology. The following default settings were used: Initial Partition prior maximal distance (P) is 1.29e-02, Distance K80 Kimura Min Slope is 1.000000. A second program, the bPTP delimitation test (https://species.h-its.org/) using Maximum Likelihood and Bayesian algorithms was used for the concatenated data to identify cryptic variabilities (Zhang et al. 2013). To visualise the relationship of several clades, statistical parsimony network methodology (TCS Network method by Clement et al. 2002) in PopART http://popart.otago.ac.nz (Leigh & Bryant, 2015) was used for haplotype network of *phyllidiid* clades of interest.

Chemical analyses

Seventy phyllidiid specimens were investigated chemically. These covered 19 species from three genera, and all three different geographic areas in North Sulawesi.

Extraction of crude extract and general experimental procedures. The ethanol storage solution was decanted and evaporated under reduced pressure. The bodies were cut into small pieces and repeatedly extracted with methanol (3 x 30 mL) and dichloromethane (1 x 30 mL), and the fractions were combined to give the crude extract.

Mass spectra were recorded on a micrOTOF-QIII mass spectrometer (Bruker) with ESI-source coupled with a HPLC Dionex Ultimate 3000 (Thermo Scientific) using an EC10/2 Nucleoshell C_{18} 2.7 µm column (Macherey-Nagel). The column temperature was 25 °C. MS data were acquired over a range from 100-3000 *m/z* in positive mode. Auto MS/MS fragmentation was achieved with rising collision energy (35-50 keV over a gradient from 500-2000 *m/z*) with a frequency of 4 Hz for all ions over a threshold of 100. HPLC begins with 90% H_2O containing 0.1% acetic acid. The gradient starts after 1 min to 100% acetonitrile (0.1% acetic acid) in 20 min. A 5 µL amount of a 1 mg/mL sample solution (MeOH) was injected at a flow of 0.3 mL/min. All solvents were LCMS grade. NMR spectra were recorded in MeOH-d_4 using Bruker Avance 300 DPX or Bruker Ascend 600 spectrometers. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0. Preparative HPLC was performed on a Merck Hitachi HPLC system equipped with a L-6200A pump, a L-4500A PDA detector, a D-6000A interface with D-7000A HSM software, a Rheodyne

7725i injection system and a Nucleodur C_{18} 5 µm Pyramid 250 mm x 10 mm column (Macherey-Nagel).

MS data acquisition and analysis. HR-LCMS analysis was performed using a 1 mg/mL LCMS grade methanol solution of the crude extracts. The obtained raw .d LCMS/MS files were converted using the DataAnalysis 4.2 software (Bruker Daltonic) to .mzXML format and uploaded to the MassIVE server (https://massive.ucsd.edu) via FileZilla (https://filezilla-project.org) FTP client.

Classical molecular network was created using the online workflow (https://ccms-ucsd.github.io/GNPSDocumentation/) on the GNPS website (http://gnps.ucsd.edu). The data was filtered by removing all MS/MS fragment ions within +/- 17 Da of the precursor m/z. MS/MS spectra were window filtered by choosing only the top six fragment ions in the +/- 50Da window throughout the spectrum. The precursor ion mass tolerance was set to 0.05 Da and a MS/MS fragment ion tolerance of 0.02 Da. A network was then created where edges were filtered to have a cosine score above 0.6 and more than three matched peaks. Feature-Based Molecular Network was created with the FBMN workflow (Nothias LF et al. 2020) on GNPS (https://gnps.ucsd.edu, Wang M et al. 2016). The mass spectrometry data were first processed with MZMINE2 (Pluskal T et al. 2010) and the results were exported to GNPS for FBMN analysis. The precursor ion mass tolerance was set to 0.05 Da and the MS/MS fragment ion tolerance to 0.05 Da. A molecular network was then created where edges were filtered to have a cosine score above 0.6 and more than four matched peaks. Furthermore, edges between two nodes were kept in the networks if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100, and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS spectral libraries (Wang et al. 2016, Horai et al. 2010). The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 6 matched peaks. The molecular networks were visualized using Cytoscape software (Shannon et al. 2003).

Isolation of pure compounds. The following protocol was used for the isolation of pure compounds: crude extracts were re-suspended in 30 mL H_2O and extracted three times with 30 mL ethyl acetate (EtAc) in a separation funnel. EtAc solubles were collected and solvent evaporated under reduced pressure. The obtained lipophilic salt-free part underwent

fractionation on a reversed phase SPE Bakerbond 2000 mg column using a stepwise gradient (50:50, 70:30, 90:10, 100:0 MeOH:H_2O v/v, 20 mL each) to obtain four fractions. In specimens Phpu15Bu14 and Phpu16Sa66 fractions SPE3 were found to be pure 3-isocyanotheonellin (18 mg) and 7-isocyano-7,8-dihydrobisabolene (14 mg), respectively. 3-isoformamidotheonellin (1 mg) was isolated from the Phpu15Bu14 SPE2 fraction using HPLC and isocratic MeOH:H2O (70:30 v/v) mobile phase. Crude salt-free extracts from *Phyllidiella nigra* specimens Phpu15Bu-6, 10, 18, 19, and 43 underwent the same SPE fractionation procedure. SPE fractions were further separated using HPLC to yield 3-isocyanobisabolane-7,10-diene and 3-isocyanobisabolane-7(14),11-diene in mixture (1 mg), epimeric 9-thiocyanatopupukeananes (1 mg) and 10-isocyano-5-cadinen-4-ol (1.4 mg). Fractions containing the same metabolites from several individually processed specimens were combined to obtain sufficient amounts for unambiguous structure elucidation with NMR.

Obtained LC-MS data was analysed with web-based cheminformatic tools (classical molecular networking, feature-based molecular networking) associated with the GNPS platform (gnps.ucsd.edu) that allow rapid compound identification via comparison of the experimental MS/MS spectra with integrated library of more than 220,000 curated spectra.

Chemical structures of known compounds could be determined after their isolation and ^{1}H and ^{13}C NMR comparison with literature values. In addition to the GNPS aided identification, structures of compounds could be putatively assigned via manual analysis of mass spectrometric data and database search (Marinlit, Dictionary of Natural Products), especially in cases where minute extract amounts, chemical instability, or high volatility hindered isolation.

Results

External identification of the specimens collected during the four expeditions allowed recognition of three genera, namely *Phyllidia* with 11 species (Figs. 4-6), *Phyllidiopsis* with seven species (Figs. 7 and 8), and *Phyllidiella* with at least 11 species (Figs. 9-12). For supplementary reasons, the two genera *Reticulidia* and *Ceratophyllidia* were included in the molecular analysis by extracting available sequences from NCBI GenBank. Some available sequences assigned to four species that were not present in our collection were also extracted

from GenBank (Table 2), i.e., *Phyllidia rueppelii* (Bergh, 1869), *Phyllidia larryi* (Brunckhorst, 1993), *Phyllidia babai* Brunckhorst, 1993, and *Phyllidiopsis cardinalis* Bergh, 1876.

The trees reconstructed from the concatenated dataset of the two mitochondrial genes 16S and CO1 using the three different model assumptions (GTR, HKY and TVM+F+I+G4) resulted in quite similar trees at generic levels; however, they differed in many ways at the species level. The tree based on the highest number of assumptions (TVM+F+I+G4), allowing different substitutional modes of the gene partitions, best reflects the results obtained from morphological analyses and is mainly used for the subsequent analysis. Figure S1 shows this tree in its full length with specimen identifiers, and Fig. 13 summarises the results of the concatenated data set by collapsing the nodes at species level. Additionally, Figs. 14 and 15 show the summarised results based on each single gene analyses, CO1 and 16S, respectively.

Four species delimitation tests were run with different algorithms and different data sets. In general, the ABGD test based on the 16S gene resulted in the most conservative species hypotheses. Interestingly, the bPTP tests when applying the concatenated data set resulted in similar species delimitation as the ABGD test using only the CO1 data set. However, the results of both bPTP tests appear to overrate the genetically identified clades as distinct species, when comparing with morphological results. We therefore based the following descriptions of species on the results of the ABGD test using the 16S gene (Fig. 13) and consulted haplotype network analyses, which supported our more conservative species hypotheses.

Phylogenetic reconstruction using the mitochondrial genes in single or in a concatenated dataset usually resulted in the monophyletic genera *Phyllidia, Reticulidia, Phyllidiella,* and *Phyllidiopsis*. However, the single sequence of a *Ceratophyllidia* was always nested within the genus *Phyllidiella* with high bootstrap support (100). The genera and species results are presented in detail below, the listing in order of the tree shown in Figs. 13 and S1. We provide detailed colour descriptions of the specimens, which were assigned to a named species or grouped in distinct clades/subclades.

Many phyllidiid species have been investigated chemically for the first time in this study: *Phyllidia elegans*, *P.* cf. *babai*, *P. haegeli*, *Phyllidiopsis burni, P. shireenae, Phyllidiella nigra, P. rudmani* and several unnamed *Phyllidiella* species. High resolution LC-ESIMS analysis provides unprecedented insights into the metabolomes of 70 specimens across the 19 phyllidiid species from three genera. The generated molecular networks based

on MS/MS spectra similarity visualise the complex chemical composition of these 70 metabolomes (see Figs.16 and S xx). All chemical suplement figures are not included in the appendix due to this chapter is still in the finalisation. A large array of natural products could be detected in individual extracts which are outlined in more detail in the species descriptions below and are summarized in Fig. 16. The majority of identified compounds belong to sesquiterpenes functionalized with isonitrile, isocyanate, formamide, thiocyanate, and isothiocyanate moieties. In ESI mass spectrometry these molecules ionize favorably under loss of the respective nitrogen containing functional group (X = CN, CNO, $HCONH_2$, SCN, CNS) (Fig. Sxx) and exhibit characteristic high intensity in-source fragment ions with *m/z* of 205.19 $[M-X]^+$ along with low intensity molecular peaks with m/z $[M+H]^+$ 232.20, 248.21, 250.21 and 264.22 that are diagnostic and allow assignment of the correspondent functional group. Interestingly, m/z values 205.19 and 232.20 are often accompanied with m/z 259.22 ions. Mass difference of 27 Da can be explained with the presence of an additional isonitrile moiety in the molecule. Bi- and even tri-functionalised diterpene isonitriles are well documented from marine sponges (Patra et al 1984; review by Emsermann et al. 2016); however, to date there are no reports of naturally occurring sesquiterpenes bearing two isonitrile moieties.

Sesquiterpene isonitriles and formamides were the most common metabolites and could be detected in almost every single chemically analysed specimen. Besides isonitrile sesquiterpenes a series of halogenated compounds with *m/z* values 266.166 [M+H] (chlorine containing) and 310.115 $[M+H]^+$ (bromine containing), with characteristic isotopic patterns could be detected in specimens across all investigated phyllidiid species and genera. MS/MS spectra indicate that these molecules share the same backbone and differ only in type of halogen attached. Intriguingly, no halogenated natural products having the mentioned above masses are known up to date. These compounds are encountered in low concentrations and despite their wide distribution, isolation of a sufficient amount for unambiguous structure elucidation was not possible.

Chemical extract composition of some investigated phyllidiids was found to be very clade specific, thus can provide chemotaxonomic evidence for species delimitation, and will be described in detail in the respective species descriptions.

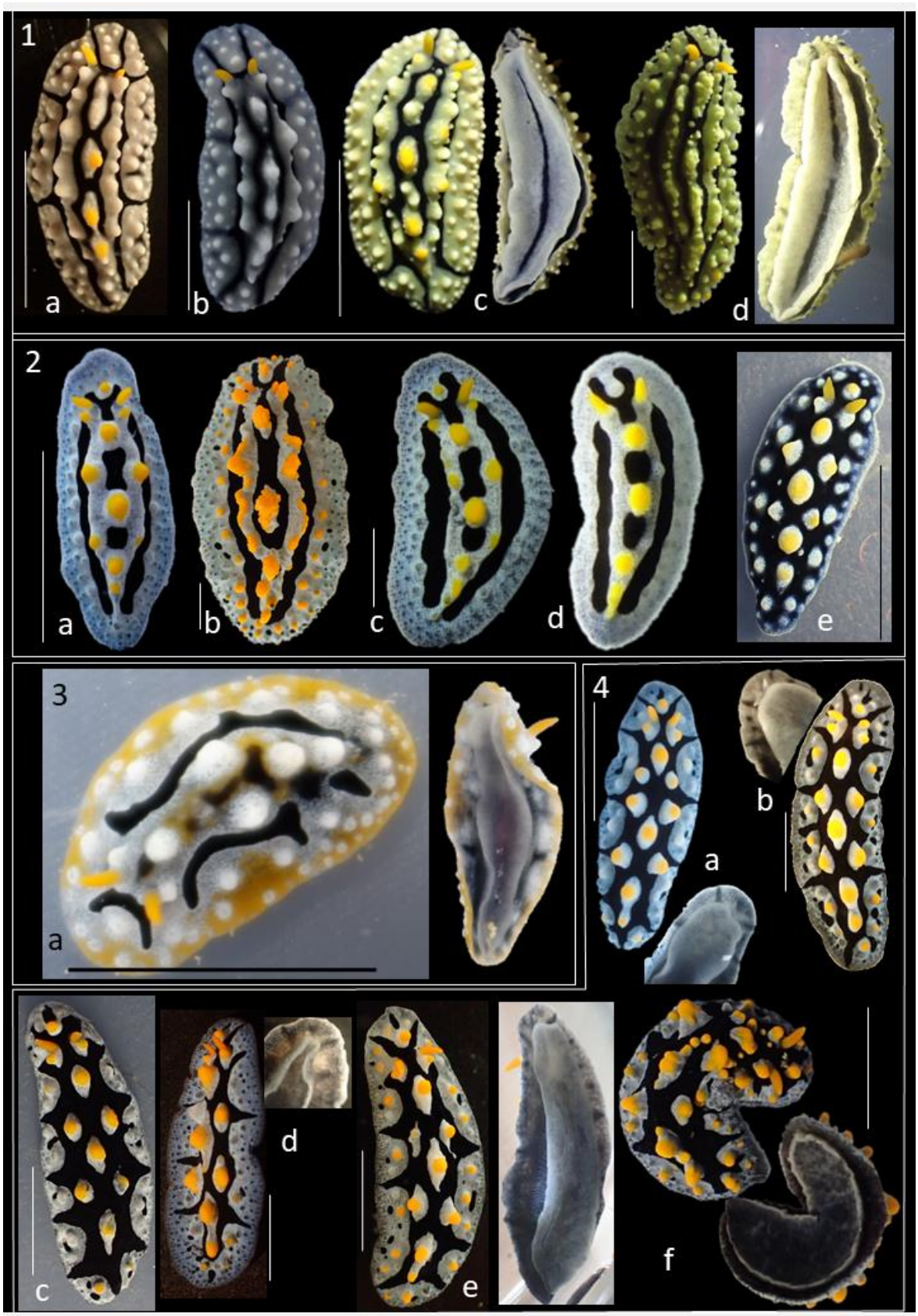

Fig. 4 *Phyllidia* species and specimens with identifiers. Bars: 10 mm. **1a-d** *Phyllidia elegans*: **a** Phel18Ba2; **b** Phel18Bu1; **c** Phel15Bu4; **d** Phaly16Bu1. **2a-e** *Phyllidia coelestis*: **a** Phco15Bu5; **b** Phco18Po1; **c** Phco15Bu12; **d** Phco15Bu16; **e** Phpic16Sa2. **3** **a** *Phyllidia* sp. 3: **a** Phex15Bu1. **4a-f** *Phyllidia picta*: **a** Phpi18Bl3; **b** Phsp18Bu1; **c** Phpi16Sa1; **d** Phsp18Ba3; **e** Phpi18Po1; **f** Phsp616Sa1.

Fig. 5 *Phyllidia* species and specimens with identifiers. Bars: 10 mm. **1a-b**: *Phyllidia exquisita*: **a** Phex17Ba1; **b** Phex18Bl2. **2a-b** *Phyllidia* cf. *babai*: **a** Phoc18Ba7; **b** Phoc16Sa3. **3a-e** *Phyllidia haegeli*: **a** Phco15Bu1; **b** Phpic16Sa4; **c** Phva16Bu2; **d** Phva16Sa51; **e** Phva16Sa19. **4a** *Phyllidia* sp. 9: **a** Phsp15Bu1. **5a-g** *Phyllidia ocellata*: **a** Phoc18Ba4; **b** Phoc15Bu1; **c** Phoc16Sa2; **d** Phoc16Sa4; **e** Phoc16Sa7; **f** Phoc17Ba1; **g** Phma16Sa1.

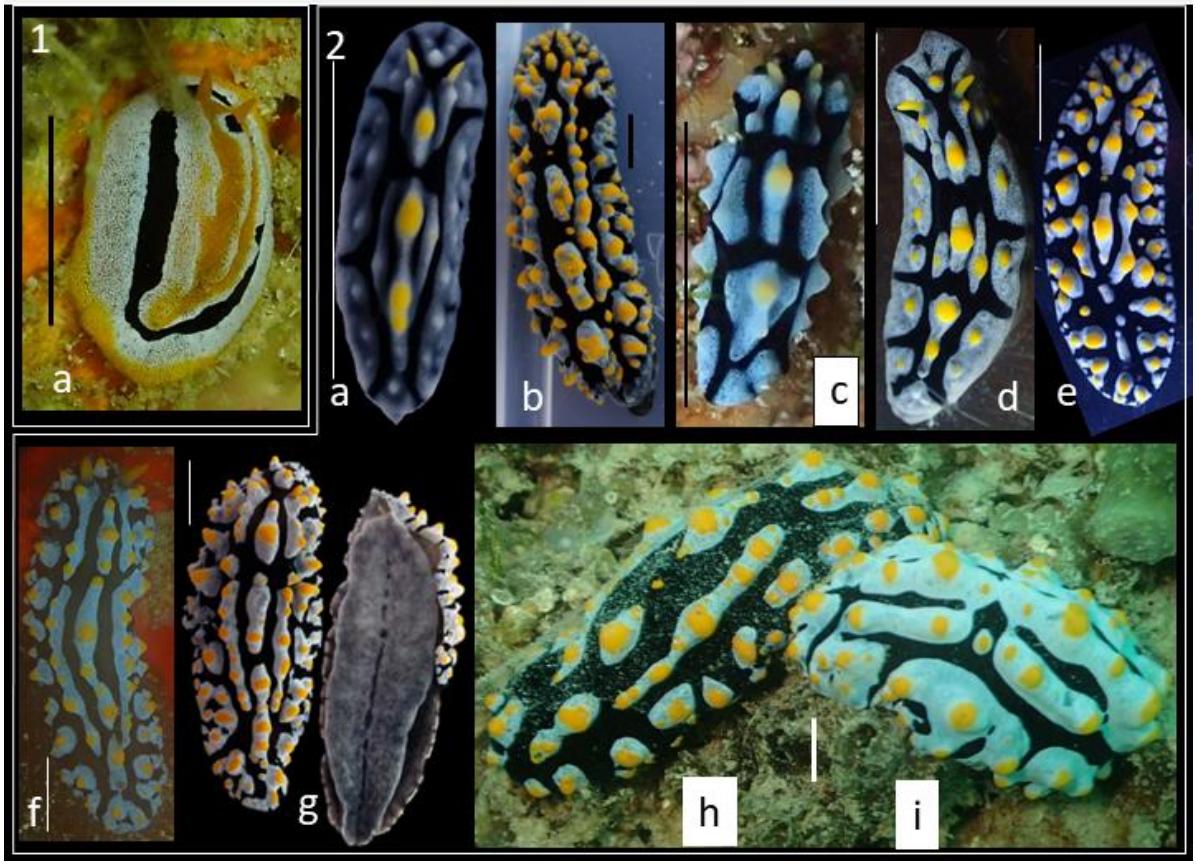

Fig. 6 *Phyllidia* species and specimens with identifiers. Bars: 10 mm. **1a** *Phyllidia* sp. a: **a.** Phsp17Bu1. **2a-i** *Phyllidia varicosa*: **a** Phel15Bu1; **b** Phva15Bu8; **c** Phel18Bu6; **d** Phel18Ko3; **e** Phva18Po1; **f** Phva18Ba1; **g** Phva15Bu2; **h** Phva16Sa37; **i** Phva16Sa38.

***Phyllidia* Cuvier, 1797**

Morphological identification resulted in 11 *Phyllidia* species (Figs. 4-6): Seven of them are recognized species: *P. elegans* Bergh, 1869, *P. coelestis* Bergh, 1905, *P. picta* Provot-Fol, 1957, *P. exquisita* Brunckhorst, 1993, *P. haegeli* Fahrner & Beck, 2000, *P. ocellata* Cuvier, 1804, and *Phyllidia varicosa* Lamarck, 1801. Two species have been illustrated as new and undescribed species in the available literature: Gosliner et al (2018) describes *Phyllidia* sp. 3 and *Phyllidia* sp. 9 (Gosliner et al. 2015), the third new species was figured by Gosliner et al. (2008: 300, top right figure only) and Venkataraman et al. (2015: pl. 75), both as *Phyllidiopsis monacha* (Yonow, 1986). The same specimen was illustrated as *Phyllidia* sp. in Eisenbarth et al. (2018), here assigned as *Phyllidia* sp. a (Phsp17Bu1, Fig. 6.1).

Molecular analyses confirmed our morphologically based assignments of the species to a large extent. Unfortunately, only CO1 genes were available in GenBank for *Phyllidia larryi* Brunckhorst, 1993 (type locality Guam), which was not present in our collections. This species does not group with *Phyllidia*, but as sister taxon to the *Phyllidia / Reticulidia* clade. This position is obtained in the tree reconstruction based only on the CO1, as well as in the

concatenated data set, and its assignment to the genus *Phyllidia* needs further investigation. Our study also confirms that species previously assigned to the genus *Fryeria* because of their ventral anal opening are part of *Phyllidia* and do not form a separate clade.

LCMS analyses revealed a range of brominated compounds that occur in several *Phyllidia* species in addition to commonly encountered sesquiterpene isonitriles. These comprise bromoindole alkaloid 5-bromotryptophan (*m/z* values 283.006 and 285.005 $[M+H]^+$, and 265.983 and 267.978 $[M-NH_3]^+$) which was detected in all *Phyllidia* species. Obtained MS data perfectly match values in the original description as *Smenospongia* sp. (Porifera) metabolites by Tasdemir et al. (2002) that allowed confident structure assignment of bromoindoles. In addition, double-charged ions of unidentified polar mono- (m/z 235.02 and 242.029 $[M+2H]^{2+}$) and dibrominated (m/z 219.035 $[M+2H]^{2+}$) compounds with retention times between 3.5 and 5.5 min. were observed in all *Phyllidia* extracts except *P. varicosa.* Some of these brominated metabolites could be detected in low amounts in the extracts of six other specimens belonging to the genera *Phyllidiopsis* and *Phyllidiella* (*Phyllidiopsis krempfi*, *Phyllidiella* sp. c subclade 1, *P. zeylanica* aucct. and *P. rudmani*).

Phyllidia elegans Bergh, 1869

The 40 specimens of *Phyllidia elegans* (Fig. 4.1a-d) are oval and broad, somewhat flattened in shape; their basic dorsal background colours are white, grey, or light green with narrow black stripes. The white granulated tubercles form three interrupted ridges along the back and are usually capped with yellow or orange. The rhinophores are golden yellow or orange, the same colour as the tubercles. The foot sole possesses a longitudinal black line, the elongated tapering oral tentacles are pale white to cream. Some of our specimens were green and not pink as described in the literature (e.g., Brunckhorst 1993) and some had no gold colouration on the dorsum but the rhinophores were gold (e.g., Fig. 4.1d). Of the 40 specimens collected from Sulawesi, we were able to sequence 34 that group with an additional 22 sequences of *P. elegans* downloaded from NCBI. Intraspecific variability is up to nearly 4 % within the monophyletic clade (Table S2 distance matrix *Phyllidia*). The metabolome of *P. elegans* is very similar to *P. coelestis* and is described in detail under the latter species.

Phyllidia coelestis Bergh, 1905

Fifty specimens of *Phyllidia coelestis* (Fig. 4.2a-e) collected around Sulawesi exhibit the typical colouration as described by Brunckhorst (1993) and Yonow (2012). The animals

all have three longitudinal black lines on the notum, and a general bluish background which is granulated. The middle line is usually interrupted by yellow-capped tubercles, but these central tubercles are encircled by the median black line in two of our 50 specimens rendering the usually interrupted line into a continuous line (Fig. 4.2b). The median line is divided by an anterior tubercle, thus forming the species-specific Y-shaped black marking in front of the rhinophores. The lateral black lines do not unite in either the anterior or posterior parts of the body. Further large and usually single rounded tubercles, capped by yellow, lie on blue ridges between the black lines. The tubercles lying more laterally are smaller and can be yellow capped or are bluish; additionally, there are tiny black flecks in this lateral blue area. One pale specimen has yellow tubercles protruding only from the central black line (Phco15Bu16, Fig. 4.2d). The species always lacks a black line on the foot. There was only one interesting and different specimen: the specimen with the identifier Phpic16Sa2 (Fig. 4.2e) differs in its more black background colouration, which somewhat resembles *Phyllidia madangensis*, or some members of *P. picta*. A dark form of *P. coelestis* was described and illustrated from Australia in Yonow (2012): externally they differed in having completely black central area, and there were differences with the internal anatomy from the typical *P. coelestis*. Some individuals of *P. coelestis* with darker colouration are illustrated on the website Sea Slug Forum (Cobb and Rudman 2008) and GBIF (2019); however, it should be emphasised that these refer only to externally identified individuals. None-the-less, 50 specimens of *P. coelestis* from Sulawesi are included in our molecular analyses with a further eight sequences retrieved from NCBI from Lizard Island, Australia (Cheney et al. 2014) and New Caledonia (Valdés 2003) which clearly group with our sequences of this species with a bootstrap value of 100 (concatenated tree, Figs. 13, S1). Intraspecific variability lies within 2 %. *P. coelestis* and *P. elegans* form a monophyletic clade supported by a bootstrap value of 99.

Metabolomic analysis of *P. coelestis* and *P. elegans* specimens collected in Bunaken National Park in 2015 demonstrated very similar characteristic chemotypes predominant in these two species (see Figures C-1, C-2), thus supporting sister taxa relationship. HR-LCMS analysis of crude extracts revealed a series of major peaks with *m/z* values that can be assigned to amphilectene-type diterpene isonitriles (m/z M+H 298.250), diisonitriles (m/z M+H 325.259) and formamides (m/z M+H 316.261), (Chart 1) that were recently reported from two Hainan *P. coelestis* populations (Carbone et al. 2019). The amphilectene diterpenes detected in *P. elegans* and *P. coelestis* form a separate cluster in the Feature-Based Molecular Network (Fig. 1). FBMN also facilitated an intriguing detection of dichloroimidic sesquiterpenes in *P. elegans* that were published previously (Bogdanov et al. 2020). Even

though found in trace amounts, dichloroimidic sesquiterpenes can provide a chemotaxonomic separation between two closely related species *P. coelestis* and *P. elegans*. Two specimens (Phco15Bu-6 and Phel15Bu-8) among chemically analysed *P. coelestis* and *P. elegans* possess an additional unknown metabolite with an m/z 404.187 $[M+H]^+$ and retention time of 13.9 min. Intriguingly, the same metabolite is detected in one specimen of the *Phyllidiella* sp. c subclade 2 (Phpu15Bu-27). Minute extract amounts prevented us from isolation and characterisation of this compound.

Phyllidia sp. 3

Two specimens grouped with one sequence available from GenBank *(*AF430363), an animal collected from 35 m depth in New Caledonia and assigned to *P. ocellata* by Valdés (2003). These three specimens form a distinct clade, confirmed by species delimitation tests, and form a sister clade to *P. elegans / P. coelestis.* One of our specimens, Phex15Bu1 (Fig. 4.3), has a similar pattern to *Phyllidia* sp. 3 in Gosliner et al. (2018): it has a bluish tinge with an irregular yellow mantle rim and two longitudinal black lines, which are slightly waved, a black curved u-shaped line in front of the rhinophores, and black marks on the midline of the dorsal midline. The tubercles are large, single, and round, not topped with yellow pigment: in fact, all tubercles are white in this species. The animal depicted in Gosliner et al (2018) only differs in so far as the yellow parts appear more orange. Unfortunately, no photograph is available of our second specimen. The clade is supported by a bootstrap value of 100 (Fig. 13) and shows no intraspecific variability (Table S2). No chemical analyses were performed for specimens of this clade.

Phyllidia picta Pruvot-Fol, 1957

Thirty-four sequences of *Phyllidia picta* were included in our molecular phylogeny, which grouped in two distinct subclades, indicating an intraspecific variation. This is confirmed by the haplotype network analysis in which the two clades are separated by 28 differing nucleotides which are shared only by specimens in one of the respective clades and not with the other (Fig.17). However, species delimitation tests using only 16S data do not support these two clades as separate species. In the first clade, all sequences from GenBank group together with sequences from our collection from North and South Sulawesi (Fig. 4.4a-c). The second clade includes specimens from our expeditions to North and Southeast Sulawesi (Fig. 4.4d-f). The intraspecific genetic variability of both clades within *P. picta* is 8.63 %). Both clades are very similar in their external morphologies, with the anal opening

lying ventrally. They resemble each other in colouration, especially with regard to the typical trapezoidal black pattern with elongations towards the edge of the mantle. The tubercles are single, the larger ones in three rows usually capped with yellow to orange. The rhinophores are also yellow to orange with one tubercle anterior to them. The oral tentacles are grey, the tips with or without yellow in both clades; the foot sole is grey to dark grey. The colouration of the living specimens matches the colouration described by Brunckhorst (1993) for *Phyllidia menindie* (Brunckhorst, 1993), a species that was subsequently synonymised with *P. picta* by Yonow (2012) after her examination of the holotype in the Natural History Museum (BMNH 1854.7.19.91). No chemical analyses were performed for this species.

Phyllidia rueppelii (Bergh, 1869)

The two clades of *Phyllidia picta* group in an unresolved relationship with a single available sequence assigned to *Phyllidia rueppelii* from Valdés (2003, 16S gene only). This species is only recorded from the Red Sea and Gulf of Oman (Valdés 2003; Yonow 2020). Like *P. picta, P. rueppelii* has a ventral anal opening but differs in having a yellow-orange margin to the mantle. Unfortunately, no CO1 sequence was available for this *P. rueppelii* specimen, nor can we confirm correct identification: *P. rueppelii* has been confused with *P. schupporum* in the same geographical range (Yonow 2020).

Phyllidia exquisita Brunckhorst, 1993

Only three specimens of *Phyllidia exquisita* were present in our collections (Fig. 5.1a, b). The main background colour is grey to white, with two or four distinct longitudinal black lines, sometimes forming a reticulate pattern across the midline. Each lateral black line forms 4-6 scallops to the mantle margin. In some specimens, some single, black, small to middle sized, round tubercles are present, highly disguised, on the black lines. The white tubercles are usually single and the larger ones capped by yellow-gold. The yellow mantle margin is characteristic of this species, mainly along the anterior rim, more seldom along the sides (Fig. 5.1a, b), or even the whole mantle margin. The anal opening lies on the greyish white part of the notum, behind the last large posterior tubercle. Our three specimens group with the only *P. exquisita* sequence available from GenBank provided by Stoffels et al. (2016) from a specimen (not illustrated) from Ternate, Indonesia.

Phyllidia cf. *babai*

Two of our specimens with the identifiers Phoc16Sa3 and Phoc18Ba7 (Fig. 5.2a, b) have a very similar colouration to *P. ocellata*, but actually group with two sequences from Stoffels et al. (2016), who assigned their sequences to *Phyllidia* cf. *babai*. This monophyletic clade is supported by a bootstrap value of 100 with an intraspecific genetic variability of 5.49 %. The animals that Stoffels et al. (2016) depicted are similar to the specimens originally described by Brunckhorst (1993) as *P. babai* with a white ground colour and only the central row of tubercles being yellow or orange. Brunckhorst also mentioned a distinct yellow margin for the newly described *P. babai*, a feature that seems to be missing in the specimens analysed by Stoffel et al. (2016). We assume that the second specimen depicted in their figure 10.e is from a preserved animal and therefore lost the yellow pigment. Our specimens differ from Stoffels's *P.* cf *babai* specimens by having a yellow/orange background colour identical to both their *P. ocellata* and our *P. ocellata*. Moreover it seems that our specimens of this clade have white lines surrounding the black patches which are always continuous between the black rings and form a central white oval around the three tubercular rows. In *P. ocellata*, the colored rings are always separated by the orange background color (see description of *P. ocellata* below). One of our *P.* cf. *babai* specimens (Phoc18Ba7, Fig. 5.2a) had an orange foot and orange oral tentacles, and not the typical cream to white ventral colouration of *P. babai*, nor the darker grey colouration of foot and oral tentacles as in *P. ocellata*. Therefore our specimens do not match *P.* cf *babai* in colour, although they group with the two sequences of Stoffel et al. (2016). They also do not match the color description of *P. babai* in Brunckhorst (1993) either. Investigation of more specimens is needed to find better distinguishing characters for this distinct clade, which we retain here as *P.* cf *babai*.

Chemical analysis of one *P.* cf. *babai* specimen (Phoc16Sa-3, Undap et al. 2019: Fig. 3B) and one *P. ocellata* specimen Phoc16Sa-7 (Fig. 5.5e) demonstrated clearly different metabolomes despite external similarity (see Figure C-3). A unique feature detected only in the metabolome of *P.* cf. *babai* among all chemically analysed phyllidiids is a series of polar brominated compounds with *m/z* values matching masses of the bromopyrrole alkaloids manzacidin A ($[M+H]^+$ 344.024 and 346.022) and manzacidin B ($[M+H]^+$ 360.017 and 362.014) (see Chart 2). These ions also form a separate cluster in the GNPS molecular network. Further, a MS^2 spectrum of a peak (m/z 403.917 M^+) with a retention time of 4.9 min and isotopic pattern of a dibrominated compound matched perfectly with the MS^2 spectrum of spongiacidin A in the GNPS library (see Fig. Sxx). Its monobrominated derivative

spongiacidin B (m/z 324.009 M^+, retention time of 3.7 min) was also detected. These alkaloids were described from Okinawan sponge species of the genus *Hymeniacidon* (Kobayashi J et al 1991, Inaba et al. 1998). Additionally, the MS2 spectrum of a dibrominated compound with an m/z 389.938 $[M+H]^+$ and retention times of 4.8 and 6.8 min matches the GNPS repository spectrum of dibromophakellin, an alkaloid from a sponge identified as *Phakellia flabellata* (Sharma and Magdoff-Fairchild 1977). Common sesquiterpene isonitriles and formamides were also detected in the *P*. cf. *babai* crude extract.

Phyllidia haegeli (Fahrner & Beck, 2000)

Phyllidia haegeli (Fig. 5.3) is represented in our collections by seven specimens. The species possesses an anal opening on the ventral side but the colouration resembles that of *P. varicosa*. The species is characterised by four black lines along three longitudinal tubercular ridges, which meet behind the rhino-tubercle, and usually meet behind the second median tubercle. Shorter horizontal black lines may originate from the longitudinal stripes and extend to the margin resembling the trapezoidal markings of *P. picta* or *P. elegans*. Single rounded tubercles on the ridges are capped by yellow, often with confluent bases, whereas the small tubercles on the blue marginal band may lack this yellow cap. One distinct yellow tubercle lies anteriorly between rhinophores. The marginal band resembles that of *P. coelestis* in being blue, somewhat granular, with tiny tubercles and flecks of black. The foot sole is grey to blue with a somewhat darker stripe / band down the middle. We assume that there was no such band remaining on the type specimen when it was examined: Fahrner & Beck (2000) clearly state that the specimen was not photographed alive (as they thought it was a *P. varicosa*, presumably with a ventral black line?) and black pigment disappeared after some time. Figure 5.3d depicts one of our *P. haegeli* specimens (Phva16Sa51) in the field to show the high degree of similarity to *P. varicosa* and the probable misidentifications that will have occurred when only photographs are examined (e.g., on internet sites such as Sea Slug Forum and iNaturalist) when not specifically searching for the position of the anal opening. In future, each specimen of *P. varicosa* will have to be carefully examined. Phylogenetic analyses support the monophyletic clade with a bootstrap value of 100 and a rather low intraspecific variability of 0.7 %.

One *P. haegeli* specimen (Phco15Bu-1, Fig. 5.3a) was analysed chemically using LC-HRMS. Its metabolomic profile is dominated by sesquiterpenes functionalised with isonitrile

and formamide moieties, whereas amphilectene type diterpenoids found in *P. coelestis* and *P. elegans* are absent.

Phyllidia sp. 9

The sister group to *P. haegeli* is a clade composed of only two specimens. One is from our collection (Phsp15Bu1, Fig. 5.4), and one is from the study of Stoffels et al. (2016: RMNH.Moll.336619, Fig. 7g) with a colouration similar to the single individual depicted by Gosliner et al. (2015: 282) under the name *Phyllidia* sp. 9. The colour of our animal is bright white, with a distinct black oval area in the middle of the mantle. This black area is disrupted by single white tubercles in three rows, which are usually capped with yellow. The yellow rhinophores arise from a broad white base encompassing the rhinophore and a rhino-tubercle in the black area. The animal depicted by Stoffels et al. (2016: Fig. 7g) does not show any yellow traces, probably because it is a photograph of the preserved specimen, and is completely white and black; the tubercles are white with white bases. This species has a ventral anal opening, like both *P. picta* and *P. haegeli*. Sister taxa relationship to *P. haegeli* is supported by a bootstrap value of 100.

Phyllidia ocellata Cuvier, 1804

Twenty-seven specimens of *P. ocellata* were collected from North Sulawesi. *Phyllidia ocellata* is considered a very variable group with many colour morphs (Pruvot-Fol 1957, Yonow 1996, Brunckhorst 1993, Gosliner et al. 2018) and differs from *P. babai* in being basically orange while *P. babai* is basically white. *Phyllidia ocellata* specimens have a typical yellow (or orange) background and large white tubercles, the lateral ones placed in the centre of black rings which are ocellated with a white line on both sides of the black ring (Fig. 5.5a-f). One of these black rings with a white tubercle is located anteriorly in front of the rhinophores and there are two or three pairs present on each side of the midline. The foot and oral tentacles are grey, the latter with yellow tips. One specimen (Phma16Sa1, Fig. 5.5g) was preliminarily assigned to *P. madangensis* based on the overall black colouration with white and yellow rounded tubercles arising from black background (Undap et al. 2019: Fig. 5E); however, our phylogenetic study clearly places this specimen within the clade of *P. ocellata* composed of 30 more *P. ocellata* sequences, and which is supported by a bootstrap value of 100. All four species delimitation tests resulted in a single species referable to *P. ocellata*, with an intraspecific variability of 5.76 %.

Previous chemical investigations of *P. ocellata* reported isolation of bicyclic sesquiterpene isonitriles (Fusetani et al. 1992, Okino et al. 1996). More recently, bicyclic and tricyclic sesquiterpene isonitriles and isothiocyanate were isolated from Australian *P. ocellata* specimens (White et al. 2015). In the crude extract of a *P. ocellata* specimen analyzed in this study several minor peaks could be assigned to sesquiterpene isonitriles, which is in accordance with published reports. Polar brominated compounds were also detected; however, these differ from those detected in *P.* cf *babai* (see above). A derivative of commonly found 5-bromotryptophane, 5-bromoabrine (m/z 297.000 and 298.996 $[M+H]^+$), was detected only in *P. ocellata*.

Phyllidia sp. a

Sister taxon of *P. ocellata* is an undescribed species, *Phyllidia* sp. a (Phsp17Bu1, Fig. 6.1). It is a unique specimen, collected from deeper areas in Bunaken Island at 25.3 m (for details see Eisenbarth et al. 2018). It differs from the other *Phyllidia* species by having only smooth and very low papillate ridges on the notum instead of tubercles. Three white bands lie on the central notum covered by these papillae, the median ridge starting behind the rhinophores along the midline. The mantle margin is yellow-gold. A smooth black band on the lateral notum forms an elongated ring. Gosliner et al. (2008: 300, top right photograph) depicts an individual identified as *Phyllidiopsis monacha*. This animal looks like our specimen and we assume that this specimen was erroneously identified as *Phyllidiopsis monacha* due to its synonymisation with *Phyllidiopsis dautzenbergi* (Vayssière, 1912) by Brunckhorst (1993). *Phyllidia monacha* has distinct tubercles which are missing in our new *Phyllidia* sp. a and the central black ring extends as black radiating lines towards the mantle margin. Phylogenetic analysis of the 16S data set revealed a sister taxa relationship of this new species with *P. ocellata*, supported by a bootstrap value of 99. Unfortunately, we were not able to retrieve the CO1 sequence of this new species; however, the species delimitation tests using 16S information clearly render *Phyllidia* sp. a as a distinct species

Phyllidia varicosa Lamarck, 1801

The most commonly collected species was *Phyllidia varicosa* with 114 specimens; these were also the largest specimens (up to 87 mm) in our collections. Smaller animals of *P. varicosa* (7 mm) resemble the whiter *P. elegans* (Fig. 6.2a) and the spikier *P. haegeli* (Fig. 5.3). They have a greater number of simple but acute ridges and the tubercles on the median ridges are capped with yellow. The oral tentacles are grey with a faint or bright yellow tip.

The foot exhibits a black stripe, which might also be broken up in black markings (Fig. 6.2g).Fahrner and Schrödl (2000) have shown that the black pigment on the foot can fade away during preservation, and thus might lead to misidentification. Adult living specimens of *P. varicosa* usually curl their lateral mantle rim underneath; thus, they look rather elongate on the substrate (Fig. 6.2f, h, i). The 130 sequences from our collection and GenBank are supported as a monophyletic group by a bootstrap value of 100. However, this species, with the highest number of sequences included in our analysis, also has the second greatest measured intraspecific genetic variability of 9.1 %.

Extracts of four *P. varicosa* specimens collected in 2015 from two locations at Bunaken National Park were analysed with LCMS. The metabolomes varied for each locality (see Figure C-4) but the major compounds detected can be assigned to sesquiterpene isonitriles. The herein studied *P. varicosa* specimens seem to lack chemotaxonomic markers that would allow the clear distinction of *P. varicosa* from other phyllidiid nudibranchs.

Phyllidia babai Brunckhorst, 1993

A single sequence is available from the studies of Stoffels et al. (2016) that was assigned to *Phyllidia babai*, and groups neither with the similarly coloured *P.* cf. *babai* nor with *P. ocellata* in our tree reconstructions. It actually represents the first offshot within the clade *Phyllidia*. Stoffels et al. (2016: Fig. 10d) described and illustrated the single specimen which is white and similar to one of their *P.* cf. *babai* specimens on the same plate (2016: Fig. 10e, f), but has more and smaller black rings and patches, as well as two black rings on the midline. We did not collect any specimens that could be assigned to this clade, and this may in fact be the "true" *Phyllidia babai*.

Reticulidia Brunckhorst, 1990

Members of the genus *Reticulidia* were not represented in our collection; however, we included sequences from two species (*R. halgerda* and *R. fungia*) from GenBank in our analysis for completeness. This monophyletic genus is sister taxon to *Phyllidia* with a bootstrap value of 100.

Fig. 7 *Phyllidiopsis* species and specimens with identifiers. Bars: 10 mm. **1a-j** *Phyllidiopsis krempfi:* **a** Phskr18Ba1; **b** Phfi16Sa2; **c** Phspi18Ba1; **d** Phpu16Sa72; **e** Phsh15Bu1; **f** Phfi16Sa1; **g** 18TMphni7; **h** Phpu15Bu37; **i** Phpu16Sa58; **j** Phpu16Sa66. **2a-d** *Phyllidiopsis burni*: **a** Phssp.a18Ba1; **b** Phpu16Sa19; **c** Phpu16Sa47; **d** Phpu16Sa55. **3a-d** *Phyllidiopsis* cf. *krempfi*: **a** Phskr18Ba2; **b** Phpu16Sa42; **c** Phpu16Sa45 **d** Phpu16Sa59.

Fig. 8 *Phyllidiopsis* species and specimens with identifiers. Bars: **1-3** 1 mm; **4** 10 mm. **1a-d** *Phyllidiopsis xishaensis*: **a** Phst16Bu1; **b** Phst16Bu2; **c** Phsxi18Ba1; **d** Phsxi18Bu1. **2a-c** *Phyllidiopsis annae*: **a** Phsan18Ba1; **b** Phsan18Ba2; **c** Phsan18Ba4. **3a** *Phyllidiopsis sphingis:* **a.** Phsph15Bu1. **4a-c** *Phyllidiopsis shireenae*: **a** 18LePhsh1; **b** Phsh16Sa2; **c** Phpi15Bu4.

Phyllidiopsis **Bergh, 1876**

The genus *Phyllidiopsis* is characterised by having fused oral tentacles that form a rectangular structure. Thirty-one species are recognised according to the WoRMS Editorial Board (2021). In our collection seven species could be distinguished, six of which can be assigned to recognised species (*P. krempfi* Pruvot-Fol, 1957, *P. burni* Brunckhorst, 1993, *P. xishaensis* (Lin, 1983), *P. annae* Brunckhorst, 1993, *P. sphingis* Brunckhorst, 1993, and *P.*

shireenae Brunckhorst, 1990). Affiliation of one clade, *Phyllidiopsis* sp. a, remains uncertain. In our analysis, *Phyllidiopsis* is monophyletic with one exception: the specimens and sequences assigned to *P. cardinalis* by Valdés (2003; AF430367) and Cheney (2014; KJ001308) branch off first within the Phyllidiidae tree when using 16S only, and in the concatenated data set (Figs. 13, 15). However, when applying only the CO1 dataset, *P. cardinalis* groups correctly with the genus (Fig. 14). Since Information on 16S was missing for KJ001308 and CO1 was missing for AF430367, the correct affiliation needs to be investigated in future with more complete data sets. Chemical analyses revealed no genus specific compounds.

Phyllidiopsis krempfi Pruvot-Fol, 1957

Our 16 specimens of *P. krempfi* (Fig. 7.1) have a pink dorsal notum with irregular black lines that meander between the tubercles. Two main longitudinal black lines merge in front of the rhinophores and run backwards where they can merge behind the anal opening or may stay separate. The rhinophores are black on the tip and posterior part, but pink on the anterior side. The hyponotum and foot sole are grey, the oral tentacles are rectangular in shape, with grey to black along the lateral grooves. *Phyllidiopsis krempfi* is very similar in its external appearance to *P. pipeki,* which was described as having two longitudinal black lines and large complex pink tubercles forming a crest on the midline. The foot sole, oral tentacles, and hyponotum are pale pink to grey. In general it is very difficult to distinguish between these two species and several of our specimens (Phskr18Ba1, Fig. 7.1a; Phspi18Ba1, Fig. 7.1c; Phsh15Bu1, Fig. 7.1e; and Phfi16Sa1, Fig. 7.1f) match the original and subsequent descriptions of *P. pipeki* (see Brunckhorst 1993; Yonow 2011). However, these specimens clearly group with typical *P. krempfi* colour morphs in our molecular analyses. Furthermore, some specimens of *P. krempfi* depicted by Stoffels et al. (2016) have oral tentacles without the black-grey colouration along the sides. These specimens of *P. krempfi* (voucher numbers 336453, 336512, and 336469) show a colouration that matches the original description of *P. pipeki*, but the tuberculate notum matches the description of *P. krempfi.* We also have four specimens with characters of *P. pipeki* on the ventral side, but characters of *P. krempfi* on the dorsal side, further supporting their synonymy. In our molecular investigations, all *P. pipeki* colour morphs grouped together with *P. krempfi* from Sulawesi (this study), Ternate and Papua New Guinea (Stoffels et al. 2016), and Terengganu Malaysia (Alqudah et.al. 2015). Based on these results, *P. pipeki* is here considered a synonym of *P. krempfi. Phyllidiopsis*

krempfi as a monophyletic group, is supported by a bootstrap value of 100 and shows an intraspecific genetic variability of 2.73 %.

Three *P. krempfi* specimens were selected for chemical analysis. Specimens Phpu16Sa-58 and Phpu16Sa-66 (Fig. 7.1i-j) from Sangihe Island had identical chemical profiles with one major metabolite (see Figure C-5). Fractionation and HPLC separation of the crude extract of Phpu16Sa-66 led to the isolation of 7-isocyano-7,8-dihydrobisabolene (see Fig. S chart) as the major metabolite. This natural product was first reported from a *Ciocalypta* sponge (Gulavita et al. 1986). The structure was unambiguously established by comparison of its ^{1}H and ^{13}C NMR spectra with published data. Intriguingly, the MS data of the main compound in the crude extract of Phpu16Sa-66 show a series of m/z values, i.e., 205.191, 232.202 and 259.215 $[M+H]^+$. The m/z 259.215 can be reasonably explained with an undescribed corresponding sesquiterpene core bearing two isonitrile functions (see Fig. Sxx) and thus, the isolated compound should be regarded as an artifact.

Interestingly, the metabolomic profile of the third *P. krempfi* specimen (Phpu15Bu-37, Fig 7.1h) collected in Bunaken Island was significantly different from the other two analyzed specimens Phpu16Sa-58 (Fig 7.1i) and Phpu16Sa-66 (Fig 7.1j) collected in Sangihe Island, ca. 200 km north of Bunaken Island. In addition to the isolated 7-isocyano-7,8-dihydrobisabolene, its extract contained several other major constituents that could be identified as sesquiterpene isonitriles based on diagnostic ions.

Phyllidiopsis burni Brunckhorst, 1993

Ten specimens are assigned to *P. burni* (Fig. 7.2), characterised by having an ovate body with a narrow but bright pink band around the mantle margin. The anterior and posterior ends of each animal have a tendency to be rather pointed. Large pink compound tubercles are present on a black background. Small single pink tubercles lie around the mantle margin. The rhinophores are predominantly black. The black anal opening is generally on the top of a tubercle. The hyponotum is dark grey with pink patches. The foot sole is grey and brighter toward the edge. The colour of our specimens is very similar to the external colouration of *Phyllidiella pustulosa*; however, *P. burni* can be distinguished by its fused oral tentacles typical of the genus *Phyllidiopsis*. The rectangular oral tentacles are pale white to pink with dark grey grooves on the outer sides.

The extracts of three investigated *P. burni* specimens (Phpu16Sa-19, Fig.7.2b; Phpu16Sa-55, Fig.7.2d; Phpu16Sa-93) revealed various isonitrile sesquiterpenes in LCMS

analysis (see Figure C-6). While chemotypes of the first two specimens are rather unspecific, the extract of the third specimen was dominated by a unique major sesquiterpene isonitrile with a retention time of 17.1 min that was not encountered in any other herein chemically investigated phyllidiid.

Phyllidiopsis sp. a

Sister to *Phyllidiopsis burni* (supported by a bootstrap value of 100 in the 16S analysis) is a clade composed of six specimens which look very similar to *P. krempfi.* This clade, *Phyllidiopsis* sp. a (Fig. 7.3), is characterised by black rhinophores with a pink to pale pink base, whereas *P. krempfi* (including the *P. pipeki* colour morphs) has rhinophores with black at the tips and posterior side, but pink on the anterior face. The boundary between the black rhinophore and the pink base runs diagonally or transversally in *P. krempfi* and not horizontally, as in *Phyllidiopsis* sp. a (Figs. 7.1 vs. 7.3). The rims of the rhinophores are angled and very low, with pustules on the posterior side. The narrow mantle edge is pink. The anal opening occurs on a white to pink tubercle. All six specimens of this clade were collected in North Sulawesi only (Lembeh Strait, Sangihe Island, and Bangka Island). Interestingly, molecular barcoding resulted only in 16S sequences and no CO1 sequence in all six specimens. Species delimitation tests based on the 16S dataset clearly indicate this clade (with a support value of 100 in the 16S tree) as a distinct species.

Phyllidiopsis clade *xishaensis / annae / sphingis / shireenae*

One monophyletic clade (with bootstrap support value of 100) consists of four *Phyllidiopsis* species, characterised by white colouration with longitudinal black lines. This clade comprises *P. xishaensis* (Lin, 1983) (Fig. 8.1), *P. annae* Brunckhorst, 1983 (Fig. 8.2), *P. sphingis* Brunckhorst, 1983 (Fig. 8.3), and *P. shireenae* Brunckhorst, 1980 (Fig. 8.4), all species supported by bootstrap values of 100. Whereas *P. shireenae* can reach a large size (more than 100 mm), the other three species are usually small, with a size of less than 30 mm (Gosliner et al. 2018). Besides the species-specific black pattern and ridge(s) on the dorsum, our seven specimens of *P. xishaensis* and one specimen of *P. sphingis* are characterised by white to yellow rhinophores. *Phyllidiopsis shireenae* has entirely pink rhinophores, *P. annae* has black rhinophores which are pink at the base. *Phyllidiopsis annae* and *P. sphingis* can be distinguished by their blue mantle colouration while *P. xishaensis* and *P. shireenae* have pale white mantle colouration. Valdés (2003) assigned one of his specimens (AF430368) from New Caledonia to *P. sphingis*, but in our study it is grouped together with two *P. annae*

sequences (one from NCBI from Philippines (Hallas et al. 2017) and our *P. annae* specimens); the misidentification is understandable as they have similar dorsal patterns. Intraspecific variability of all four species is low, with a maximum of 1.84 % in *P. annae*. However, the number of included specimens was also low with a maximum of 11 specimens in *P. xishaensis* (intraspecific variability 1.34 %).

Only one species of this *Phyllidiopsis* clade was investigated chemically. The crude extracts of two analysed *P. shireenae* specimens contained sesquiterpenes typical for Phyllidiidae, and did not provide any species-specific chemical cues.

Phyllidiella **Bergh, 1869**

According to our morphology and preliminary molecular-based results, we were able to identify six species of *Phyllidiella*: *P. pustulosa* (Cuvier, 1804), *P. nigra* (van Hasselt, 1824), *P. zeylanica* (auctt. not the original description by Kelaart, 1958, but used by authors in error, see below), *P. rudmani* Brunckhorst, 1993, *P. albonigra* (Quoy & Gaimard, 1832), and *P. hageni* Fahrner & Beck, 2000, and at least ten clades or subclades that could not be assigned to a named species. The four clades and three subclades are numbered consecutively 1 to 7 (Figs. 9-12). One unexpected result in our tree was the position of the single specimen of an unidentified *Ceratophyllidia* species from Philippine Islands (Hallas et al. 2017). It is placed within *Phyllidiella*, which would render the genus *Phyllidiella* paraphyletic if the specimen was correctly identified to genus. All clades of *Phyllidiella*, including the un-named clades, were supported by high bootstrap values.

Phyllidiella pustulosa (Cuvier, 1804)

Our results strengthen the need to formulate adequate diagnostic characters for *P. pustulosa*, the type species of the genus *Phyllidiella*, as described and illustrated by Cuvier (1804). Only then can we characterise the other clades to distinguish them from the nominal species and solve the *P. pustulosa* "species complex" (see Stoffels et al. 2016, Bogdanov et al. 2020). All eight synonymisations by Brunckhorst (1993) must be reconsidered in light of these results. However, of the type material of *P. pustulosa* only one syntype is available that was selected as lectotype by Brunckhorst (1993: 49). Pruvot-Fol (1957) re-described the material of *Phyllidiella pustulosa* deposited by Cuvier in the Muséum nationale d'Histoire naturelle in Paris, as did Brunckhorst (1993), and our re-analysis of this lectotype did not reveal any further information. The single animal is completely white with no traces of any

black pigment. Brunckhorst (1993) revised the genus and its species, based on his material as well as some museum specimens. He described *P. pustulosa* as having an elliptical to oval shape, with a black dorsal background, rounded tubercles of various numbers grouped into clusters of two or three, and a pink margin. This description refers to the same set of characters previously described and illustrated by Cuvier (1804); these descriptions match the majority of our specimens in the first *Phyllidiella* clade in our tree (Figs. 13 and S1), and so we assign the nominal name *P. pustulosa* to this clade, comprising 74 specimens from our collection: the animals all have a black background with a narrow white mantle margin (Fig. 9.1a-d). The tubercles are rather small and conical, and they usually cluster in two, three or more in confined tubercular fields. Their colour can vary from pale pink to dark pink, and even green. The rhinophores are black; the black anal opening lies in a black field. The hyponotum is grey, with pale pink patches, and the gills are dark grey-pink. The foot sole is pale grey to white, as are the oral tentacles, which have a dark grey line along the lateral grooves. With regards to the structure of the tubercles, most of our specimens also match one of the type materials of *Phyllidia nobilis* Bergh, 1869 which had been synonymised with *P. pustulosa* (paralectotypes designated by Brunckhorst 1993). Examination of the paralectotype material deposited in Copenhagen, comprising three specimens (NHMD-633656, Fig. 2.2; NHMD-633657, Fig. 2.3; NHMD-633658, Fig. 2.4b), revealed that the tubercles cluster in groups of three or more on a black background and the mantle has a white band along the rim. These specimens all appear to have median clusters with a clear separation from the lateral clusters. Examination of the lectotype of *P. pustulosa* (MNHN, Paris, IM-2000-35147) shows the same tubercular dorsal morphology as *P. nobilis*. Unfortunately the black pigmentation was lost and therefore a comparison of color patterns not possible. Our material is here identified as the true *P. pustulosa* and groups with most other sequences retrieved from GenBank under the name *P. pustulosa*. The 94 concatenated sequences from our collection and from GenBank cluster with a support value of 96 and are sister to *Phyllidiella* cf. *pustulosa* with a support value of 100. Intraspecific genetic variability is 9.87 %.

Numerous chemical studies of *P. pustulosa* have been published (Okino et al. 1996, Kassühlke et al. 1991, Hirota et al. 1998, Wright 2003, Manzo et al. 2004, Lyakhova et al. 2010, Jomori et al. 2015, White et al. 2017, Sim et al. 2020) as well as our recent publication (Bogdanov et al. 2020), highlighting the chemical variability that is found in nudibranchs commonly encountered and identified as *P. pustulosa*. Eight *P. pustulosa* specimens were utilised in the chemical analysis: LCMS revealed three main chemotype variants within the 'true' *P. pustulosa* (see Figures C 7-9). As expected from previous reports, all investigated

specimens possess sesquiterpene isonitriles. The first chemotype is found in specimens Phpu15Bu-14 and Phpu16Sa-5 (from Bunaken National Park and Sangihe Island), both lying within the first branch of the clade. The major constituent 3-isocyanotheonellin was isolated from the crude extract of Phpu15Bu-14 with its formamide derivative (Gulavita et al. 1986) and the structures were confirmed with ^{1}H and ^{13}C NMR experiments. The second chemotype is characterised by having unidentified isonitriles in addition to 3-isocyanotheonellin and is shared by three specimens (Phpu16Sa-3, Phpu16Sa-4, and Phpu16Sa-6) collected in Sangihe Island and grouping in a subclade of *P. pustulosa* supported by a bootstrap value of 100. The third chemotype is found in specimens Phpu15Bu-8 (Fig. 9.1a), Phpu15Bu-9, and Phpu16Sa-52 from different localities (Bunaken National Park and Sangihe Island), and belonging to different subclades. Intriguingly, the observed chemotypes represent the subclades within the true *P. pustulosa* rather than the collection locality. Fig. 18 shows the relationship of these clades within *P. pustulosa* in a network analysis. The clades are separated by few shared mutations, but also confirm the closer relationship of specimens sharing the same chemotype.

Phyllidiella cf. *pustulosa*

Twenty specimens group in a separate clade (with a bootstrap value of 98) that sister to *P. pustulosa*, and this relationship is supported by a bootstrap value of 100 (Fig. 9.2). Members of *Phyllidiella* cf. *pustulosa* are characterised by a black mantle covered in short, conical, compound, pink tubercles which have a somewhat broader base than the tubercles in all other members assigned to *P. pustulosa*. This also renders the specimens lighter, because the black lines are narrower. These are arranged in longitudinal and diagonal lines, forming an X-shaped pattern in the first cluster on the medial dorsum. A narrow pink line runs along the mantle margin. The rhinophores are black with pale white-pink bases, their openings lying at the transition of a black line and a posterior pink tubercular field, and there are no rhinophoral sheaths. The anal opening lies in the connection of the two longitudinal black lines. Members of this clade have white or pale pink oral tentacles and foot sole. The colour pattern somewhat resembles the pattern of *Phyllidiella lizae* but analysis of the holotype and the paratypes of *P. lizae* (Australian Museum, C159493; Fig. 2.7-2.9) allows the clear differentiation of our material from this species. Examination of the type material of *P. lizae* results in the following external characters: a white to pale pink background of the notum with a narrow cross-like black pattern; the margin is white; the foot and oral tentacles are also pale pink. *Phyllidiella lizae* is also characterised by low pink rhinophoral sheaths that are absent in specimens of *P.* cf. *pustulosa*. It also appears that the tubercular field between the

rhinophores is continuous in *P. lizae* but not in *P.* cf. *pustulosa*. One of the available *P. lizae* sequences (AF430365) retrieved from GenBank and identified by Valdés (2003) is clearly misidentified; unfortunately, there is no description or photograph of this particular specimen available. Our *P.* cf. *pustulosa* seems to match with one syntype and one paralectotype of *P. nobilis* (NHMD 91773, Fig. 2.1; NHMD-633658a, Fig. 2.4).

The species delimitation tests of the concatenated dataset as well as of CO1 clearly distinguish between *Phyllidiella pustulosa* and *Phyllidiella* cf. *pustulosa,* the members of the latter united with a support value of 98. However, the ABGD test using only the 16S data set considers these two clades as one species (bootstrap value 100). The maximum distance between *P. pustulosa* and *P.* cf. *pustulosa* using the CO1 dataset is less than 9 %; this genetic distance value is lower in comparison to other interspecific genetic distances of Phyllidiella species (lowest minimum values between *P. pustulosa* and *P. nigra* is 9.77 %, but in most cases higher than 10 % table S4, distance matrix *Phyllidiella*), thus not providing adequate evidence for separate entities. Nevertheless, we consider *P.* cf. *pustulosa* as a separate subspecies because of the consistent external colour pattern, despite its broad distribution around Sulawesi. Two chemically analysed specimens (Phpu16Sa-76, Phpu16Sa-79) each represent a branch of *P.* cf. *pustulosa*. LCMS analysis shows very different extract compositions in these two specimens (Figure C-10) but both dominated by sesquiterpene isonitriles and their derivatives, which nevertheless differ from those of the true *P. pustulosa*.

Using the haplotype network analyses of CO1 and plotting chemical results on this network (Fig. 18) highlights discrepancies in the results between the various methods. In the network analysis, *P.* cf. *pustulosa* cannot be considered as an independent species from *P. pustulosa* because several specimens that form a subclade of *P. pustulosa* in the tree actually cluster with *P.* cf. *pustulosa*. This subclade is still unique as it shares at least 18 nucleotide states, thus distinguishing it from *P.* cf. *pustulosa*. However, it does not share unique nucleotide states only with *P. pustulosa*. Chemical studies clearly distinguish *P.* cf. *pustulosa,* but chemistry also renders specimens of this particular subclade (Phpu15Bu-8 and Phpu16Sa52) as part of the *P. pustulosa* clade and not of *P.* cf. *pustulosa*. All these data indicate an ongoing process of speciation within *Phyllidiella pustulosa,* but also indicate the lack of clear species boundaries.

Fig. 9 *Phyllidiella* species and specimens with identifiers. Bars: 10 mm. **1a-d** *Phyllidiella pustulosa*: **a** Phpu15Bu8; **b** Phpu15Bu33; **c** Phpu18Ba12a; **d** Phpu17Ba2. **2a-d** *Phyllidiella* cf *pustulosa*: **a** Phpu18Ko7; **b** Phpu18Ba3; **c** Phpu18Ko8; **d** Phpu18Sm1. **3a-f** *Phyllidiella nigra*: **a** Phpu15Bu7; **b** Phni18Po1; **c** Phpu18Ko9; **d** Phpu18Ba19; **e** Phpu17M3; **f** Phpu17Bu1. **4a-d** *Phyllidiella* sp. a**: a** Phpu18Po2; **b** Phpu18Bu4; **c** Phpu16Sa9; **d** Phpu16Sa1.

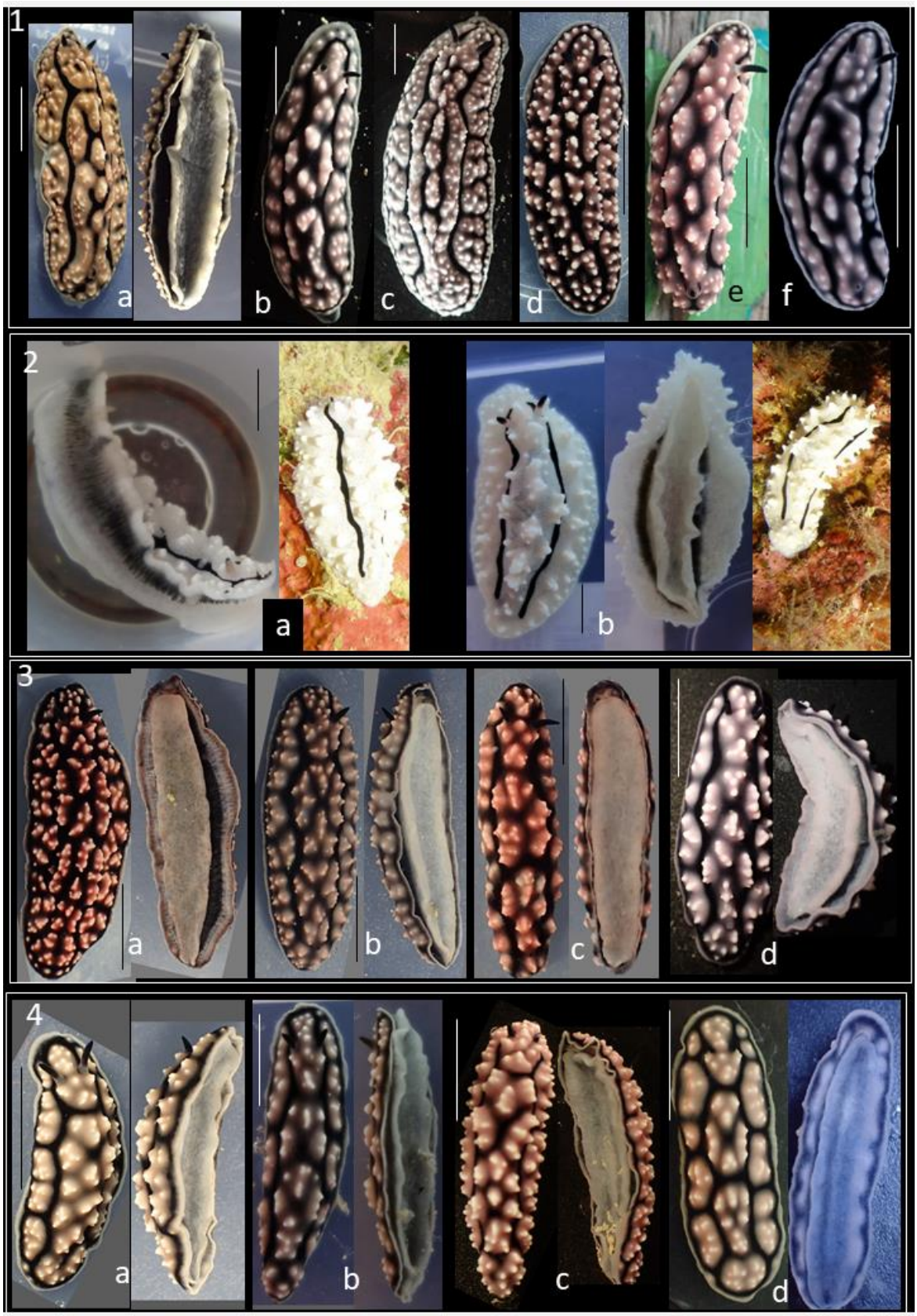

Fig. 10 *Phyllidiella* species and specimens with identifiers. Bars: 10 mm. **1a-f** *Phyllidiella zeylanica* auctt.: **a** Phan16Bu1; **b** Phli18Bu1; **c** Phze18Bu1; **d** Phpu15Bu41; **e** Phpu18Bl4; **f** Phpu15Bu25. **2a-b** *Phyllidiella rudmani*: **a;** Phpi15Bu2; **b** Phpi15Bu5. **3a-d** *Phyllidiella* sp. b: **a** Phpu16Sa25; **b** Phpu16Sa39; **c** Phpu16Sa25; **d** Phpu18Bu5. **4a-d** *Phyllidiella* sp. c subclade 1: **a** Phli16Sa8; **b** Phpu15Bu35; **c** Phli18Ba3; **d** Phpu18Po4.

Fig. 11 *Phyllidiella* species and specimens with identifiers. Bars: 10 mm. **1a-e** *Phyllidiella* sp c subclade 2: **a** Phpu18Ba12; **b** Phan15Bu3; **c** Phli16Sa5; **d** Phli18Ba1; **e** Phpu16Sa11. **2a-f** *Phyllidiella albonigra*: **a** Phpu15Bu5; **b** Phpu16Bu8; **c** Phpu16Bu3; **d** Phpu15Bu21; **e** Phpu16Sa32; **f** Phpu17Ba4. **3a-d** *Phyllidiella* sp. d: **a** Phpu18Po1; **b** Phpu18Ba1; **c** Phpu18Bl5; **d** Phpu18Po3. **4a-d** *Phyllidiella* species complex e: **a** Phpu15Bu28; **b** Phpu16Sa35; **c** Phpu15Bu39; **d** Phpu18Bl2.

Fig. 12 *Phyllidiella* species and specimens with identifiers. Bars: 10 mm. **1a-d** *Phyllidiella hageni*: **a** Phllsp15Bu1; **b** Phru18Ko1; **c** Phpu16Sa30; **d** Phha18Ko1.

Phyllidiella nigra (van Hasselt, 1824)

All 25 collected specimens of *Phyllidiella nigra* (Fig. 9.3a-f) exhibit a greater extent of black background compared to *P. pustulosa* and most clades. Additionally, the mantle margin is black in contrast to the white rim in all subclades of *P. pustulosa* and *P.* cf *pustulosa*. The specimens have irregular, single or coalesced, rounded tubercles distributed all over the mantle. The coalesced tubercles are smaller and usually conical, whereas the single tubercles are relatively large and round. Their colour can vary between white to pink and green. The rhinophores are black; the black anal opening exits from the black notum, sometimes in-between two small tubercles. The foot sole is grey to black. The oral tentacles are black; however, they can also be pale pink with dark grey-black tips or with dark lateral grooves. Originally, *P. nigra* was described as having tall pink-red rounded tubercles irregularly dispersed on the dorsal notum. The oral tentacles were black and the foot sole was grey. We also collected animals that have the rather reticulate black pattern typical for *P. pustulosa* (e.g., Fig. 9.3a), but all our specimens group together with the eight *P. nigra* sequences retrieved from GenBank (see Table 2) with a bootstrap value of 100.

LC-HRMS analysis of *Phyllidiella nigra* (Phpu15Bu-7, Fig. 9.3.a; Phu15Bu-10, Phpu15Bu-18, and Phpu15Bu-19) shows that all four specimens share the same major metabolites present as sesquiterpenoids (Figure C-11). Relatively large extract amounts allowed fractionation and subsequent isolation and structure elucidation of five known sesquiterpenes, i.e., two bisabolene-type isonitriles (Manzo et al. 2004, Iwashima et al. 2002), 10-isocyano-5-cadinen-4-ol (Hirota et al. 1998), and an epimeric mixture of 9-thiocyanatopupukeananes (Yasman et al. 2003). However, the major metabolites were highly volatile or unstable and repeated isolation attempts failed.

Phyllidiella sp. a

The clade *Phyllidiella* sp. a is composed of 29 specimens and contains 26 specimens from our collections (Fig. 9.4) that are externally very similar to the sister clade *P. zeylanica* auctt. (not the original description by Kelaart, 1958 but used by authors in error), described in more detail below. Some specimens of *Phyllidiella* sp. a have tuberculate ridges, and some have small compound and rounded tubercles on the notum. A white band is also present along the mantle margin, typical of most *Phyllidiella* species. The rhinophores are black, but the base is red in nearly all specimens (Fig. 9.4a-d). All members of *Phyllidiella* sp. a have two or three black X-shaped lines on the middle of the dorsal notum. The species is further characterised by a grey to black anal opening on a black part of the notum. The oral tentacles and the foot sole and hyponotum are grey, but have dark grey on the tips or lateral grooves. The clade *Phyllidiella* sp. a is supported by a bootstrap value of 100. However, molecular analyses provide evidence of three subclades within *Phyllidiella* sp. a. Two subclades are composed of our specimens and include one further sequence retrieved from GenBank (Cheney et al. 2014: KJ001310 from Lizard Island; assigned to *P. pustulosa*). Network analyses (Fig. 19) clearly show the distinctiveness of the two clades, and thus confirm species delimitation when using the CO1 data set only.

Two further sequences retrieved from GenBank identified as *Phyllidiella pustulosa* by Wollscheid-Lengeling et al. (2001; AF249232, GBR, Australia) and Valdés (2003; AF430366, New Caledonia) form a sister clade to the two subclades mentioned above. The separation of this sister clade as a distinct species is not supported by our species delimitation test using the 16S data and we therefore consider these two specimens as part of our *Phyllidiella* sp. a. We could not include these two specimens in our haplotype network analyses (Fig. 19) because CO1 data are lacking.

LCMS analysis of the crude extracts obtained from four specimens of *Phyllidiella* sp. a revealed a high degree of variation. We could not identify any unique features except for presence of several non-polar metabolites (retention times more than 20 minutes, Fig. C-13), most probably sesqui- and diterpene isonitriles and sesquiterpene thiocyanates with m/z 264.18 $[M+H]^{+\cdot}$. However, Phpu16Sa9 and Phpu16Sa90 showed a higher similarity to each other than to the two other investigated specimens PhpuSa24 and PhpuSa26, thus reflecting the closer relationship of these two specimens, also according to molecular data (Figs. 13, S1 phylogenetic tree) and network analysis (Fig. 19).

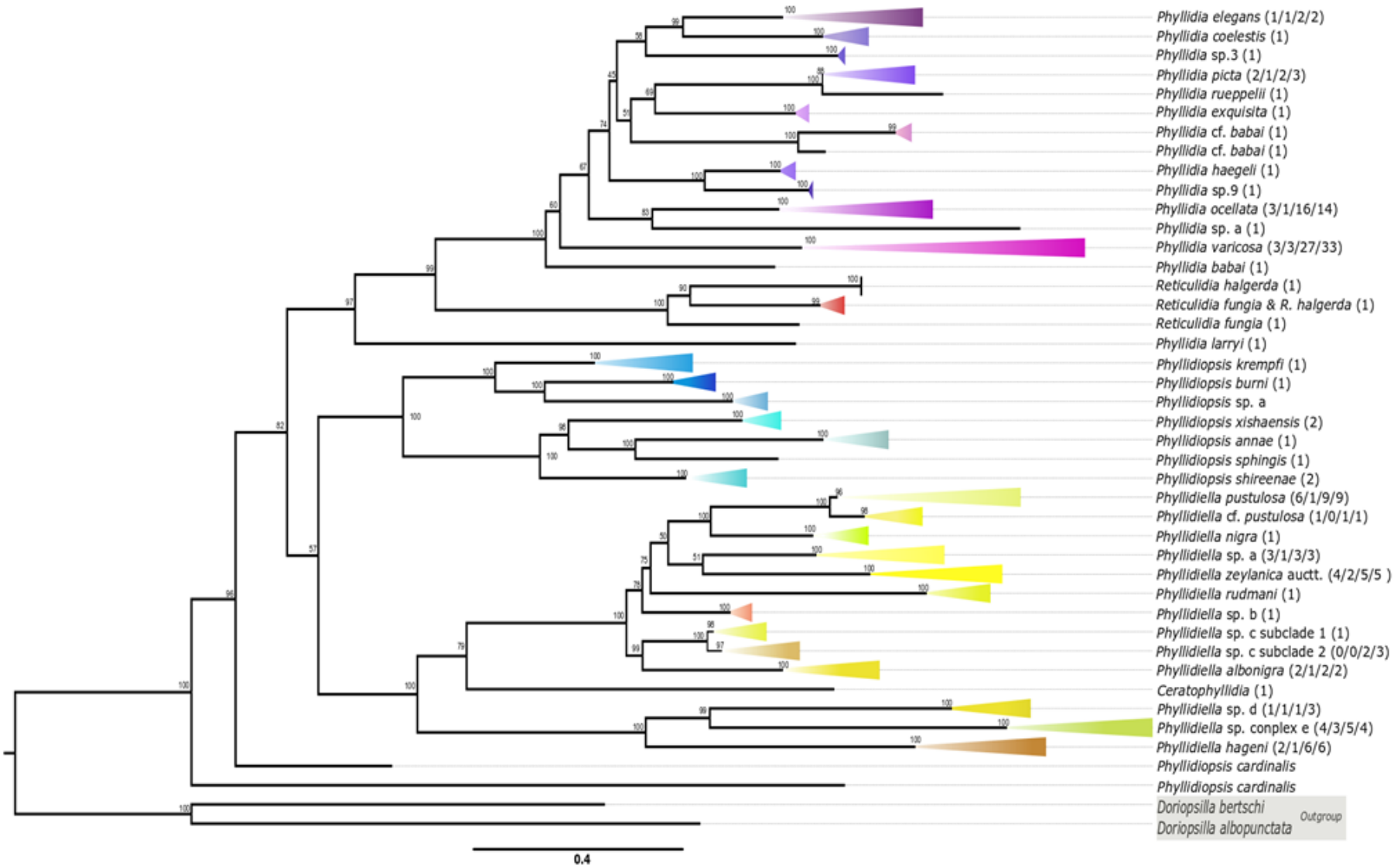

Fig. 13 ML tree of Phyllidiidae based on the concatenated dataset of partial CO1 and 16S sequences. Terminal taxa on species level collapsed. Numbers at nodes are bootstrap values. The genera are marked with the same colours as Figure S1.

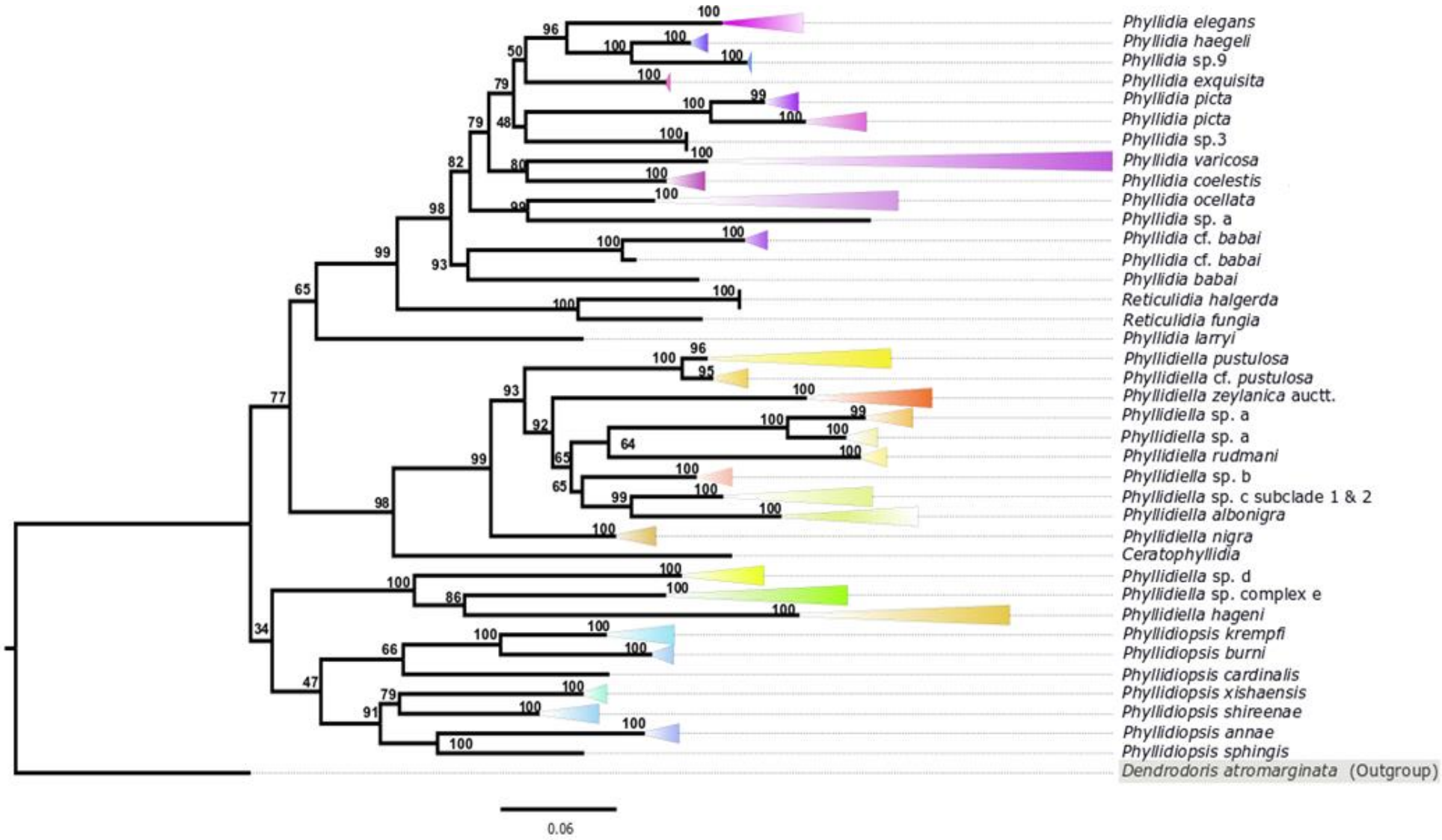

Fig. 14 ML tree of Phyllidiidae based on partial CO1 sequences. Terminal taxa on species level collapsed. Numbers at nodes are bootstrap values. The genera are marked with the same colours as Figure S1.

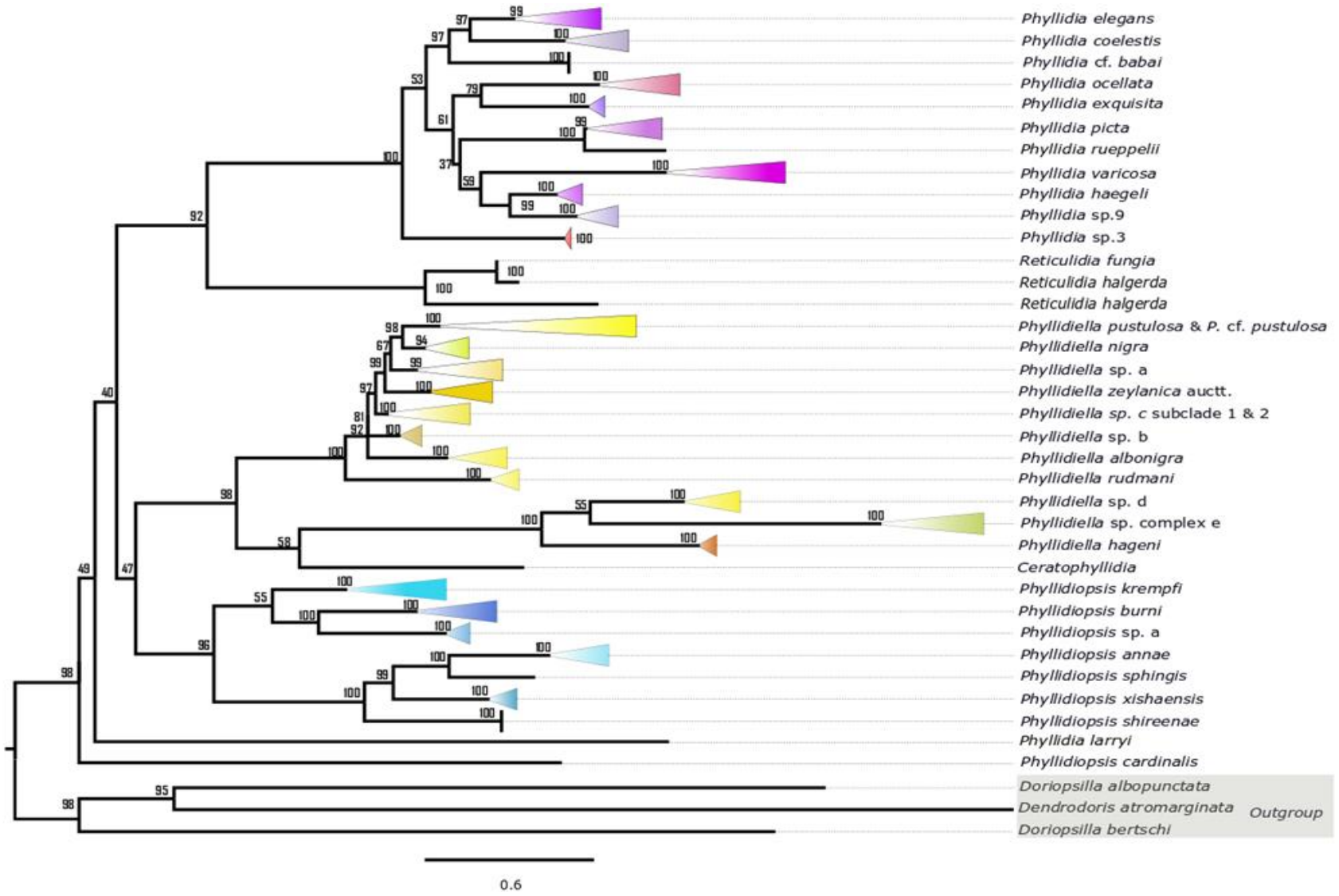

Fig. 15 ML tree of Phyllidiidae based on partial 16S sequences. Terminal taxa on species level collapsed. Numbers at nodes are bootstrap values. The genera are marked with the same colours as Figure S1.

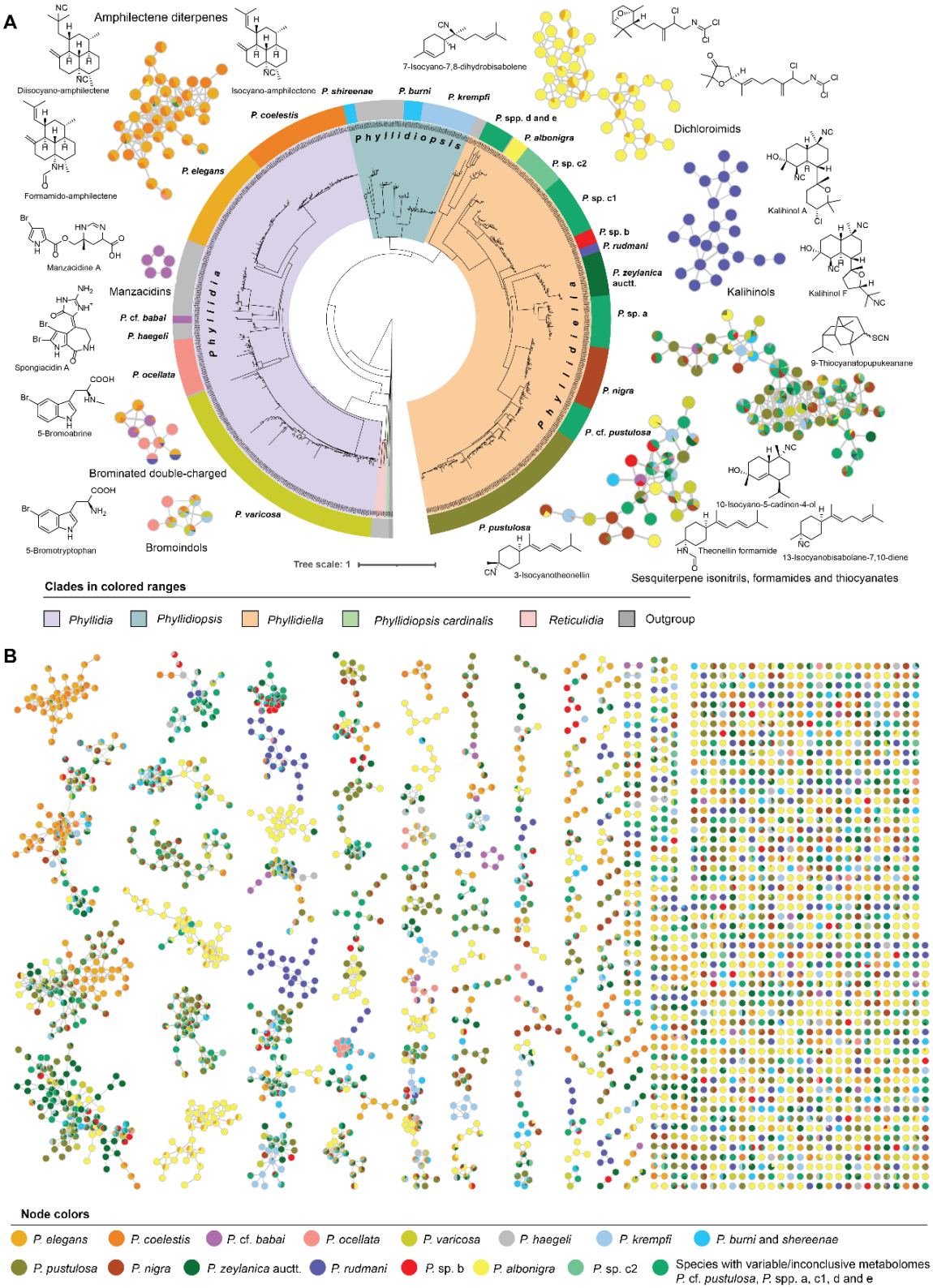

Figure 16. Phylogeny of Phyllidiidae and chemical analysis of 70 individually preserved phyllidiid specimens from three genera (*Phyllidia*, *Phyllidiopsis*, and *Phyllidiella*) and 19 species. **A**. Coloured clusters were obtained with classical or feature-based molecular networking and depict families of secondary metabolites characteristic for certain clades, e.g., brominated alkaloids for the genus *Phyllidia*, amphilectene-type diterpenes for *P. coelestis* and *elegans*, kalihinol-type diterpenes for *P. rudmani*, as well less specific secondary metabolites (sesquiterpene isonitriles and derivatives) that were detected throughout the Phyllidiidae. The chemical structures were established either with NMR after compound isolation, or were assigned basing on HR-LCMS/MS analysis, MarinLit database search, and comparison with reference spectra in the GNPS repository. **B.** Visualisation of the chemical space as present in phyllidiid extracts using classic molecular networking. Each node represents a parent ion (molecule) and based on similarities in fragmentation patterns, the nodes are connected with edges producing clusters/compound families. Nodes are colour-coded according to the legend and are displayed as pie charts when compounds are detected in more than one species.

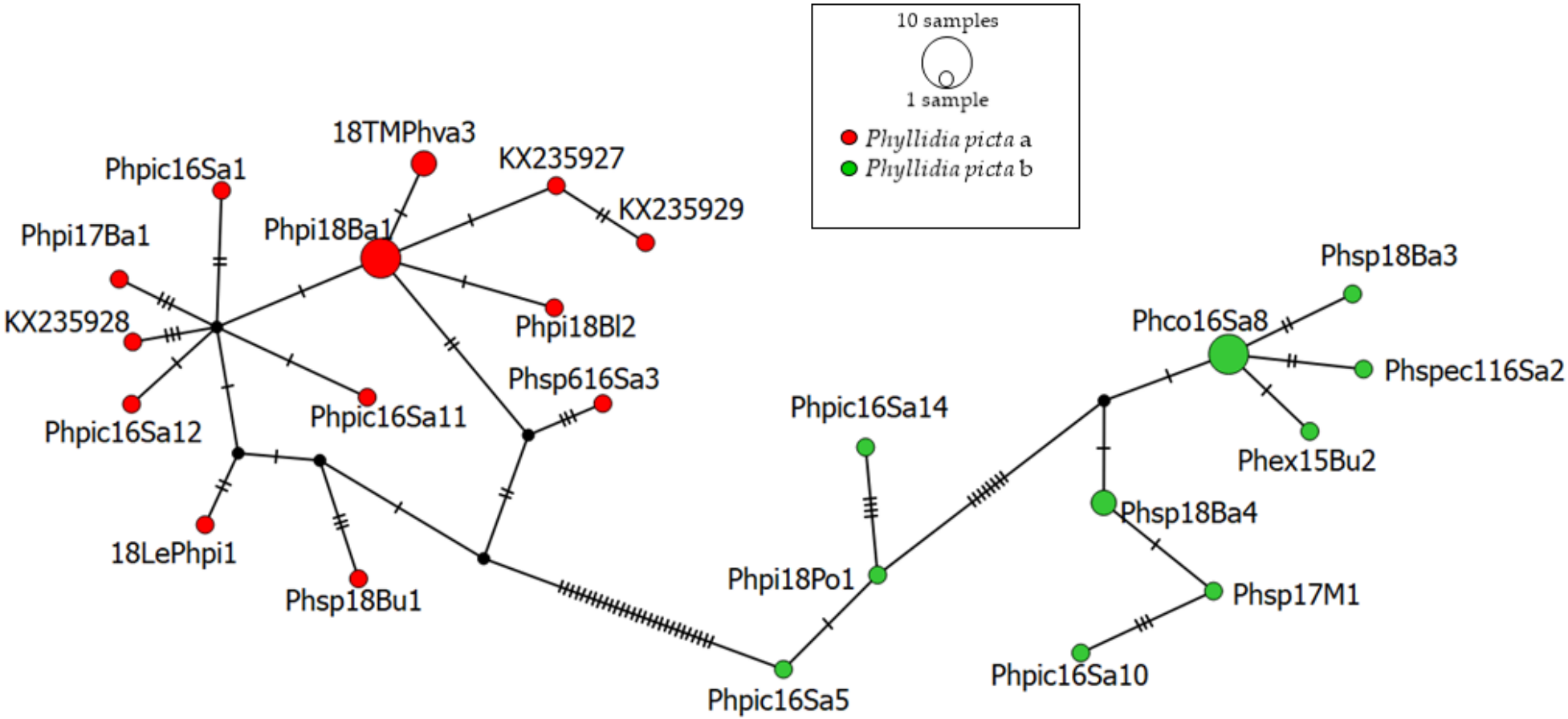

Fig. 17 Haplotype network of *Phyllidia picta* including all CO1 sequences. Note the separation of the two clades by nearly 30 mutational steps. The two clades are coloured red and green, respectively.

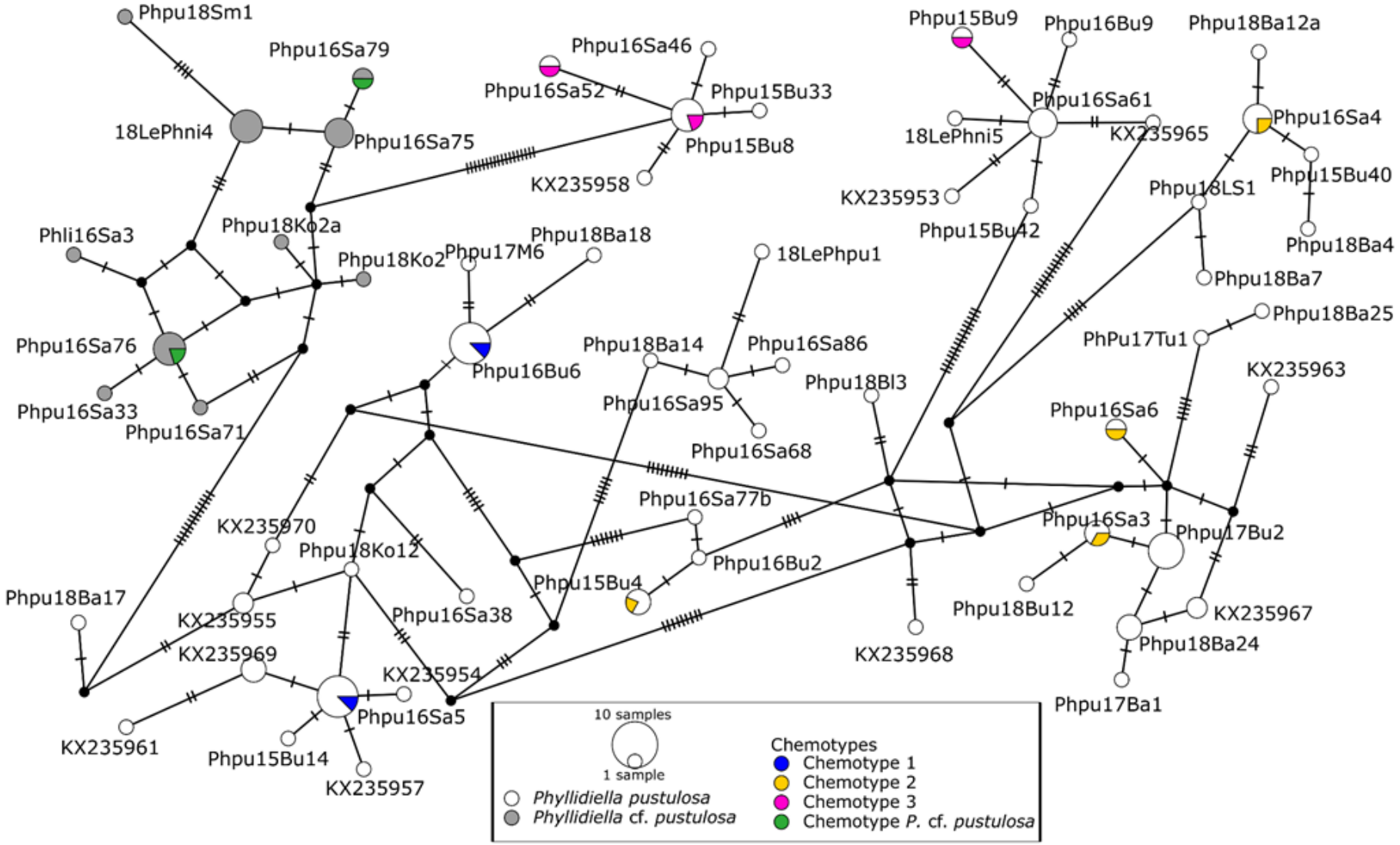

Fig. 18 Haplotype network of *Phyllidiella pustulosa* (white circles) and *P.* cf. *pustulosa* (grey circles) based on the CO1 gene, with the four described chemotypes (coloured circles). Note the closer relationship of specimens with similar chemotypes, except chemotype 3 (purple), which is shared by distantly related specimens.

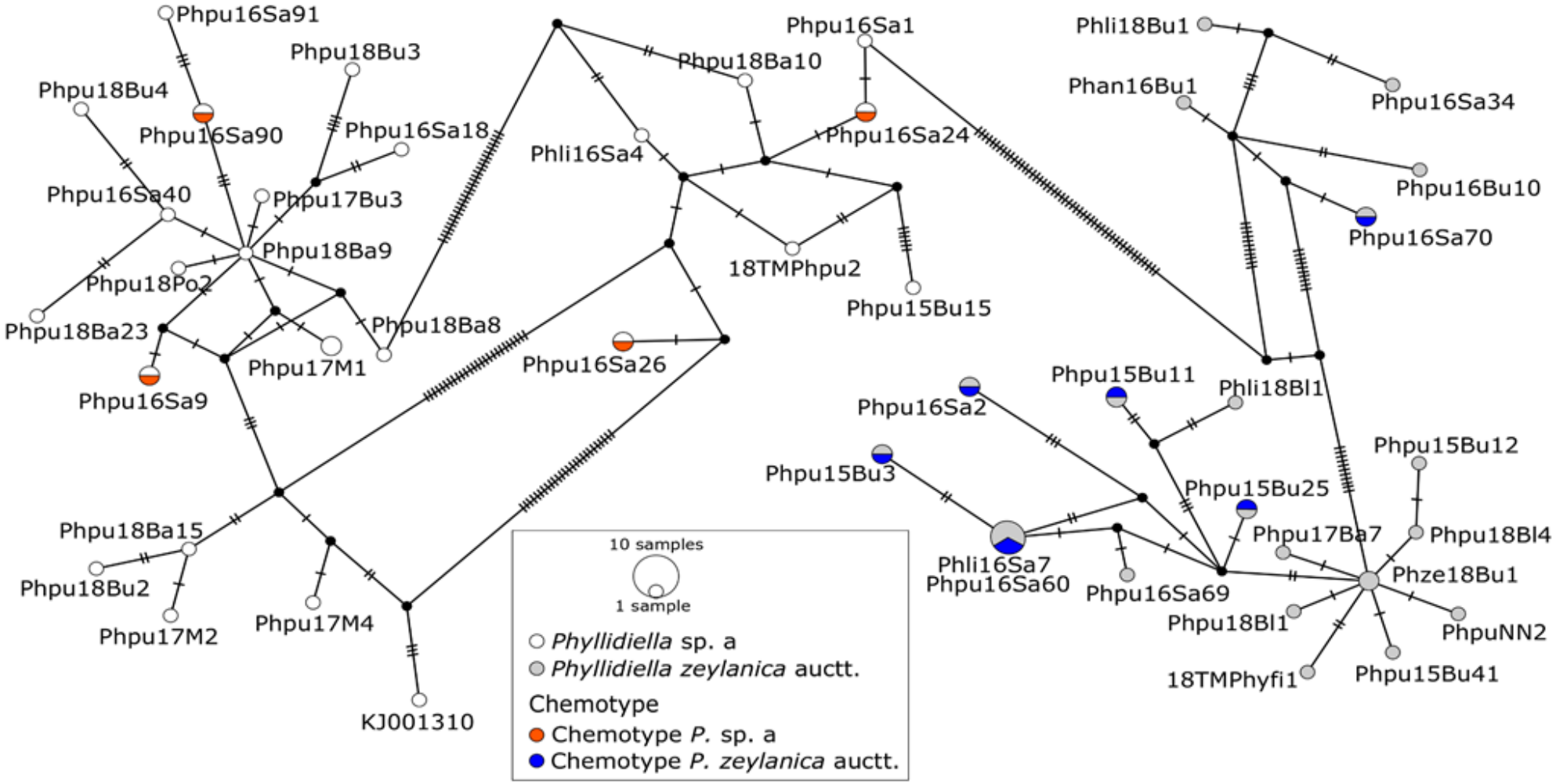

Fig. 19 Haplotype network of *Phyllidiella* sp. a (white circles) and *Phyllidiella zeylanica* auctt. (grey circles) based on the CO1 gene. Note the closer relationship of specimens with similar chemotypes (coloured orange and blue).

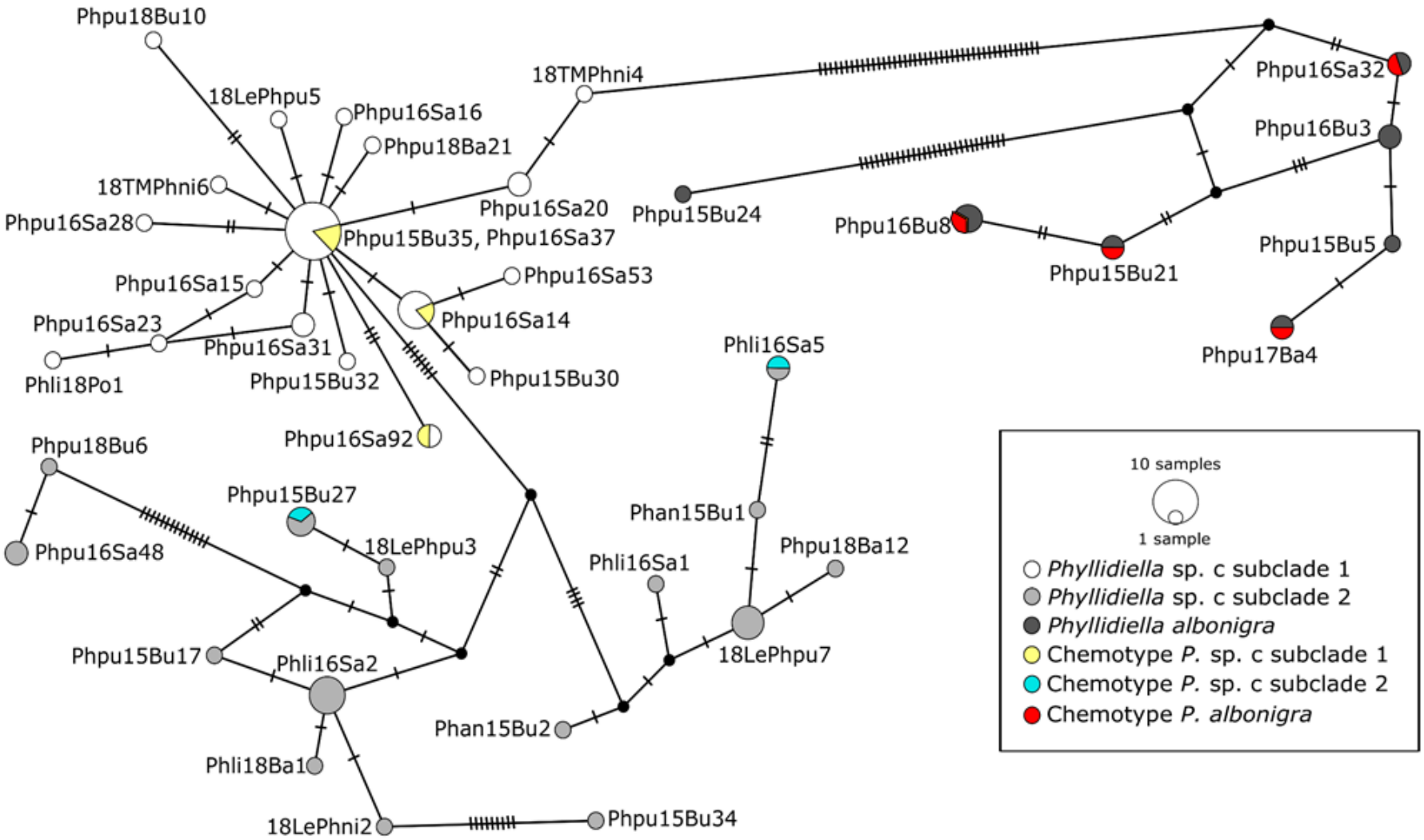

Fig. 20 Haplotype network from concatenated sequences of *Phyllidiella* sp. c with both subclade 1 (white circles) and subclade 2 (light grey circles) and *Phyllidiella albonigra* (dark grey circles), based on the concatenated dataset. Note that different chemotypes (in different colors) are shared by more closely related specimens.

Phyllidiella zeylanica auctt.

Twenty-four specimens group in a clade tentatively named *Phyllidiella zeylanica* auctt. These specimens (Fig. 10.1) vary in their background colour from grey to pink. Many specimens have the tubercles arranged into longitudinal series; however, some specimens display single conical tubercles which can vary in size (e.g., Phli18Bu1, Phze18Bu1, Phpu15Bu41, Phpu18Bl4, Phpu15Bu25; Fig. 10.1a-f). A few have small rounded compound tubercles. The black lines can be arranged in very different ways, forming a reticulate pattern, but the pattern is basically a linear one surrounding larger central tubercular fields (Fig. 10.1a-f). The pink/white margin is followed by a black submarginal line. The rhinophores are black with white base. In contrast to many other species, the anal opening is large in all specimens, placed on grey to black ground, and surrounded by a ring of tubercles; it has a pink outer opening and black on the inner surface of the anal opening. By this unique character, it can be easily distinguished from *Phyllidiella* sp. a, which is characterised by a grey to black anal opening on a black area of the notum. The oral tentacles are white or pale pink with faded grey on the lateral grooves. Their outer appearance matches the description of *Phyllidiella zeylanica* of Brunckhorst (1993), but not the original description of *P. zeylanica* by Kelaart (1859: type locality Sri Lanka, figured by Eliot 1906: Pl. 52, Fig. 10). The original description clearly states that there are two black rings and one central black stripe. The two rings are narrower than the central stripe with the outermost ring being the narrowest one. Burn (1970) also described and illustrated specimens of *P. zeylanica* (sensu Kelaart) from Gulf of Kutch (India) with three black circular bands on the notum; *P. zeylanica* (sensu Kelaart) was also described and illustrated by Yonow (2012 and references therein) based on specimens from the Maldives, Seychelles, Thailand, and Chagos with two or three circular lines. Therefore, *P. zeylanica* was certainly misidentified in several publications, including Brunckhorst (1993), Dominguez (2007), and Gosliner et al. (2015, 2018).

Unfortunately several species were synonymised with *P. zeylanica* by Brunckhorst (1993) based on black lines which coalesce in the posterior part of the body and pink compound tubercles which form longitudinal ridges and will need re-examination in light of misidentifications and putative synonymies: *P. catena* (Provot-Fol, 1956), *P. seriata* (Pruvot-Fol, 1957), *P. empelia* (Yonow, 1984), and *P. rosans* (Bergh, 1873). According to Brunckhorst (1993) and Yonow et al. (2012), *P. rosans* clearly differs from *P. zeylanica* by having smooth continuous concentric ridges instead of distinct tubercles forming high ridges. The animals in our clade are also not like *Phyllidiella empelia* (Yonow 1984), varying in

details such as the numbers of black rings around the lateral mantle (which may be an ontogenetic variation), whether or not the black lines are united around the anal opening, the position of the black line surrounding the rhinophores, the presence of a central black line or band, and the anterior tubercles directly surrounding the rhinophores which may form an angular rectangle-like line instead of a curved line. *Phyllidiella empelia* (Sri Lanka) and *P. catena* Pruvot-Fol, 1956 (Mayotte, Mauritius) are species only recorded from the Indian Ocean while the type locality of *P. seriata* Pruvot-Fol, 1957 is not known; however, these three species were synonymised with *P. zeylanica* auctt. by Brunkhorst (1993). Thus we cannot assign this clade to any of the above species without molecular evidence of these nominal Indian Ocean species. We urgently need more investigation involving these species to clarify the difficult taxonomy of this group. Our molecular analyses render *Phyllidiella zeylanica* auctt. as sister taxon to *Phyllidiella* sp. a with a very low support value of 51. This result reflects the contradicting results in the single gene analyses. A joined network analysis of *Phyllidiella* sp. a and *P. zeylanica* auctt. (Fig. 19) also clearly shows the distinctiveness of these two species with 50 separating mutational steps, despite their similar colorations.

Crude extracts of seven *P. zeylanica* auctt. specimens were analysed with LCMS. The chemotypes found showed a high degree of variation (see Figure C-12) with dominant peaks attributable to sesquiterpene isonitriles by mass spectrometry. Careful inspection of the feature-based molecular network pointed out a few features that were detected in all investigated *P. zeylanica* auctt. specimens, e.g., ions with m/z 262.16 (M+H) and retention times of 14.6 min and 15.8 min match the accurate masses of bicyclic unsaturated isothiocyanate sesquiterpenes (see Fig. Sxx) reported from various sponges (Alvi et al. 1991, Adinolfi et al. 1977). These features are very rare among the 70 chemically analysed phyllidiids and were detected in only five other specimens belonging to three genera, *Phyllidia varicosa*, *Phyllidiopsis krempfi, Phyllidiopsis burni,* and *Phyllidiella* sp. c subclade 2 in trace amounts. Small specimen size and resulting low extract amounts hindered us from isolation and unambiguous structure elucidation of the extract constituents.

Phyllidiella rudmani Brunckhorst, 1993

Two specimens in our collection could be confidently assigned to *P. rudmani* based on the pale white to bright pink dorsum with two characteristic narrow black lines on the lateral mantle (Fig. 10.2a-b). These lines do not unite in the anterior or posterior parts of the body in our two specimens. The isolated tubercles in-between these two lines are tall and multi-

compound; a few small rounded single tubercles occur between these tall complex tubercles. The rhinophores are pink on the basal half and black on the upper clavus. A pink anal opening looks like a small tubercle lying in-between the two black lines. The hyponotum is white to bright pink whereas the gill lamellae are black, only interrupted by the pink genital opening. The oral tentacles are entirely pink. Two sequences identified as *P. rudmani* and *P. lizae* from GenBank grouped with our specimens (bootstrap value 100): one specimen from Raja Ampat, Indonesia (336589) included in the analyses by Stoffels et al. (2016) looks like our specimens of *P. rudmani.* No information on the external features is available for the *P. lizae* specimen from Cheney et al. (2014) collected from Lizard Island, Australia.

Crude extract of one *Phyllidiella rudmani* specimen (Phpi15Bu-2, Fig. 10.2a) was submitted to LCMS analysis. A number of unique clusters in the molecular network (see Figure Sxx) indicated secondary metabolites that are found exclusively in this specimen. Careful inspection of the mass spectrometry data and marine natural product database search (Antimarin) allowed assignment of the major constituents of the complex extract (see Figure C-14). Unlike the other chemically analysed Phyllidiidae, *P. rudmani* possesses kalihinol-type diterpenes (see Fig. 16 and Sxx) known from sponges *Acanthella cavernosa* and *A. pulcherrima* (Chang et al. 1984, Patra et al. 1984, Braekman et al. 1994, Rodriguez et al. 1994, Hirota et al. 1996, Wolf and Schmitz 1998 as *Phakellia*).

Phyllidiella sp. b

Eight specimens are characterised by a range of external appearances (Fig. 10.3a-d); no specific or distinguishing character permits an unproblematic identification. The notum of the animals possesses compound tubercles which are rather pointed. In general, the black lines form a reticulate pattern with X-shaped black lines in the middle of the tubercular groups on the central notum. The rhinophores are black with a white base, and low pink-rimmed openings. Most specimens have a pink foot sole, but one specimen has a foot sole that is grey in the middle and pink on the sides. Another (Fig. 10.3d) has rings of alternating dark grey and light grey around a darker grey central band on the sole. The oral tentacles are pink with dark grey apices and/or lateral grooves. Specimen Phpu16Sa25 (Fig.10.3a) has dark pink oral tentacles. The clade is confirmed by a bootstrap value of 100.

Of the eight specimens in *Phyllidiella* sp. b, five were analyzed with LCMS. Despite a high level of external diversity, the observed chemical profiles were very similar (see Figure C-15), thus strengthening molecular results uniting these specimens. Many of the major peaks

observed in UV-chromatograms could be attributed to sesquiterpene isonitriles and formamides. Additionally, a prominent peak with the retention time of 19.3 min. that can be attributed to a diterpene alcohol with five degrees of unsaturation (*m/z* values 289.249 $[M+H]^+$ and 271.239 $[M-H_2O]^+$) was detected in all analysed *Phyllidiella* sp. b. This feature was also found in *P. zeylanica* aucct. (see above) and in one specimen of *Phyllidiella* sp. c subclade 2 (see below).

Phyllidiella sp. c

Phyllidiella sp. c consists of two different subclades (subclades 1 and 2) which are genetically not considered separate species, but are nevertheless morphologically different and described below as two separate entities.

Phyllidiella sp. c subclade 1 comprises 32 specimens. The black pattern of the specimens consists of two longitudinal lines and one outer ring (Fig. 10.4a-d). The obvious margin is pink. The inner lines unite with the outer one anteriorly in front of the rhinophores and posteriorly, and are connected to each other by several black X-shapes. There is no black band between the rhinophores, and the tubercular clusters in front of and behind the rhinophores, are continuous. A number of pink compound tubercles lie between these black lines forming short ridges arranged in clusters in the central part of the notum and in the lateral parts. The long pointed rhinophores are black with white to pink or red bases. The anal opening arises on the black part of the notum. The hyponotum is grey with pink-white patches corresponding to the dorsal tubercles and ridges, the foot sole is white to grey and often with two distinct darker longitudinal lines; the oral tentacles are whitish pink with black along the lateral grooves. Some of these characters are similar to parts of the original description of *P. granulata,* but future molecular analyses including specimens from the type locality are needed.

All 24 specimens of *Phyllidiella* sp. c subclade 2 have one outer black ring and two lateral black lines (Fig. 11.1), similar to subclade 1. The outer ring and the inner longitudinal lines unite anteriorly and posteriorly, similar to subclade 1. The inner lines also connect to each other transversely, forming two central tubercular areas with a black mark in the middle of these regions, not X-shaped as in subclade 1. The pink anal opening lies in a pink field, not in a black area as in subclade 1. There is a very obvious white-pink margin. In the central part of the notum, the compound pink tubercles are usually arranged in a circular form, with only four specimens exhibiting tubercles arranged in elongate but short ridges interrupted by black

and smooth areas (Fig. 11.1e). The compound lateral tubercles are arranged in curved or straight series, and separated by black lines. The rhinophores are predominantly black with a white base but in Phpu18Ba12 (Fig. 11.1a) the rhinophores have a red base. The hyponotum is pink with grey patches caused by the dorsal coloration showing through, the gills are grey to black, the foot sole is white to pinkish grey, and the outer side of the oral tentacles are black.

The specimens somewhat match the original description of *P. melanocer*a, but our specimens have a distinct black submarginal line. There are also vague similarities with *P. annulata* (Brunckhorst 1993) but this species was described with the absence of a white margin and an overall black coloration with a high number of pink rings (4–14) on the notum (Brunckhorst 1993). Re-investigated type material of *P. annulata* (Fig. 3.1-5) indicates similarities to the description of the holotype and paratype (only) of *P. melanocera* (Fig. 2.6) in all other respects. In *Phyllidiella* sp. c all members from both subclades 1 and 2 lack the black stripe between the rhinophores that is characteristic for most *Phyllidiella* species, and therefore the pale pink tubercular field is continuous. The anal opening also is not in a black area as usual, but lies between two compound tubercles in a pink to grey field.

Molecular evidence, including haplotype network analyses (Fig. 20), does not strongly separate *P.* subclade 2 from *P.* subclade 1. Both clades are united with a bootstrap support of 100. Species delimitation tests also reject subclade 2 as a separate species from subclade 1, except when applying bPTP algorithms. Within the network analysis based on CO1, only seven mutational steps are present between the two clades.

Four members of *Phyllidiella* sp. c subclade 1 (Phpu15Bu-35, Fig.10.4b; Phpu16Sa-14, Phu16Sa-37, and Phpu16Sa-92) and two members of subclade 2 (Phli16Sa-5, Fig. 11.1c, and Phpu15Bu-27) were chemically investigated. Metabolomes found in both subclades exhibited a high degree of variation rendering *Phyllidiella* sp. c "difficult" from a chemical point of view, thus reflecting the discrepancy when comparing morphological and molecular data (see Figures C-16 and C-17). The crude extracts contained, like many other phyllidiids, various sesquiterpenes with usual functionalities, i.e., isonitriles and formamides. We consider two major sesquiterpene isonitriles with retention times of 18.2 min and 18.4 min as a unifying chemical feature of subclade 1. Even though these metabolites are found in varying amounts in all four subclade 1 specimens, they could not be otherwise detected in the two analysed specimens of subclade 2. Crude extracts of both members of *Phyllidiella* sp. c subclade 2 had different chemical profiles (see Fig. C-17) which also differed from subclade 1. Interestingly,

the chemical profile of Phpu15Bu-27 (subclade 2) was identical with those found in one specimen of *Phyllidia coelestis* (Phco15Bu-6) and one specimen of *P. elegans* ((Phel15Bu-8). All three specimens did not contain the usually observed sesquiterpenes, but several amphilectene-type sesquiterpenes with an unknown major metabolite with an m/z 404.187 $[M+H]^+$. In contrast, the extract of the other *Phyllidiella* sp. c subclade 2 specimen (Phli16Sa5) is composed of sesquiterpene isonitriles and corresponding formamides with a major compound eluting at 18.1 min in LCMS analysis. This compound is probably 7-isocyano-7,8-dihydrobisabolene, also isolated from a *P. krempfi* specimen. Both *Phyllidiella* sp. c subclades seem to lack obvious chemotaxonomic markers other than individual isonitrile compositions and thus are difficult to clearly separate from other phyllidiids. This high degree of variability makes chemical characterisation of *P.* sp. c on subclade level, as well as on species level, inconclusive and do not contribute to a taxonomic solution

Phyllidiella albonigra Quoy & Gaimard, 1832

All nine members of *Phyllidiella albonigra* (Fig. 11.2) are more elongated in shape than the other clades, with rather pointed ends and a greater proportion of black background to the mantle than other species except *P. nigra*, and a narrow pink/green/white band along the mantle margin. The dense complex tubercles are small and compound, arranged in small clusters on the notum and their colour varies from pink to green and grey. The rhinophores are black with a white base. The black anal opening arises on black background. This pattern most resembles the external appearance of the type material of *P. albonigra* (type locality Tonga islands): the original description depicts a specimen that is also rather black with irregular clusters of small tubercles. We therefore remove *P. albonigra* from synonymy with *P. pustulosa* (see Brunckhorst 1993) and reinstate it here. *Phyllidiella albonigra* is clearly separate from other *Phyllidiella* species (bootstrap value of the clade is 100). Minimum genetic differences varied from 10 % from *Phyllidiella* sp c up to 17 % from *P. rudmani*. Its sister taxa relationship with *Phyllidiella* sp. c is also supported in the tree reconstruction based on the concatenated data set and CO1 (bootstrap 99), but is not resolved in the 16S analysis. Haplotype analysis of this species, together with *Phyllidiella* sp. c provides further evidence of its distinctiveness (Fig. 20).

The chemical analysis of *P. albonigra* was described in detail in a recent publication (see Bogdanov et al. 2020). We were able to isolate very rare dichloroimidic sesquiterpenes and their derivatives from one specimen (Phpu15Bu-21) of this clade. These dichloroimids

were identified as the major extract constituents in three out of four analysed members of *Phyllidiella albonigra*. Importantly, all chemically studied specimens were collected at different localities around North Sulawesi during the course of three years. Whereas the chemical profiles (UV chromatograms obtained during LCMS analysis) of two specimens, i.e., Phpu15Bu-21 (Fig. 11.2d) and Phpu16Sa-32 (Fig. 11.2e), were identical, the chromatogram of a third specimen (Phpu17Ba-4, Fig. 11.2f) was very different at first glance (see Figure C-18). However, after obtaining pure compounds from the in-depth analysed specimen, the different metabolomes could be explained by the presence of degradation products of the chemically unstable dichloroimids. Intriguingly, no dichloroimids or derivatives thereof could be detected in the crude extract of the fourth analysed specimen Phpu16Bu-8 (Fig. 11.2b), which was smaller (30 mm) compared to the other 3 specimens (38 to 80 mm). Chemical analyses thus provide further evidence that *P. albonigra* is a separate species and can be distinguished from its closest relative *Phyllidiella* sp. c. (Fig. 18).

Phyllidiella sp. d

The ten specimens united in *Phyllidiella* sp. d (Fig. 11.3a-d) are externally quite different from each other. They have a notum covered by clusters of low, conical tubercles surrounded by a network of narrow black lines. Sometimes, these lines form an X-shape on the central part of the notum. In one specimen (Fig. 11.3c), the tubercles in the central part are arranged in a hexagonal pattern. There is a black line just anterior to the rhinophores. A white-pink mantle margin is present in all specimens. Each rhinophore arises from a compound pink to white/grey tubercle and is surrounded by a distinct sheath. The oral tentacles are white to pink with black tips. Two of our specimens (Phpu18Po3, Fig. 11.3c and Phpu18Bl5, Fig. 11.3d) resemble specimens of *Phyllidiella nobilis* illustrated from Thailand by Bergh (1902). This clade is supported by the highest bootstrap values in all analyses (100).

Phyllidiella species complex e

This clade comprises a species complex of probaby four different species, represented here by one or two specimens each (Fig. 11.4a-f). We could not find any external characters that are unique to this clade, which most resembles *Phyllidiella* sp. d. Nevertheless, based on our molecular analyses, this clade is a distinct evolutionary lineage supported by a bootstrap value of 100, as are all four species, confirmed by the species delimitation tests using the 16S data set. To assign any of these species to a certain nominal species is not yet possible due to the low number of specimens, and intraspecific variability cannot be assessed either.

One member of this species complex (Phpu16Sa50) was analysed chemically. The chemical profile is dominated by sesquiterpene isonitriles. Its overall composition resembles most closely that found in *Phyllidiella nigra*. However, thiocyanates, typical for many phyllidiids, were not detected in the extract of Phpu16Sa50. The extract lacks distinctive chemotaxonomic markers and since it is the only investigated specimen of the *Phyllidiella* species complex sp. e, further metabolomic studies are required to characterise this evolutionarily distinct clade.

Phyllidiella hageni Fahrner & Beck, 2000

Five specimens of *Phyllidiella hageni* collected at several localities in Sulawesi (Fig 12.1a-d) show the species' diagnostic external appearance as described by Fahrner & Beck (2000) and subsequently by Domínguez et al. (2007). However, the background is green in our material and not pink, and the oral tentacles are white without black tips; however, in all other colouration, our animals match the original and subsequent descriptions and illustrations. Two black longitudinal lines run the length of the mantle. Tubercles are rather flat and single, but sometimes clustered together. Black lines connecting the longitudinal lines meander between these groups of tubercles. A thin black line runs around the mantle margin. The rhinophores are black with a bright green base arising from a low rhinophoral sheath on green notum. The green anal opening is disguised between the green tubercles on green ground. The hyponotum, gills, foot sole, and oral tentacles are pale white (as described by Domínguez et al. 2007) to bright green. Fahrner & Beck (2000) based their description on three specimens from Lombok and Domínguez et al (2007) described a further two specimens from Papua New Guinea in detail, both with detailed figures. Differing, the four specimens in our collection have a green background instead of the pink colouration as originally and subsequently described in the living specimens and lack the black tips of the oral tentacles of the original description. Some specimens with simple black lines, also typical for *P. rudmani*, can be distinguished from the latter by their tubercles: the tubercles of *P. rudmani* are tall and sparse, while those of *P. hageni* are low and densely distributed. One specimen from Raja Ampat (Stoffels et al. 2016: Fig. 10i, KX235944) was assigned to *Phyllidiopsis fissuratus*, but clusters within our *P. hageni* sequences and was probably misidentified. Despite the very distinctive fused oral tentacles in *Phyllidiopsis*, which are separate in *Phyllidiella*, these can contract to a smaller unit during fixation, thus leading to generic misidentification. Fahrner & Beck (2000), Domínguez et al. (2007), and Stoffels et al. (2016 as *P. fissuratus*, RMNH.Moll.336590) described *P. hageni* as having a pink dorsum, but *P. hageni* specimens

from our expedition groups with the specimen from Stoffels et al. (2016, RMNH.Mol.num 336590). Pink notal tubercles for *Phyllidiella hageni* should no longer be a defining character due to variability of dorsal colour. The clade is supported by a bootstrap value of 100 and shows the highest interspecific generic distances between 15 to 25 %.

Ceratophyllidia Eliot, 1903

Ceratophyllidia is only represented by one sequence obtained from GenBank (Hallas et al. 2017, voucher CASIZ181247 from Beatrice, Philippine Islands), and groups within the genus *Phyllidiella*. The genus can be easily distinguished from *Phyllidiella* species by the shape of the swollen tubercles which are stalked (Brunckhorst 1993). Because of the lack of data for this genus within our data set, we cannot assess this unexpected relationship of *Ceratophyllidia* with *Phyllidiella* in detail, assuming generic identification is correct.

Discussion

Phylogenetic results on the genera and species in our study

Taxonomy and systematics of the Phyllidiidae has been considered a difficult issue mainly due to the high number of very similarly coloured species, the lack of clearly distinct morphological characters, and/or the lack of proper species descriptions (Brunckhorst 1989a, 1989b; Stoffels et al. 2016; Yonow 1986; 1996). Broader comparative morphological studies with cladistic analyses have led to the synonymisation of the previously accepted genera *Fryeria* Gray, 1853 and *Reyfria* Yonow, 1986 with *Phyllidia*, leaving the family with six accepted genera (*Phyllidia, Phyllidiella, Phyllidiopsis, Ceratophyllidia,* and *Reticulidia).*Few studies on the Phyllidiidae have included molecular data (Valdés 2003; Aliqudah et al. 2015; Hallas et al. 2017) and usually these studies only included a few sequences, with a focus on deeper phylogenetic relationships, and it is not clear whether identifications were correct or if the sequence identities in NCBI are reliable, as shown in this work. Stoffels et al. (2016) were the first to provide deeper insights into intrageneric relationships and to provide information about some problematic groups, e.g., *Phyllidiella pustulosa,* which is now known to be represented by several clades. The provision of images of their specimens made the interpretation of sequences assigned to their clades very valuable, and we therefore follow their example and provide images for many specimens, especially those with aberrant colouration. Stoffels et al. (2016) discussed the relationships of some phyllidiidid genera, comparing their CO1 results with a few 16S results obtained from Valdés (2003) and Cheney

et al. (2014) as well as on morphologically based cladograms by Brunckhorst (1993) and Valdés (2003). Our trees are based on both mitochondrial genes and confirm the synonymisation of *Fryeria* with *Phyllidia* as per Gosliner (2002), and the sister-taxon relationship of *Phyllidia* with *Reticulidia*, but our trees show a sister-taxon relationship between *Phyllidiella* and *Phyllidiopsis* with low bootstrap support, in contrast to previous studies (Valdés, 2003; Cheney et al. 2014; Stoffels et al. 2016). Furthermore, the only sequences assigned to the type species of the genus *Phyllidiopsis, P. cardinalis* Bergh, 1876, does not group with all other *Phyllidiopsis* species, rendering the genus paraphyletic. If we interpret the identification of the sequences *P. cardinalis* by Valdés (2003; AF430367, New Caledonia) and Cheney (2014; KJ001308, Queensland, Australia) as an error, we do not need to reconsider all other *Phyllidiopsis* species as members of a different genus. However, if their identifications were correct, then all other *Phyllidiopsis* species need to be assigned to a different/new genus. To clarify the relationships between genera, more data on *Ceratophyllidia* and *Phyllidiopsis*, especially properly identified type species, need to be included and analysed with nuclear genes. Support values for species groups are also often very low, and therefore relationships within the genera are not reliable at present. Similar results were recently obtained by Layton et al. (2018, 2020) for *Chromodoris* within the Chromodorididae. These authors suggested that their study group experienced a recent radiation, thus lacking a clear diversification of the analysed gene. This might be the same case for the Phyllidiidae and here, we aim to clarify some species identities in order to reveal the diversity of phyllidiidids in North Sulawesi, and we do not imply any relationships between the species.

Species identification

For separating species, the barcode gap of a certain percentage of genetic differences between the species is considered a valuable boundary for identification in many organism groups (Hebert et al. 2003; Pentinsaari et al. 2016; Puillandre et al. 2012). In our study, intrageneric minimum values between the species were similar in the genera *Phyllidia, Phyllidiopsis,* and *Phyllidiella*, with minimum values of 8.82 % differences between *Phyllidia exquisita* and *P. haegeli*, 8.52 % between *Phyllidiopsis krempfi* and *P. burni*, and 8.83 % between *Phyllidiella* sp. b and *Phyllidiella* sp. c subclade 1. Maximum interspecific values were up to 25 % with the highest value of 25.25 % between two *Phyllidiella, P. rudmani* and *Phyllidiella* sp. complex e. Intraspecific genetic variability was lower than 4 % for most species/clades, except *Phyllidia picta* that had a maximum distance value of 8.63 %, *P.* cf

babai (5.49 %), *P. ocellata* (5.76 %), *P. varicosa* (9.1 %), *Phyllidiella pustulosa* (9.87 %), *Phyllidiella zeylanica* auctt. (5.48 %), *Phyllidiella* sp. c subclade 2 (6.18 %), and *Phyllidiella* sp. e (7.83 %) (Table S4 with distance matrices).

By including similarities in nucleotide composition, which is taken into consideration in the haplotype network analyses, additional information is provided beyond the genetic distance. This can help in distinguishing species, especially when intraspecific genetic variability is broad and maximum values are close to or even overlapping with interspecific genetic distances. This is the case in, for example, the species *Phyllidia picta*, where intraspecific genetic variability was up to 8.63 % in our study (Table S2). The haplotype network analysis (Fig. 17) confirmed the existence of two different clades which seem to be separated by 28 mutational steps. Problematic cases mainly appeared within the genus *Phyllidiella*. The *P. pustulosa* clade showed a maximum of 9.87 % genetic difference within the clade and minimum interspecific distance to *P.* cf. *pustulosa* of only 5.5 % (Table S4). This value might still be considered as a separate species, as indicated by the species delimitation tests when only using CO1 gene; however, these tests split *P. pustulosa* into many more species. The haplotype network analyses clearly show that these subclades share nucleotide states and thus contradict the species concept based on CO1 only. One group which clusters here with *P.* cf. *pustulosa* (and therefore differs from our phylogenetic analysis) still shows a typical chemistry observed in *P. pustulosa*. This specific situation might be caused by hybrid introgression and analysis of nuclear genes are necessary for further insights into the ongoing speciation processes.

With regard to the closely related *Phyllidiella* sp. c subclades 1 and 2, the maximum intraspecific genetic variability was 1.45 % and 1.27 %, respectively; however, the minimum interspecific genetic distance between these two clades was only 2.93 %. This value is far below the typical interspecific genetic distances observed between the other *Phyllidiella* species, which was a minimum of 8.83 % between *Phyllidiella* sp. b and *Phyllidiella* sp. c subclade 1. This indicates that both subclades 1 and 2 genetically belong to the same species, despite the differences in their external appearances and metabolomes. Looking at the minimum values between the various species, the largest distance was observed between *Phyllidiella rudmani* and *Phyllidiella* species complex e.

Using molecular data in different methodological approaches (phylogenetic as well as haplotype network analyses), and additionally using chemical compounds to characterise clades, we were able to identify variations within species (clades) and show that colour

patterns, thought to be species specific, can occur repeatedly in a number of species, and that in many cases molecular investigation is necessary for correct assignment. Nevertheless, most species can be identified based on the combination of external morphological characters, with important specific and generic distinguishing morphological and colour features, such as the position and colour of the anal opening, the shape of the tubercles, the colour of the rhinophores, the presence of a rhinophoral rim, or the form of the oral tentacles. Very often, the overall black colour pattern is also characteristic; however, there may be limitations in identification based only on colour. In contrast to the black patterns, a variation of the background color (green or pink to white) might result from different food items (Brunckhorst 1993), or variations in water quality. A change in colouration during ontogeny is also possible and could lead to wrong species assignments. Similar intraspecific color variation by mimicking other species has been shown in the family Chromodorididae: colour morphs within the same species, as well as similarly coloured species, might occur sympatrically (Padula et al. 2016; Layton et al. 2018, 2020; Oskars & Malaquias 2020), leading to the conclusion that identification might be limited when based only on colour patterns. Layton et al. (2018) discussed Müllerian mimicry in *Chromodoris* sea slugs with aposematic colouration as one explanation. However, the various colour patterns, especially in the genus *Phyllidiella*, which might be obvious for scientific identification, is probably not distinct enough for a natural predator. We assume that hybrid introgression might have occurred or still is occurring, resulting in an introgression of colour morphs into various clades and subclades, as recently shown for *Chromodoris* species by analysing genomes (Layton et al. 2020). This would explain a certain variability within a species or clade, as well as the repeated occurrence of distinct colour patterns from other clades. Hybrid introgression is increasingly found in gastropods (e.g., Costa et al. 2020; Prockow et al. 2021), highlighting the complex nature of speciation, and genomic evidence was provided only recently for the first time for another nudibranch genus, the genus *Chromodoris* (Layton et al. 2020). These questions can only be addressed by future nuclear genome analyses of phyllidiids.

Chemical analyses

Chemical analyses of phyllidiids collected from Sulawesi using high resolution LCMS, metabolomic tools (GNPS platform), and "classical" isolation and structure elucidation of secondary metabolites aimed to provide chemotaxonomic evidence of clade entities and/or species as revealed by molecular analysis combined with morphological characters. Since phyllidiids sequester their secondary metabolites from sponges they prey on, the extract

composition is directly dependent on the respective food source. Specialisation on a certain prey with morphological adaptation is regarded as a major force that drives evolution in Heterobranchia (Cimino & Ghiselin 1999, Wägele 2004). Precise food sources of many phyllidiids are unknown and metabolomic analyses are an indirect way to provide information on this important ecological factor. A complex chemical profile of a phyllidiid extract can indicate either a broader array of food or a complex metabolome of a specific food source. Extracts composed of either only few or rarely encountered compounds certainly indicate a restricted food array. We often do not know to what extent chemical composition of the same sponge species may vary, or if and how nudibranchs might chemically alter (= metabolise) acquired chemicals. Additionally, collected specimens were stored in ethanol under field conditions with exposure to environmental factors, such as light and high temperature. Unstable secondary metabolites might thus degrade during the first days after fixation. Taking in account these difficulties associated with metabolomic analyses, some investigated phyllidiids nevertheless were found to have highly specific extract composition, demonstrating the feasibility of a chemotaxonomic approach as an additional character in species delimitation.

The most commonly detected secondary metabolites across the three analysed phyllidiid genera are sponge-derived sesquiterpene isonitriles and related compounds, which is in accordance with previous chemical investigations of phyllidiid nudibranchs (Burreson et al. 1975, Kassühlke et al. 1991, Fusetani et al. 1992, Okino et al. 1996, Hirota et al. 1998, Wright 2003, Manzo et al. 2004, Lyahova et al. 2010, Jomori et al. 2015, White et al. 2017, Sim et al. 2020). Despite extensive chemical studies on marine heterobranchs (see recent reviews Avila 2020, Avila et al. 2020), sesquiterpene isonitriles are known only from phyllidiids and two dorid nudibranchs, *Cadlina luteomarginata* (Thompson et al 1981, Burgoyne et al. 1993) and *Hexabranchus sanguineus* (Zhang et al. 2007). Both species are considered as generalists feeding on many different sponges.

In addition to the typical array of sesquiterpenes throughout the Phyllidiidae, we detected specific compounds, or compound compositions, that allow chemical characterisation of some species or genera. A common feature that is detected across all chemically analysed *Phyllidia* species is the presence of rather polar brominated natural products. Whereas some of them are unique and were found only in *P*. cf. *babai*, we detected a range of brominated compounds that occur in several *Phyllidia* species. One of these, bromoindole alkaloid 5-bromotryptophan was detected in all *Phyllidia* species. However, its

derivative 5-bromoabrine was detected only in *P. ocellata*. Double-charged ions of unidentified polar mono- and dibrominated compounds were observed in all *Phyllidia* extracts except those of *P. varicosa.* Other than in *Phyllidia,* some of these brominated metabolites were detected in low amounts in the extracts of only six other specimens (all three *Phyllidiopsis krempfi* specimens, and one specimen each of *Phyllidiella* sp. c subclade 1, *P. zeylanica* aucct. and *P. rudmani*).

Within the genus *Phyllidia*, extracts of *P. coelestis* and *P. elegans* showed very similar characteristic chemotypes with major metabolites being diterpene isonitriles, which were rarely found in other phyllidiids, thus uniting these two species and confirming sister taxa relationship. Unusual dichloroimidic sesquiterpenes described from *Phyllidiella albonigra* in our previous work (Bogdanov et al. 2020) were detected in very low amounts in *P. elegans*, providing a chemical clue for the separation from *P. coelestis*. Brominated alkaloids spongiacidin A and B, are here reported from a nudibranch (*Phyllidia* cf. *babai*) for the first time and differentiates this species from the very similarly colored *P. ocellata*. Within the genus *Phyllidiella*, *P. albonigra* and *P. rudmani* were found to have the most characteristic metabolomes. *Phyllidiella rudmani* was analysed chemically for the first time and contained an array of kalihinol-type diterpenes not detected in any other phyllidiid in this study. Rare dichloroimidic sesquiterpenes were found to be a characteristic feature of *P. albonigra*, and were detected in three out of four specimens collected from the three different areas of our collections: Sangihe Island, Bunaken Island, and Bangka Island. However, these special compounds were absent in the fourth investigated smaller animal from Bunaken Island.

We could not identify such obvious chemical features for the other investigated phyllidiids that would allow a clear separation/identification of the species based on chemical analysis only. Rather than possessing compounds of a specific chemical class, the extracts of many phyllidiids are characterised by varying compositions of sesquiterpene isonitriles and related compounds (thiocyanates, isothiocyanates, and formamides). Data obtained with high resolution LC-ESIMS coupled with a UV/Vis diode array detector (DAD) provides deep insights into the chemical extract composition, but is limited to UV absorbance, molecular mass/formula, fragmentation pattern, and retention time and can be used mainly for identification of known molecules. Currently, MarineLit database has 54 entries for sesquiterpene isonitriles with a molecular formula $C_{16}H_{25}N$, 33 for formamides ($C_{16}H_{27}NO$) and 60 for thio- and isothiocyanates ($C_{16}H_{25}NS$). The major fragmentation is the loss of the functional group that results in a fragment with exactly the same mass for all listed above

compounds. Thus, laborious fractionation of crude extracts, isolation of pure compounds, and their structure elucidation with NMR is ideally required for metabolome characterisation of the majority of phyllidiid species. Extract amount is a limiting factor for such in-depth chemical analysis and it could be performed on only three species, *Phyllidiella pustulosa*, *P. nigra*, and *Phyllidiopsis krempfi*. Isolated and unambiguously identified sesquiterpenes were different in each species, providing evidence that an individual assembly of structurally closely related compounds might be very characteristic for certain species.

Interestingly, there is a similarity of compounds within the subclades of *P. pustulosa*, or the two subclades of *Phyllidiella* sp. a, or *Phyllidiella* sp. c but with distinct differences between the respective subclades, indicating a specialisation within the various subclades that is not associated with localities. For example, the four *P*. sp c subclade 1 specimens possessed sesquiterpene isonitriles that were not found in the two specimens of subclade 2. Unfortunately, we do not know enough about food specificity or about the metabolomes of the food organisms. Our data indicate that speciation of phyllidiids began as a generalist feeding on a broader array of sponge species, with subsequent specialisation on certain food organisms during the speciation processes, thus restricting the number of compounds and exhibiting the more specialised compound composition of the particular food. The chemical profiles given here allow the identification of specific sponge species in several cases (P. ocellata, *P. rudmani* feeding on *Acanthella* species).

We assessed geographic variations of metabolomes by investigation of (usually) several specimens from different localities for each targeted species. In most of the clades, no geographic associations were detected, with few exceptions. *Phyllidiopsis krempfi* was investigated including two specimens from Sangihe Island and one specimen from Bunaken Island. The two specimens from Sangihe, with nearly the same chemical profile, differed considerably from the third specimen collected from Bunaken Island. This “Sangihe” chemotype was found in four other specimens belonging to *P. zeylanica* aucct. (Phpu16Sa60, Phpu16Sa70), *Phyllidiella* sp. a (Phpu16Sa1), and *Phyllidiella* sp. c clade 2, all species with high metabolome variability. However, the number of analysed individuals was low, and therefore the assumption of geographic differences is preliminary.

Novel species / clades

We were able to assign many of our specimens to formally described species but we clearly show that several phyllidiid species are new to science, and in need of formal

descriptions, or available names found. Within *Phyllidia*, our collection now comprises nine described species, but four species are recognised as unnamed (including *P*. cf. *babai*). Within the genus *Phyllidiopsis*, our collection comprises six described species (excluding the dubious *P. cardinalis*), and one species remains unnamed. The species *Phyllidiopsis krempfi* and *Phyllidiopsis pipeki* seem to be indistinguishable on a molecular basis and *P. pipeki* is here considered a junior synonym of *P. krempfi*: we have specimens across the full range of external morphologies, including a mixture of characters from both species in one and the same animal. Within *Phyllidiella*, 14 genetically distinct species can be separated based on slight differences in colouration, on molecular data, and, in some instances, on the presence of chemical compounds. Chang & Willan (2015) already recognised at least nine clades within the *P. pustulosa* complex based on the mitochondrial genes CO1 and 16S; Stoffels et al. (2016) were also able to identify cryptic varieties of *P. pustulosa*. Our much larger dataset including many more species clearly shows that many cryptic species and subspecies exist with colour patterns similar to the nominal *P. pustulosa*. As a consequence we no longer employ the term *Phyllidiella pustulosa* species complex in order to avoid the hypothesis of a monophyletic clade. However, we emphasise here, again, that the interspecific relationship of the species and clades was not the goal of this study and probably cannot be solved with these two mitochondrial genes. We were able to assign members of our collection to five described species, including *P*. cf. *pustulosa* within the nominal species *P. pustulosa*, but at least nine clades are recognised as currently unnamed species. This includes the species we call *P. zeylanica* auctt, as well as the species complex forming clade e comprising four species.

We are able to resurrect *Phyllidiella albonigra* that had been synonymised with *P. pustulosa* by Brunckhorst (1993). We recognise the species often identified as *Phyllidiella zeylanica* as distinct and different from the true *P. zeylanica* Kelaart and herein label it *P. zeylanica* auctt. We were also able to identify several problematic assignments of sequences in GenBank, which are listed in Table 2 together with their NCBI accession numbers, sources, and collection localities.

We are also aware that many misidentified specimens are figured throughout the literature, some of which may match our clades.

Problems with museum material

Our re-investigation of museum material clearly revealed the problems of imprecise old descriptions, the loss of holotypes, the subsequent designation of syntypes into lectotypes up

to synonymisation of available names in previous revisions. Our study clearly shows the necessity of molecular barcoding to verify species identities as well as careful re-examination of the literature and museum material. Unfortunately, our attempts to barcode more recently collected type material did not provide sequences that could be used to assign our unnamed clades to the respective types, and thus to an available name. Colour, apart from black melanin, is not preserved in the type materials, and often this melanin has faded (Fahrner & Schrödl 2000). Thus, even identification of specific colour features on e.g., the mantle rim, oral tentacles, or foot, was not possible and hindered a possible assignment of our material to available type material. Further problems occurred in syntypes where the individual animals appear to belong to different species (e.g., *Phyllidiella nobilis*). Future analyses, and hopefully the development of methodologies to analyse old and formalin-preserved specimens, will solve some of these problems.

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Chapter 6

General Discussions

Subjects of this thesis were to perform a monitoring study on sea slug diversity and investigate the taxonomy of the family Phyllidiidae (Nudibranchia). The sea slug diversity project was part of several monitoring projects focused in North Sulawesi, Indonesia (Bunaken National Park, Sangihe Island, Lembeh Strait and Bangka Archipelago). My study covered the Bangka Archipelago. During these studies (not only mine), the high number of phyllidiid specimens was interesting, and therefore the second part of my thesis focussed on the family Phyllidiidae. This taxon is known to be difficult with regard to taxonomy and systematics. I used mitochondrial genes 16S and CO1 for molecular barcoding, and then used the same genes for a phylogenetic analysis and species identification to finally revise some species. The difficulties of the taxonomy of Phyllidiidae was tackled by morphological and molecular investigation. Additionally, we used the presence of chemical compounds to separate and characterize species. Toxins as secondary metabolites were originally isolated for potential drug lead detection; however, in my study it was used for the first time in sea slugs as a kind of chemical taxonomy, and the chemotype results provided additional characters that confirmed to some extent our phylogenetic and species delimitation test results.

Sea Slug Diversity in North Sulawesi

Sulawesi is unique in its history. It was a part of the Eurasian plate influenced by subduction and volcano activities (Burung Indonesia 2014). Sulawesi Sea was very close to the boundary of the North Pangea plate and the Australian plate (South Pangea, now called Papua Island) and thus part of the neotethys (https://web.archive.org/web/20080516030745/http://www.palaeos.com/Mesozoic/Triassic/MidTrias.html). These boundaries are described as Wallace and Weber imaginary lines which separate different types of terrestrial organisms. However, the marine organisms are potentially less affected by this situation and are mixing due to the currents. Sulawesi Sea can be considered as the melting point between Indo-Pacific Ocean and Indian Ocean and this is enforced by the Indonesian Throughflow circulation (ITF). Because of this special geographic situation, Sulawesi, as well as other islands of the Pacific Ocean that lie in the coral triangle

(Solomon Islands, Philippines, Timor) are known as the hotspots of coral diversity including organisms associated with the coral reef ecosystems.

Several studies show that North Sulawesi is the hotspot of diversity not only on the terrestrial but also in the marine ecosystems. Several local Indonesian studies were performed in Sulawesi, Several studies are more general on various ecosystems: e.g., on coral reef communities (Ginoga et al. 2015; Tito et al. 2005; Kase et al. 2019), mangroves (Schaduw 2016), and seagrass beds (Kamaruddin et al, 2016), or plankton (Ruga et al. 2014). Others are more group specific and cover vertebrates, from the ancient Coelacanth (http://lipi.go.id/berita/coelacanth-ikan-purba-yang-tersisa/855; Sudarto et al. 2010), fishes (Setiawan et al. 2013; Patty et al. 2015) up to other common vertebrates like sea turtles (Kasenda et al. 2013), Many studies focus on certain invertebrate groups, like ascidians (Opa et al. 2020), crustaceans (Waisaley et al. 2019), echinoderms (Yusron 2012; Rompis et al. 2013), and Molluscs (Rangan 2010, Dajoh et at. 2020; Ukar et al. 2020). Even the ecology of sea slugs is covered by some studies (Kambey 2011; Purba et al. 2013; Pungus et al. 2017; Ompi et al. 2019; Marpaung et al. 2019). However, sea slugs' diversity is certainly understudied. Interestingly, citizen scientists contribute to our knowledge on Sea slug distribution in North Sulawesi, e.g., in the Sea Slug Forum (www.seaslugforum.edu).

Seven sea slug collection events from this project in four areas, Bunaken National Park (2015-2017), Sangihe Island (2016), Lembeh Strait (2018) and Bangka Archipelago (2017-2018), resulted in five diversity publications (Kaligis et al. 2018, Eisenbarth et al. 2018, Undap et al. 2019, Ompi et al. 2019 and Papu et al. 2020). Number of expeditions, natural conditions (e.g. El Niño events, see Goddard et al. 2018, Undap et al. 2019), and number and experience of personnel are reasons for lower species number results in Sangihe Island (23 species, one collection event) and in Lembeh Strait (27 species, one collection event). Including published data (from Tonozuka 2003 and Sea Slug Forum) numbers accumulate to a total of 91 sea slug species in Lembeh Strait. However, no published data is available from Sangihe Island.

A total of 259 species of sea slugs is recorded in North Sulawesi from 2015 to 2018 from our expeditions. This is the first large diversity study in Sulawesi. When we add all reports from the citizen scientists and guidebook of Nudibranchia (Tonozuka 2003, Burghard et al. 2015 and Sea Slug Forum), all recorded species from North Sulawesi sum up to 335. The underwater photographers and citizen scientists' data is additionally important for providing information to the diversity databases; however, the taxonomists are needed to

investigate and describe the specimens properly. Integrating the metadata to the global data is also very important. Integrative data in the Diversity data banks (e.g. Diversity workbench) are useful tools to monitor diversity and indirectly monitor the environment quality and then can form a base for the government to make environmental decisions.

Around 37% (96 out of 259) of the collected species are new to science and shows that sea slugs diversity is understudied in this area. It reveals North Sulawesi as a hotspot of sea slug species, but also that it is not so extensively explored compared with Philippine Islands, with 1006 species recorded by Gosliner et al. in 2015). Many organisms in this area, not only sea slugs, are rarely known or undescribed. Moreover, according to our results on species accumulation, sea slug species recorded from North Sulawesi is still increasing and not indicating a saturation, thus the maximum amount of sea slugs species in this region cannot be estimated yet, and certainly is much higher than recorded so far.

Bunaken National Park (BNP), Bangka Archipelago (BA) and Lembeh Strait (LS) are adjacent sampling locations with a distance of about 30 km. Few species in those locations have an overlapping distribution between these three areas. Those three locations have different substrates that might be the reason for lack of overlapping species distribution. BA and LS have similar substrates influenced from the adjacent volcanoes with black sand and volcanic rocks, whereas BNP is dominated by coral reefs and white coral sands (Poli 2018). However, Bangka Island is part of a rather shallow archipelago, whereas Lembeh Strait is a strait with a flow through system from deeper areas. These environmental differences affect the presence or absence of food organisms and thus the presence or absence of many sea slug species. Since BNP and LS are separated by BA, the species overlap between BNP and LS is lower than the overlap between BA to the two other locations. Whitten et al (2002) in their figure 2.4 shows less mixed surface water flow from LS to BNP. Contrary to the very frequent surface current flows from LS to BA or BNP or BA. In this case, surface currents are of great importance for the larvae dispersal.

Chapter 3 focuses on the sea slug diversity in Bangka Archipelago (BA) which I particularly investigated. The expedition to BA resulted in 149 species. 484 specimens were collected in total. The Nudibranchia ascidian feeder *Nembrotha* (Doridina, Polyceridae) shows the highest records in BA. Whereas closely related bryozoan feeders, like *Polycera* (Doridina, Polyceridae), were not collected there. When diving or snorkelling there, the high number of tunicate species is also very obvious, whereas bryozoans are much less in numbers. Besides ascidian feeders, we also record sponge feeders and egg feeders among the sea slugs.

Further interesting findings were e.g., the sacoglossan *Thuridilla gracilis* which showed four different rhinophore variations in colour, indicating cryptic speciation in this species, as was recently shown for this species by (Martín-Hervás et al. (2019).

Sea slugs' diversity studies are important to monitor the environment's health and climate change (Nimbs et al. 2016). Not only are sea slugs highly influenced by a change of the food availability, due to environmental changes. Loss of competitors can cause an outbreak, which was documented for the sea slug *Pleurobranchaea* sp. by Farias et al. (2015). Globally, sea slugs diversity is higher in the Indo-Pacific (Burghardt 2006; Gosliner 1992) compared to other areas, like the South Polar Sea (Wägele 1987), Red Sea (Yonow 2018), Mediterranean Sea (Crocetta et al. 2013), Carribean Sea (Camacho-García et al. 2014), and Atlantic ocean in general (Garcia & Bertsch 2009). In several cases, species are endemic to these regions.

Sea slugs compensate the shell loss by producing toxins by *de novo* synthesis or more often by sequestering them with their food, and use them for the defense system for fish or predator deterrent (Böhringer et al. 2017). Because of the different origins, the toxins differ to a great extent in the various sea slugs. The source of the toxic compound can come from sponges or octocorals that are associated with microorganism e.g. bacteria and dinoflagellate (Blunt et al. 2017; Ponder et al. 2020; Bogdanov et al. 2020). Very often, the compounds are distributed not only in the mantle, but also in the gonad and the digestive gland, as was shown for *Pleurobranchaea maculata.* Furthermore, the compounds can also be found in the larvae and egg mass (Blunt et al. 2017). Transfer mechanism to the egg mass is unknown. These compounds make the sea slugs also very interesting for humans, as the extract of the toxin is also a potential for drug lead discovery. The compounds can be used as antiinflammation, antihistamine, antibacterial, antiviral and anticancer agents (Jacob et al. 2014; Fisch et al. 2017). A variety of chemical compounds are also recorded from Phyllidiidae, rendering them also very interesting for pharmaceutical studies. The major compound found so far is sesquiterpenoids (Bogdanov et al. 2020).

Phyllidiidae (Doridina- Nudibranchia)

Most sea slugs have slimy soft bodies to avoid predation. Sea slugs can hide in the corals, or mimic their substrate in colour and form (Ponder et al. 2020). Toxins can be released in the mucus that also prevents predation. However, members of the Phyllidiidae are forming thick spicule nets in their body for additional protection. The calcium carbonate spicules fill

a lot of space in the animal body. It makes them hard, and thus less flexible to hide in small crevices, but protects them in various ways: Calcium carbonate as a mineral provides no nutritional value for a potential predator and is inconvenient for digestion (Penney, 2008). These spicules, together with the toxins, make phyllidiids thus very unattractive as prey and that is probably the reason why they are active during the daytime (diurnal) and also often very conspicuously in colour, thus making them very visible in the reef system.

Besides the defense system, the taxonomy of Phyllidiidae is also of interest (Ritson-Williams 2007). Only a few phylogenetic analyses addressed this group. Identification of the various species is often difficult, because the colouration of many species is quite similar. This renders this family as a very difficult one (Stoffel et al. 2016). My study presented in chapter 5 is the first that includes hundreds of phyllidiid specimens. The huge phylogenetic tree includes 29 species from our expeditions comprising 11 species of *Phyllidia,* 7 species of *Phyllidiopsis,* and 11 species of *Phyllidiella.* Additional 6 species extracted from GenBank are also included. The large tree consisting of 587 concatenated sequences (CO1 and 16S) provided evidence for genus and species relationship and also about the presence of cryptic species. The three included genera (*Phyllidia, Phyllidiella and Phyllidiopsis*) all of them covered by several species, are monophyletic. However, the only sequence of *Ceratophyllidia* species that was published by Hallas et al. (2017) is nested within *Phyllidiella*. Future studies including more members of *Ceratophyllidia* have to be performed to clarify this problem. Following taxa revealed to be problematic and were discussed in detail in the manuscript (chapter 5): *Phyllidia babai, Phyllidiella pustulosa,* and *Phyllidiopsis krempfi.* Several further problems were not addressed in the manuscript and are mentioned here.

Brunckhorst (1993) has synonymised two species with the nominal species *P. ocellata*: *Phyllidia undula* Yonow, 1986 and *Phyllidia multituberculata* C. R. Boettger, 1918. *P. ocellata, P. undula* and *P. multituberculata* are distributed in different geographic areas. *Phyllidia ocellata* var. *undula* is common in the Red Sea. *P. multituberculata* is common in the Indian Ocean (GBIF Secretariat 2019d), while specimens of *Phyllidia ocellata* which are described with rings on the notum are very common in the Pacific Ocean. Yonow (1996) refused to synonymyse *P. undula* with *P. ocellata* because of the different size of oesophagus, shape of pharyngeal bulb, pharyngeal muscle and geography. Unfortunately, no molecular data of *P. undula* and *P. multituberculata* are available yet in GenBank to confirm Brunckhorst's or Yonow's statement.

Stoffel et al (2016) name one clade in their tree as *P.* cf. *babai* based on the one specimen with external features that are quite the same as *P. babai*. However, this clade does not group with *P. babai* in their study. Two of our specimens are very similar externally with one specimen of *Phyllidia* cf. *babai* from Stoffel et.al (2016; Fig. 10e). Though they clustered in *P.* cf. *babai,* those specimens are also very similar to *Phyllidia ocellata* to a great extent. I was able to find distinguishing features for the specimens in this clade: they differ from *P. ocellata* by having a yellow foot, and single large sized tubercles surrounded by white and black rings, and the white rings are connected with the side of other rings. In *P. ocellata*, these white rings never touch and are separated.

The monophyletic genus *Phyllidiopsis* is split into two lineages with 100% bootstrap. First lineage is with distinct tubercles. This lineage comprises three species: *P. krempfi, P. burni* and a new species (*Phyllidiopsis* sp. a). *P.* sp. a shows characters of the other two species: it is externally very similar to *Phyllidiopsis krempfi*, but the rhinophores pattern is similar to *P. burni*. The second *Phyllidiopsis* lineage is with less tubercles or even lack tubercles. The dorsal notum exhibits one or several ridges. This lineage consists of the four species *P. xishaensis, P. annae, P. sphingis* and *P. shireenae.*

Brunckhorst's (1993) original and Yonow's (2011) subsequent description of *Phyllidiopsis pipeki* mention distinct externally dorsal features but the species also resembles in several characters to *P. krempfi.* Both species differ so far in the colour of notum background and the tip of the oral tentacle. However, in my phylogenetic tree, members with the typical colouration of *P. pipeki* cluster with members of *P. krempfi.* Thus, *P. pipeki* is considered as a junior synonym of *P. krempfi.*

Phyllidiella pustulosa **and other** ***Phyllidiella***

P. pustulosa which is one of the oldest described phyllidiid species has been recorded from the Pacific Ocean, Indian Ocean and Red Sea (GBIF Secretariat 2019f) and thus shows the broadest distribution of all *Phyllidiella* species. Brunckhorst (1993) synonymized several species with *Phyllidia pustulosa* Cuvier, 1804: *Phyllidia verrucosa* Hasselt, 1824, *Phyllidia albonigra* Quoy & Gaymard 1831, *Phyllidia nobilis* Bergh 1869, *Phyllidia spectabilis* Collingwood, 1881, *Fryeria variabilis* Collingwood, 1881, *Phyllidia rotunda* Eliot, 1904, *Freyeria pustulosa* Grey, 1853, and *Phyllidia melanocera* Yonow, 1986. *Phyllidiella nobilis* (Bergh, 1869) was mentioned very often in old literature and was also collected from many localities in Indo-Pacific Ocean. However, not all syntypes match the original description and

part of the type material represents different species. Brunckhorst synonymysed the holotype of *P. melanocera* (Yonow, 1986) (Holotype: Fig. 10F) with *P. pustulosa* (Bruckhorst 1993). However, a specialist in phyllidiids, Nathalie Yonow, rejects this synonymisation and considers *P. melanocera* as an endemic Red Sea phyllidiid species (pers. comm). The specimens figured in her publication under Figs. 10H and 10I (Yonow 1986) are considered by her as juveniles of *P. melanocera*. We did not have a specimen of *P. melanocera* in our collection and were not able to solve this question. Brunckhorst (1993) also synonymised *P. spectabilis* with *P. pustulosa.* This result was confirmed by us based on the external morphology. In the phylogenetic tree, specimens assigned by us in preliminary identification to *Phyllidiella pustulosa,* as well as sequences assigned to *P. pustulosa* and taken from GenBank, group in five different clades, which do not form one monophyletic group. They are nested between the distinct and nominal species of *Phyllidiella nigra, P. zeylanica* auctt., *P. albonigra, P. rudmani* and *P. hageni.* At the moment, we cannot finally conclude, whether the positions of the *Phyllidiella* species in the tree and relationships are correct. Several problems have to be mentioned here. Kück & Wägele (2015) described how plesiomorphic characters can provide a false signal in the algorithms. This might lead to wrong relationships also in my tree. It also seems probable that hybrid introgression has happened in the past or is still ongoing and species delimitation is not distinct enough. By an exchange of mitochondrial DNA from different “species" might lead to the similar colour forms in the various species. This was already mentioned by Ballard & Whitlock (2003). To solve this problem, nuclear genes need to be investigated. But in overall, the chemical types of the species exhibit different configurations of compounds (Bogdanov et al. 2020) and these chemo types confirmed in several cases our molecular species delimitations. These integrative methods are supporting the correct and objective distinction of Phyllidiidae species, and solving the *Phyllidiella* species complex to some extent.

Our manuscript as represented in chapter 4 and 5 can only be the beginning of the *Phyllidiella* revision. Not all new clades of *Phyllidiella* are solved in this paper with regard to species assignments and the clades are open to be further discussed with available nominal species. However, for most of the species no sequences are available yet and comparison can only be made with the sometimes very sketchy original descriptions. I also showed that in literature many specimens are misidentified and this contributes to the chaos in the taxonomy and systematics of the Phyllidiidae. One of the distinct clades in our tree could be clearly assigned to the type material of *Phyllidiella albonigra,* which was synonymised by Brunckhorst (1993) with *P. pustulosa.* Interestingly, this clade contains a unique metabolome

configuration (Bogdanov et al. 2020) which is not found in other clades of *Phyllidiella*. Therefore, the species name *Phyllidiella albonigra* is resurrected in our study.

One unsolved question is, what drives speciation in *Phyllidiella* with so many clades similar in colour. Very often specimens of these clades co-occur, therefore it cannot be geographic isolation. Phyllidiids are sponge feeders, which may have different compounds and thus attract the sea slug larvae of the respective clades and guide them to settle down on their respective sponge food (Faucci et al. 2006). Therefore, it seems more likely that specialization on different sponge species drives speciation.

Due to the encountered difficulties with regard to the type material and the problems in the external investigation, we suggest for the next studies to take pictures from all sides, dorsal, ventral and lateral, in live stage, *in situ* and laboratory scale. These pictures will be useful for the future, because sea slugs usually lose their colour and often also the shape of external structures, when preserved. This would help in correct identification and also future revisions.

Chapter 7

General Conclusion

Summarizing all our diversity studies performed within the IndoBio project, where I was involved to a great extent, 259 sea slugs species have been collected from four localities, comprising 215 species from Bunaken National Park (BNP), 23 species from Sangihe Island (SI), 23 species from Lembeh Strait (LS) and 149 species from Bangka Archipelago (BA). Only a few species are overlapping in distribution between these four regions. This indicates that the habitats in these regions, which are separated only by 30 to 200 km, are highly diverse in their structure, and that we have by far not reached a final number of species that live in these areas. 96 species in our collections are new to science. More collection events in North Sulawesi will certainly result in many more species and also more undescribed species.

The phylogenetic tree analyses of the family Phyllidiidae (Nudibranchia), which is the most abundant taxon of sea slugs in North Sulawesi, revealed monophyly of the genera *Phyllidia*, *Phyllidiopsis* and *Phyllidiella*; however, with queries to be solved in the future. Our tree comprises 32 species based on specimens from our collection plus additional sequences of seven species extracted from the GenBank. *Ceratophyllidia* clustered in the large clade of *Phyllidiella*. However, it is the only sequence available from GenBank. Now, we need more analyses to show whether *Ceratophyllidia* is within the clade of *Phyllidiella* and therefore the genus *Ceratophyllidia* should be synonymized, or whether the single sequence was misidentified. Future work with further specimens collected and properly documented with pictures, will also show, how many of the already described phyllidiid species match to our tree, and which ones are valid. The many misidentified phyllidiid specimens in literature (especially in the chemical literature) will be a challenge for these analyses.

According to the chemical analyses (done by Dr. Alexander Bogdanov) of specimens throughout the various phyllidiid clades, no new compounds were found in Phyllidiidae. However, the combination of morphological, molecular and metabolome analyses provide evidence that the metabolome can be used to distinguish a few species and confirms the external morphology and molecular descriptions. Thus, chemotaxonomy can work at least in this case.

We suggest resurrecting the old name *Phyllidiella albonigra* that was synonymised as *Phyllidiella pustulosa* for one of the clades in the Phyllidiidae tree. *Phyllidiopsis pipeki* is a morphological variation of *Phyllidiopsis krempfi*, and therefore synonymized with the latter.

References

Adinolfi, M., De Napoli, L., Di Blasio, B., Iengo, A., Pedone, C., Santacroce, C. (1977) The Absolute Configuration of the Axane Sesquiterpenes from the Sponge *Axinella cannabina*. *Tetrahedron Letters, 32,* 2815-2816.

Alqudah, A.; Saad, S. B.; Hadry, N. F.; Susanti, D. (2015). Identification and Phylogenetic Inference in Different Mollusc Nudibranch Species via Mitochondrial 16S rDNA. *Brazilian Journal of Biological Sciences, 2(4),* 295–302.

Alqudah, A.A.; Shahbudin, S.; Deny, S.; Hadry, N.F.; Khodzori, M.F.A.; Yusof, M.H.; Rani, M.H. (2016). Observations on nudibranch behaviour patterns under laboratory conditions. *Jurnal Teknologi. 78(11),* 167–171. DOI: 10.11113/.v78.6639.

Alvi, K. A., Tenenbaum, L., Crews, P. (1991) Anthelmintic Polyfunctional Nitrogen-Contaning Terpenoids from Marine Sponges. *Journal of Natural Products, 54 (1),* 71-78.

Ando, K.; Sasaki, H.; Hosokawa, T.; Nawata, Y.; Iitaka, Y. (1975) Molecular structure of 4-O-ethyl ascofuranone. *Tetrahedron Lett.*, 11, 887-890.

Araki, Y.; Awakawa, T.; Matsuzaki, M.; Cho, R.; Matsuda, Y.; Hoshino, S.; Shinohara, Y.; Yamamoto, M.; Kido, Y.; Inaoka, D. K.; Nagamune, K.; Ito, K.; Abe, I.; Kita, K. (2019). Complete biosynthetic pathways of ascofuranone and ascochlorin in *Acremonium egyptiacum*. *Proc. Natl. Acad.* Sci. U. S. A., 116, 8269-8274.

Astrin, J. J. & Stüben, P. E. (2008). Phylogeny in Cryptic Weevils: Molecules, Morphology and New Genera of Western Palaearctic Cryptorhynchinae (Coleoptera: Curculionidae). *Invertebrate Systematics, 22(5),* 503–22.

Atmadipoera, A. S.; & Mubaraq, G. L. (2017). Structure and variability of Indonesia throughflow at Sulawesi Sea. *Jurnal Kelautan Nasional, 11(3),* 159-174.

Avila, C.; Núñez-Pons, L.; Moles, J. (2018). In *Chemical Ecology; The Ecological Impacts of Marine Natural Products;* Puglisi, M. P., Becerro, M. A., Eds.; CRC Press/Taylor and Francis Group: Boca Raton, pp 71-163. Avila C. (2020). Terpenoids in Marine Heterobranch Molluscs. *Marine Drugs, 18,* 162. Doi: 10.3390/md18030162

Avila C., Angulo-Preckler C. (2020) Bioactive Compounds from Marine Heterobranchs. *Marine Drugs, 18,* 657. Doi: 10.3390/md18120657

Ballard, J. W. O.; & Whitlock, M. C. (2003). The incomplete natural history of mitochondria. *Molecular Ecology*, *13*, 729–744. Doi: 10.1046/j.1365-294X.2003.02063.x.

Barletta, G. & Melone, G. (1976). Nudibranch del Promontorio de Portofino (Genova) (Gastropoda: Nudibranchia). *Natura* 67*(3-4),* 203-236.

Bergh, R. (1904). The Danish Expedition to Siam 1899-1900: I. Gastropoda opisthobranchiata. *det kongelige danske videnskabernes selskabs skrifter* https://www.biodiversitylibrary.org/item/107830#page/5/mode/1up [accessed 22 December 2020]

Bogdanov, A.; Papu, A.; Kehraus, S.; Cruesemann, M.; Wagele, H.; & König, G. M. (2020). Metabolome of the *Phyllidiella pustulosa* species complex (Nudibranchia, Heterobranchia, Gastropoda) Reveals Rare Dichloroimidic Sesquiterpene Derivatives from a Phylogenetically Distinct and Undescribed Clade. *Journal of Natural Products, 83(9)*, 2785–96.

Böhringer, N.; Fisch, K. M.; Schillo, D.; Bara, R.; Hertzer, C.; Grein, F.; Eisenbarth, J-H.; Kaligis, F.; Schneider, T.; Wägele, H.; König, G. M.; & Schäberle. T. F. (2017). Antimicrobial Potential of Bacteria Associated with Marine Sea Slugs from North Sulawesi, Indonesia. *Frontiers in Microbiology, 8*, 10-92**.** Doi: 10.3389/fmicb.2017.01092.

Bouchet, P.; Rockroi, J-P.; Hausdorf, B.; Kaim, A.; Kano, Y.; Nützel, A.; Parkvaev, P.; Schrödl, M.; Strong, E.E. (2017). Revised classification, nomenclator and typification of gastropod and monoplacophoran families. *Malacologia, 61(1–2),* 1–526, doi:10.4002/040.061.0201.

Brodie, G.D.; Brodie, J.E. (1995). Species diversity and habitat selection in opisthobranch gastropods on two adjacent reefs in Fiji. *South Pacific, J. Nat. Sci., 14*, 97–113.

Brunckhorst, D. J. (1993). The Systematics and Phylogeny of Phyllidiid Nudibranchs (Doridoidea). *Records of the Australian Museum, Supplement 16,* 1–107.

Brust, A.; Garson, M.J. (2003). Advanced precursors in marine biosynthetic study. Part 3: The biosynthesis of dichloroimines in the tropical marine sponge *Stylotella aurantium*. *Tetrahedron Lett., 44*, 327–330.

Burghardt, I.; Carvalho, R.; Eheberg, D.; Gerung, G.; Kaligis, F.; Mamangkey, G.; Schrödl, M.; Schwabe, E.; Vonnemann, V.; Wägele, H. (2006). Molluscan diversity at Bunaken National Park, Sulawesi. *J. Zool. Soc. Wallacea*, *2*, 29–43.

Burghardt, I.; Gosliner, T.M. (2008). *Phyllodesmium rudmani* (Mollusca: Nudibranchia: Aeolidoidea), a new solar powered species from the Indo-West Pacific with data on its symbiosis with zooxanthellae. *Zootaxa, 1308*, 31–47.

Burghardt, I.; Wägele, H. (2014). The symbiosis between the 'solar-powered' nudibranch *Melibe engeli* Risbec, 1937 (Dendronotoidea) and *Symbiodinium* sp. (Dinophyceae). *J. Moll. Stud.***,** *80*, 508– 517. Burgoyne, D. L., Dumdei, E. J., Andersen, R. J. (1993) Acanthenes A to C: A Chloro, Isothiocyanate, Formamide Sesquiterpene Triad Isolated from the Northeastern Pacific Marine Sponge *Acanthella* sp. and the Dorid Nudibranch *Cadlina luteomarinata*. *Tetrahedron, 49(21),* 4503-4510.

Burn, R. (1970). *Phyllidia* (*Phyllidiella*) *zeylanica* Kelaart, a rare nudibranch from the Indian Subcontinent. *Memoirs of the National Museum of Victoria* (18 May), 37–41.

Burnham, K. P.; & Anderson, D. R. (2009). *Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach, Second Edition*. New York, USA: Springer-Verlag.

Burreson, B. J.; Scheuer, P. J.; Finer, J.; Clardy, J. (1975). 9-Isocyanopupukeanane, a Marine Invertebrate Allomone with a New Sesquiterpene Skeleton. *Journal of American Chemical Society 97(16),* 4763–4764.

Burung Indonesia (2014). Hotspot keanekaragaman hayati Wallacea. *Critical Ecosystem Partnership Fund report*.

Camacho-Garcia, Y. E.; Pola, M.; Carmona, L.; Padula, V.; Villani, G.; Cervera, J. L. (2014). Diversity and distribution of the heterobranch sea slug fauna on the Caribbean of Costa Rica. *Cahiers de Biologie Marine 55*, 109-127.

Carbone, M.; Ciavatta, M. L.; Manzo, E.; Li, X.-L.; Mollo, E.; Mudianta, I. W.; Guo, Y.-W.; Gavagnin, M. (2019). Amphilectene Diterpene Isonitriles and Formamido Derivatives from the Hainan Nudibranch *Phyllidia Coelestis*. *Mar. Drugs*, *17*, 603. https://doi.org/10.3390/md17110603.

Carolan, J. C.; Murray, T. E.; Fitzpatrick, Ú.; Crossley, J.; Schmidt, H.; Cederberg, B.; McNally, L.; Paxton, R. J.; Williams, P. H.; Brown, M. J. F. (2012). Colour Patterns Do Not Diagnose Species: Quantitative Evaluation of a DNA Barcoded Cryptic Bumblebee Complex. *PLoS ONE, 7(1),* e29251. https://doi.org/10.1371/journal.pone.0029251

Cattaneo-Vietti, R.; Angelini, S.; Gaggero, L.; Lucchetti, G. (1995). Mineral composition of nudibranch spicules. *Journal of Molluscan Studies. 61,* 331–337.

Chang, Y-W.; Willa, R. C.; Mok, H-K. (2013). Can the morphology of the integumentary spicules be used to distinguish genera and species of phyllidiid nudibranchs (Porostomata: Phyllidiidae)? *Molluscan Research, 33(1),* 14–23, http://dx.doi.org/10.1080/13235818.2012.754144

Cheney, K. L.; F. Cortesi, M. J. How, N. G. Wilson, S. P. Blomberg, A. E. Winters, S. Umanzör, and N. J. Marshall. (2014). Conspicuous visual signals do not coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology, 27(4),* 676–87.

Cimino, G.; Ghiselin, M. T. (1999). Chemical defense and evolutionary trends in biosynthetic capacity among dorid nudibranchs (Mollusca: Gastropoda: Opisthobranchia). *Chemoecology, 9,* 187-207.

Cimino, G.; Gavagnin, M. (2006). *Molluscs.* From chemo-ecological study to biotechnological application. Springer-Verlag Berlin Heidelberg, Germany.

Clement, M.; Snell, Q.; Walker, P.; Posada, D.; & Crandall, K. (2002). TCS: Estimating gene genealogies. *Parallel and Distributed Processing Symposium, International Proceedings, 2,* 184.

Cobb, G.; Rudman B. (2008). Comment and Re: *Phyllidia coelestis* mating. *[Message in] Sea Slug Forum.* Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/21754.

Cockey, C. (2013). The Wallace Line. https://mistralmtn.blogspot.com/2013/10/the-wallace-line.html [Accessed 28 December 2020]

Coleman, N. (1989). *Nudibranchs of the South Pacific, Vol. 1*. National Library of Australia, Queensland, Australia.

Coleman, N. (2001). *Nudibranchs Encyclopedia, Catalogue of Asia/Indo Pacific Sea Slugs, 2nd edition.* Edited by G. Cobb and D. Mullins. New South Wales, Australia: Thomas lone, Masalai Press and Tim Hochgrebe, Underwater Australia.

Coleman, N.; Cobb, G.; Mullins, D. (2015). *Nudibranchs Encyclopedia, Catalogue of Asia/Indo Pacific Sea Slugs*, 2nd ed. Masalai Press: Oakland, California USA, pp. 312, ISBN 978-0-9714127-9-8.

Costa W. J. E. M.; Vilardo, P.; Katz, A. M. (2020). Sympatric sister species with divergent morphological features of psammophilic catfishes of the south-eastern Brazilian genus *Microcambeva* (Siluriformes: Trichomycteridae). *Zoologischer Anzeiger*, *285*, 12-17. https://doi.org/10.1016/j.jcz.2020.01.001.

Crocetta, F.; Zibrowius, H.; Bitar, G.; Templado, J.; Oliverio, M. (2013). Biogeographical homogeneity in the eastern Mediterranean Sea - I: the opisthobranchs (Mollusca: Gastropoda) from Lebanon. *Mediterranean Marine Science*, *14/2*, 403-408. DOI: http://dx.doi.org/10.12681/mms.404.

Debelius, H. (2007). *Nudibranchs of the World*. IKAN Unterwasser-Archiv, Frankfurt, Germany. ISBN13: 9783939767060.

Diepenbroek. M.; Glökner, F.O.; Grobe, P.; Güntsch, A.; Huber, R.; König-Ries, B.; Kostadinov, I.; Nieschulze, J.; Seeger, B.; Tolksdorf, R.; Triebel, D. (2014). Towards an integrated biodiversity and ecological research data management and archiving platform: The German Federation for the Curation of Biological Data (GFBio). In *Proceedings. GI, Informatik 2014 - Big Data Komplexität meistern, Stuttgart, Germany, January 2014;* Plödereder, E., Grunske, L., Schneider, E., Ull, D., Eds. Köllen Verlag: Bonn, Germany, pp. 1711–1724.

Domínguez, M.; Quintas, P.; & Troncoso, J. S. (2007). Phyllidiidae (Opisthobranchia: Nudibranchia) from Papua New Guinea with the Description of a New Species of Phyllidiella. *American Malacological Bulletin, 22(1),* 89–117.

Eisenbarth, J.-H.; Undap, N.; Papu, A.; Schillo, D.; Dialao, J.; Reumschüssel, S.; Kaligis, F.; Bara, R.; Schäberle, T.F.; König, G.M.; Yonow, N.; Wägele. H. (2018). Marine Heterobranchia (Gastropoda, Mollusca) in Bunaken National Park, North Sulawesi, Indonesia—A follow-up diversity study. *Diversity*, *10*, 127. https://doi.org/10.3390/d10040127.

Emsermann, J.; Kauhl, U.; Opatz, T. (2016). Marine Isonitriles and Their Related Compounds. *Marine Drugs, 14(1),* 16.

Epstein, H.E.; Hallas, J.M.; Johnson, R.F.; Lopez, A.; Gosliner, T.M. (2019). Reading between the lines: Revealing cryptic species diversity and color patterns in *Hypselodoris* nudibranchs (Mollusca: Heterobranchia: Chromodorididae). *Zool. J. Linn. Soc., 186,* 116–189. doi:10.1093/zoolinnean/zly048.

Fahrner, A. & Beck. L. A. (2000). Identification Key to the Indo-Pacidic Species of the Nudibranch Family Phyllidiidae Rafinesque 1814, Including the Description of Two New Species. *Archive für Molluskenkunde, 128(1–2),* 189–211.

Fetzer, I. (2004). Reproduction strategies and distribution of larvae and juveniles of benthic soft-bottom invertebrates in the Kara Sea (Russian Arctic). The influence of river discharge on the structure of benthic communities: A larval approach. *Ph.D. Thesis*, Bremen University, Bremen, Germany. Available online: http://hdl.handle.net/10013/epic.21857.d001 (accessed on 28 January 2020).

Fisch, K.M.; Hertzer, C.; Böhringer, N.; Wuisan, Z.G.; Schillo, D.; Bara, R.; Kaligis, F.; Wägele, H.; König, G.M.; Schäberle, T.F. (2017). The potential of Indonesian Heterobranchs Found around Bunaken Island for the Production of Bioactive Compounds. *Marine Drugs, 15,* 384, doi: 10.3390/md15120384.

Fusetani, N.; Wolstenholme, H. J.; Shinoda, K.; Asai, N.; Matsunaga, S.; Onuki, H. & Hirota, H. (1992). Two sesquiterpene isocyanides and a sesquiterpene thiocyanate from the marine sponge *Acanthella* cf. *cavernosa* and the nudibranch *Phyllidia ocellata*. *Tetrahedron Letters 33,* 6823–6826.

García, F.J.; Bertsch, H. (2009). Diversity and distribution of the Gastropoda Opisthobranchia from the Atlantic Ocean: A global biogeographic approach. *Scienta Marina*, *73*, 153–160, doi:10.3989/scimar.2009.73n1153.

Garson, M.J.; Simpson, J. (2004). Marine isocyanides and related natural products – structure, biosynthesis and ecology. *Natural Product Reports, 21,* 164–179.

GBIF Secretariat (2019a). *Ceratophyllidia papilligera* (Bergh, 1890). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-23.

GBIF Secretariat (2019b). Nudibranchia. GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-22.

GBIF Secretariat (2019c). *Phyllidia coelestis* Bergh, 1905 in GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-02-23.

GBIF Secretariat (2019d). *Phyllidia ocellata* Cuvier, 1804. GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-12.

GBIF Secretariat (2019e). Phyllidiidae. GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-22.

GBIF Secretariat (2019f). *Phyllidiella pustulosa* (Cuvier, 1804). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-24.

GBIF Secretariat (2019g). *Phyllidiopsis dautzenbergi* (Vayssière, 1912). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-23.

GBIF Secretariat (2019h). *Phyllidiopsis sinaiensis* (Yonow, 1988). GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 2021-03-24.

Giglio, V. J.; Luiy, O. J.; Schiavetti, A. (2015). Marine life preferences and perceptions among recreational divers in Brazilian coral reefs. *Tourism Management, 51*, 49-57.

Ginoga, D.; Katili, D. Y.; Papu. A. (2015). Kondisi Tutupan Karang di Desa Ratatotok Timur Kabupaten Minahasa Tenggara. *Jurnal MIPA 4(2),* 14-19. DOI: 10.35799/jm.5.1.2016.11193

Gosliner, T. M. (1992). Biodiversity of tropical opostobranch gastropod faunas. *Proceedings of the Seventh International Coral Reef Symposium, Guam, 2*, 702-709.

Gosliner, T. M.; Behrens, D. W.; & Valdés Á. (2008). *Indo-Pacific Nudibranchs and Sea Slug, A field guide to the world's most diverse fauna*: Sea Challengers Natural History Books, Washington, USA.

Gosliner, T. M.; Behrens, D. W.; & Valdés Á. (2015). *Nudibranch & Sea Slug Identification: Indo-Pacific*. First. Florida, USA: New World Publications, Inc., Jacksonville, FL, USA.

Gosliner, T.; Á. Valdés, & Behrens, DW. (2018). *Nudibranch & Sea Slug Identification Indo-Pacific.* New World Publications, Jacksonville, FL, USA.

Golestani, H.; Crocetta, F.; Padula, V.; Camacho, Y.; Langeneck, J.; Poursanidis, D.; Pola, M.; Yokes, M.B.; Cervera, J.L.; Jung, D.W.; Gosliner, T.M.; Araya, J.-F.; Hooker, Y.; Schrödl, M.; Valdes, A. (2019). The little *Aplysia* coming of age: From one species to a complex of species complexes in *Aplysia parvula* (Mollusca: Gastropoda: Heterobranchia). *Zoological Journal of the Linnean Society, 187*, 279–330, doi:10.1093/zoolinnean/zlz028/5509942.

Goodheart, J. A.; Bleidißel, S.; Schillo, D.; Strong, E. E.; Ayres, D. L.; Preisfeld, A.; Collins, A. G.; Cummings, M. P.; Wagele, H. Comparative morphology and evolution of the cnidosac in Cladobranchia (Gastropoda: Heterobranchia: Nudibranchia). *Frontiers in Zoology*, *15*, 43.

Gosliner, T.M. (1992). Biodiversity of tropical opisthobranch gastropod faunas. In *Proceedings of the Seventh International Coral Reef Symposium, Guam, Micronesia,* 22–27 June 1992, 702–709.

Gosliner, T.M.; Valdés, A.; Behrens, D.W. (2015). *Nudibranch & Sea Slug Identification Indo-Pacific;* New World Publications: Jacksonville, FL, USA, pp. 408.

Gosliner, T.M.; Valdés, A.; Behrens, D.W. (2018). Nudibranch & Sea Slug Identification Indo-Pacific; New World Publications: Jacksonville, FL, USA, pp. 451.

Gulavita, N. K., de Silva, E. D., Hagadone, M. R., Karuso, P., Scheuer, P. J., Van Duyne, G. D. (1986). Nitrogenous Bisabolene Sesquiterpenes from Marine Invertebrates. *Journal of Organic Chemistry, 51*, 5136-5139.

Haber, M.; Cerfeda, S.; Carbone, M.; Calado, G.; Gaspar, H.; Neves, R.; Maharajan, V.; Cimino, G.; Gavagnin, M.; Ghiselin, M.T.; Mollo, E. (2010). Coloration and defense in the nudibranch gastropod *Hypselodoris fontandraui*. *Biology Bulletin, 218,* 181–188, doi: 10.2307/25664519.

Hagadone, M. R.; Burreson, B. J.; Scheuer, P. J.; Finer, J. S.; & Clardy, J. (1979). Defense Allomones of the Nudibranch *Phyllidia varicosa* Lamarck, 1801. *Helvetica Chimica Acta, 62(7),* 2484–94.

Hallas, J. M.; Chichvarkhin, A.; & Gosliner, T. M. (2017). Aligning evidence: concerns regarding multiple sequence alignments in estimating the phylogeny of the Nudibranchia suborder Doridina. *Royal Society. Open Science, 5,* 171095. http://dx.doi.org/10.1098/rsos.171095

Hanwell, M.D.; Curtis, D. E.; Lonie, D.C.; Vandermeersch. T.; Zurek, E.; Hutchison, G. R. (2012). Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics, 4 (1),* 17.

Hebert, P. D. N.; Cywinska A.; Ball, S. L.; & deWaard J, R. (2003). Biological Identifications through DNA Barcodes. *Proceedings of the Royal Society London B, 270,* 313-321.

Hirota, H.; Okino, T.; Yoshimura, E.; Fusetani, N. (1998). Five New Antifouling Sesquiterpenes from Two Marine Sponges of the Genus *Axinyssa* and the Nudibranch *Phyllidia pustulosa*. *Tetrahedron, 54,* 13971–13980.

Hoang, D. T.; Chernomor, O.; von Haeseler, A.; Minh, B. Q & Vinh, L. S. (2017). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *BioRxiv, 35(2),* 518–22.

Horai, H., Arita, M., Kanaya, S. *et al.* (2010) MassBank: a public repository for sharing mass spectral data for life sciences. *Journal of Mass Spectrometry, 45(7),* 703-714.

Huson, D. H.; Richter, D. C.; Rausch, C.; Dezulian, T.; Franz, M.; Rupp, R. (2007). Dendroscope: An interactive viewer for large phylogenetic trees. *BMC Bioinformatics 8,* 460.

Huson, D. H.; & Scornavacca, C. (2012). Dendroscope 3: An Interactive Tool for Rooted Phylogenetic Trees and Networks. *Systematic Biology, 61(6),* 1061–1067.

Inaba, K., Sato, H., Tsuda, M., Kobayashi, J. (1998). Spongiacidins A-D, New Bromopyrrole Alkaloids from *Hymeniacidon* Sponge. *Journal of Natural Products, 61*, 693-695.

Iwashima, M., Terada, I., Iguchi, K., Yamori, T. (2002) New Biologically Active Marine Sesquiterpenoid and Steroid from the Okinawan Sponge of the Genus *Axinyssa*. *Chemical and Pharmaceutical Bulletin, 50(9),* 1286-1289.

Jacob, A. S.; Simon, O. R.; Wheatle, D.; Ruddock, P.; McCook, K. (2014). Antihistamine effect of a pure bioactive compound isolated from slug (*Diplosolenodes occidentalis*) material. *The West Indian Medical Journal 63(5),* 401-7. doi:10.7727/wimj.2013.269.

Jasaimut, S.; Prapbai, S.; Tancharoen, C.; Yuenyongsawad, S.; Hannongbua, S.; Kongsaeree, P.; Plubrukarn, A. (2013). Bridged Tricyclic Sesquiterpenes from the Tubercle Nudibranch *Phyllidia coelestis* Bergh. *Journal of Natural Product, 76,* 2158–2161.

Johannes, R. E. (1963). A Poison-Secreting Nudibranch (Mollusca: Opisthobranchia). *Veliger 5*:104–105.

Jomori, T.; Shibutani, T.; Ahmadi, P.; Suzuka, T.; Tanaka, J. (2015). A New Isocyanosesquiterpene from the Nudibranch *Phyllidiella pustulosa*. *Natural Product Communications, 10(11),* 1913–1914.

Kaligis, F.; Eisenbarth, J.H.; Schillo, D.; Dialao, J.; Schäberle, T.F.; Böhringer, N.; Bara, R.; Reumschüssel, S.; König, G.M.; Wägele, H. (2018) Second survey of heterobranch sea slugs (Mollusca, Gastropoda, Heterobranchia) from Bunaken National Park, North Sulawesi, Indonesia—How much do we know after 12 years. *Marine Biodiversity Records*, *11*, 1–20, https://doi.org/10.3390/d10040127.

Kalyaanamoorthy, S.; Minh, B. Q.; Wong, T. K. F.; von Haeseler, A.; & Jermiin, L. S. (2017). ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature Methods, 14(6),* 587–89.

Kamaruddin, Z. S.; Rondonuwu, S. R.; Maabuat, P. V. (2016). Keragaman lamun (Seagrass) di pesisir desa Lihunu pulau Bangka kecamatan Likupang kabupaten Minahasa Utara, Sulawesi Utara. *Jurnal MIPA Unsrat online 5(1),* 20-24.

Kasamesiri, P.; Meksumpun, S.; & Meksumpun, C. (2011). Spicule network patterns of *Phyllidia varicosa*. *Science Asia, 37(2),* 160. doi:10.2306/scienceasia1513-1874.2011.37.160.

Kase, A; Manembu, I.; Schaduw J. (2019). The condition of Mantehage island coral reefs of North Minahasa district, North Sulawesi province. *Jurnal Pesisir dan Laut Tropis 7(3),* 208. DOI: 10.35800/jplt.7.3.2019.24466

Kasenda, P.; Boneka, F. B.; Wagey, B. T. (2013). Lokasi bertelur penyu di pantai timur kabupaten Minahasa provinsi Sulawesi Utara. *Jurnal Pesisir dan Laut Tropis* 1(2).

Kassühlke, K. E.; Potts, B. C. M.; Faulkner, D. J. (1991). New nitrogenous sesquiterpenes from two Philippine nudibranchs, *Phyllidia pustulosa* and *P. varicosa*, and from a Palauan sponge, *Halichondria* cf. *lendenfeldi*. *Journal of Organic Chemistry, 56,* 3747–3750.

Kobayashi, J., Kanda, F., Ishibashi, M., Shigemori, H. (1991). Manzacidins A-C, Novel Tetrahydropyrimidine Alkaloids from the Okinawan Marine Spone *Hymeniacidon* sp. *Journal of Organic Chemistry, 56,* 4574-4576.

Korshunova, T.; Martynov, A.; Picton, B. (2017). Ontogeny as an important part of integrative taxonomy in tergipedid aeolidaceans (Gastropoda: Nudibranchia) with a description of a new genus and species from the Barents Sea, *Zootaxa, 4324(1),* 1–22.

Larkin, M.F.; Smith, S.D.A., Willan, R.C.; Davis, T.R. (2018). Diel and seasonal variation in heterobranch sea slug assemblages within an embayment in temperate eastern Australia. *Marine Biodiversity*, *48*, 1541–1550. doi: 10.1007/s12526-017-0700-9.

Lasut, M. T.; Weber, M.; Pangalila, F.; Rumampuk, N. D. C.; Rimper, J. R. T. S. L.; Warouw, F.; Kaunang S. T.; Lott, C. (2017). *Proceedings of the International Conference on Microplastic Pollution in the Mediterranean Sea*. In: Cocca M., Di Pace E., Errico M., Gentile G., Montarsolo A., Mossotti R. (eds). Springer Water. Springer, Cham. pp 107-113. https://doi.org/10.1007/978-3-319-71279-6_15.

Layton, K. K. S.; Gosliner, T. M.; Wilson, N.G. (2018). Flexible color check patterns obscure identification and mimicry in Indo–Pacific *Chromodoris* nudibranchs (Gastropoda: Chromodorididae). *Molecular Phylogenetics and Evolution, 124*, 27–36.

Layton, K. K. S.; Carvajal, J. I.; Wilson, N. G. (2020). Mimicry and mitonuclear discordance in nudibranchs: New insights from exon capture phylogenomics. *Ecology and Evolution, 10(21)*, 11966-11982. https://doi.org/10.1002/ece3.6727

Leigh, J. W.; Bryant, D. (2015). Popart: Full-feature Software for Haplotype Network Construction. British Ecological Society. *Methods in Ecology and Evolution. 6(9)*, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lyakhova, E. G.; Kolesnikova, S. A.; Kalinovskii, A. I.; Stonik, V. A. (2010). Secondary metabolites of the Vietnamese nudibranch mollusk *Phyllidiella pustulosa*. *Chemistry of Natural Compounds, 46(4)*, 534–538.

Macri, G. (1986). Notes and remarks on *Phyllidia pulitzeri* Pruvot- Fol, 1962. *La Conchiglia 18(208-209)*, 3-5.

Manzo, E.; Ciavatta, M. L.; Gavagnin, M.; Mollo, E.; Guo, Y. W.; & Cimino, G. (2004). Isocyanide Terpene Metabolites of Phyllidiella Pustulosa, a Nudibranch from the South China Sea. *Journal of Natural Products, 67(10)*, 1701–1704.

Martín-Hervás, M.R.; Carmona, L.; Malaquias, M.A.E.; Krug, P.J.; Gosliner, T.; Cervera, J.L. (2019). Integrative Systematics of the Genus *Thuridilla* Bergh, 1872 (Mollusca, Gastropoda, Heterobranchia) reveals a cryptic radiation of polymorphic sea slug species. In: *Proceedings of World Congress of Malacology*, California Academy of Sciences: Pacific Grove, CA, USA, 11–16 August 2019; pp. 119. https://www.calacademy.org/sites/default/files/final_abstract_volume_2019_1.pdf

Martynov, A.V.; Korshunova, T.A. (2012). Opisthobranch molluscs of Vietnam (Gastropoda: Opisthobranchia); In *Benthic Fauna of the Bay of Nhatrang, Southern Vietnam 2*; Britayev, T.Z., Pavlov, D.S., Eds.; KMK Scientific Press: Moscow, Russia, pp. 142–257.

Matsuda, S.B.; Gosliner, T.M. (2018). Glossing over cryptic species: Descriptions of four new species of *Glossodoris* and three new species of *Doriprismatica* (Nudibranchia: Chromodorididae). *Zootaxa, 4444*, 501–529, doi:10.11646/zootaxa.4444.5.1.

Mayr, E. (1981). Biological classification: Toward a synthesis of opposing methodologies. *Science, 214*, 510-516.

Minh, B. Q.; Nguyen, M. A. T.; & Von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution, 30(5)*, 1188–1195.

Minh, B. Q, Trifinopoulos, J.; Schrempf, D.; & Schmidt, H. A. (2017). MANUAL Phylogenomic Software by Maximum Likelihood.

Moles, J.; Wägele, H.; Cutignano, A.; Fontana, A.; Ballesteros, M.; & Avila, C. (2017). Giant embryos and hatchlings of Antarctic nudibranchs (Mollusca: Gastropoda: Heterobranchia). *Marine Biology, 164 (114)*. DOI 10.1007/s00227-017-3143-8.

Musman, M.; Tanaka, J.; Higa, T. (2001). New sesquiterpene carbonimidic dichlorides and related compounds from the sponge *Stylotella aurantium*. *Journal of Natural Products, 64*, 111-113.

Naturalis Biodiversity Center, Repository, 4. Available online: https://repository.naturalis.nl/#filter=all:rumphius%20biohistorical (accessed on 9 February 2019).

NCBI Resource Coordinators. (2018). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research. 4,46 (D1)*: D8-D13. doi: 10.1093/nar/gkx1095. PMID: 29140470; PMCID: PMC5753372.

Neusser, T. P.; Fukuda, H.; Jörger, K. M.; Kano, Y.; Schrödl, M. (2011). Sacoglossa or Acochlidia? 3D reconstruction, molecular phylogeny and evolution of Aitengidae (Gastropoda: Heterobranchia). *Journal of Molluscan Studies, 77*, 332-350. doi:10.1093/mollus/eyr033.

Nguyen, L. T.; Schmidt, H. A.; Von Haeseler, A.; & Minh, B. Q. (2015). IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution*, *32(1)*, 268–74.

Nikitenko, E.; Ereskovsky, A.; Vortsepneva, E. (2020). Ontogenetic dynamics of the subepidermal spicule complex in Nudibranchia (Gastropoda): the case of *Onchidoris muricata, Zoology*. https://doi.org/10.1016/j.zool.2020.125886

Nimbs, M.J.; Larkin, M.; Davis, T.R.; Harasti, D.; Willan, R.C.; Smith, S.D.A. (2016). Southern range extensions for twelve heterobranch sea slugs (Gastropoda: Heterobranchia) on the eastern coast of Australia. *Marine Biodiversity Records, 9*, 1–12, doi: 10.1186/s41200-016-0027-4.

Nimbs, M.J.; Smith, S.D.A. (2018). Beyond Captricornia: Tropical sea slugs (Gastropoda, Heterobranchia) extend their distributions into the Tasman Sea. *Diversity*, *10*, 99, doi: 10.3390/d10030099.

Nothias, L. F.; Petras, D.; Schmid, R. (2020) Feature-based molecular networking in the GNPS analysis environment. *Nature Methods, 17*, 905–908. https://doi.org/10.1038/s41592-020-0933-6

Odum, H.T. (1951). Nudibranch spicules made of amorphous calcium carbonate. *Science 114*, p. 395.

Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. (1996). New antifouling sesquiterpenes from four nudibranchs of the family Phyllidiidae. *Tetrahedron*, *52*, 9447–9454.

Ompi, M.; Lumoindong, F.; Undap, N.; Papu, A.; Wägele, H. (2019). Monitoring marine Heterobranchia in Lembeh Strait, North Sulawesi (Indonesia), in a changing environment. *AACL Bioflux, 12*, 664–667.

Oskars, T. R.; & Malaquias, M. A. E. (2020). Systematic revision of the Indo-West Pacific mangrove-associated snails of the genus Bakawan (Cephalaspidea: Haminoeidae). *Journal of Molluscan Studies 86(4),* 1-19. Doi: 10.1093/mollus/eyaa012

Padula, V.; Bahia, J.; Stöger, I.; Camacho-Garcia, Y.; Malaquias, M.A.E.; Cervera, J.L.; Schrödl, M. (2016). A test of color-based taxonomy in nudibranchs: Molecular phylogeny and species delimitation of the *Felimida clenchi* (Mollusca: Chromodorididae) species complex. *Molecular Phylogenetics and Evolution, 103*, 215–229.

Palumbi, S. S. R.; W.; Mcmillan, O.; & Grabowski. G. (2002). *The Simple Guide to PCR. 96822(808)*, 1–45.

Papu, A.; Undap, N.; Martinez, N. A.; Segre, M. R.; Datang, I. G.; Kuada, R.R.; Perin, M.; Yonow, N.; & Wägele, H. (2020). First Study on Marine Heterobranchia (Gastropoda, Mollusca) in Bangka Archipelago, North Sulawesi, Indonesia. *Diversity, 12(2),* 1-72.doi: 10.3390/d12020052.

Paringgi E.; Mamuaja, J.; Rampengan, R.; Ompi, M.; Roeroe, K. A.; Rembet, U. (2018). Sebaran spasial foraminifera bentik pada terumbu karang Pulau Bunaken Sulawesi Utara. *Jurnal Pesisir dan Laut Tropik 6(1),* 33.

Pasternak, G.; Galil, B.S. (2012). An established population of the alien sea slug *Elysia grandifolia* Kelaart, 1858 (Mollusca, Opisthobranchia, Elysiidae) off the Mediterranean coast of Israel, *BioInvasions Record, 1(3),* 221–223.

Patra, A.; Chang C. W. J.; Van Duyne, G. D.; Matsumoto, G. K.; Scheuer, P. J.; Clardy, J. (1984). An Unprecedented Triisocyano Diterpenoid Antibiotic from a Sponge. *Journal of American Chemical Society, 106,* 7981-7983.

Patty, W.; Manu, G.; Reppie, E.; Dey, L. N. (2015). Coral Fish Communities on the Biorock Artificial Reef in Coastal Waters of Siladen Island, Manado, North Sulawesi. *Jurnal Perikanan (J. Fish. Sci.) XVII (2),* 73-78.

Penney, B. K. (2008). Phylogenetic comparison of spicule networks in cryptobranchiate dorid nudibranchs (Gastropoda, Euthyneura, Nudibranchia, Doridina). *Acta Zooogica, 89,* 311–329.

Pentinsaari, M.; Salmela, H.; Mutanen, M.; Roslin, T. (2016). Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Scientific Reports, 6(1),* 35275. DOI: 10.1038/srep35275.

Pinheiro, C. E. G.; & Freitas, A. V. (2014). Some possible Cases of Escape Mimicry in Neotropical Butterflies. *Neotropical Entomology 43,* 393-398. https://doi.org/10.1007/s13744-014-0240-y

Pluskal, T.; Castillo, S.; Villar-Briones, A.; Orešič, M. (2010). MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based

molecular profile data. *BMC Bioinformatics 11*, 395. https://doi.org/10.1186/1471-2105-11-395.

Pola, M.; Cervera, J. L.; Gosliner, T. M. (2008). Revision of the Indo-Pacific genus *Nembrotha* (Nudibranchia: Doridídae: Polyceridae), with a description of two new species, *Scienta Marina*, *72(1)*, 145–183, doi:10.3989/scimar.2008.72n1145.

Poli, D. (2018). *North Sulawesi Ecology: Coral triangle*. CORALEYE-Indonesian reef research outpost (Presentation).

Ponder W. F.; Lindberg, D. R.; Ponder, J. M. (2020). *Biology and Evolution of the Mollusca*, Volume two. Taylor & Francis Group, 419-525.

Ponti, M.; Fratangeli, F.; Dondi, N.; Reinach, M.S.; Serra, C.; Sweet, M.J. (2016). Baseline reef health surveys at Bangka Island (North Sulawesi, Indonesia) reveal new threats. *PeerJ*, *4*, e2614; doi: 10.7717/peerj.2614.

Proćków, M.; Kuźnik-Kowalska, E.; Pieńkowska, J.; Żeromska, A.; Mackiewicz, P. (2021). Speciation in sympatric species of land snails from the genus Trochulus (Gastropoda, Hygromiidae). *Zoologica Scripta 50*:16-42. DOI: 10.1111/zsc.12458

Pruvot-Fol, A. (1957). Révision de La Famille Des Phyllidiidae. *Journal de Conchyliologie, 2(97)*, 104–35.

Puillandre, N.; Lambert, A.; Brouillet, S. & Achaz. G. (2012). ABGD, Automatic Barcode Gap Discovery for Primary Species Delimitation. *Molecular Ecology*, *21(8)*, 1864–77.

Purohit, A.L. (2006) Possible *Aplysia vaccaria* from Santa Catalina Island. *[Message in] Sea Slug Forum.* Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/17246. Accessed on 29 March 2021.

Putz, A.; König, G. M.; Wagele, H. (2010). Defensive strategies of Cladobranchia Gastropoda, Opisthobranchia). *Natural Product Reports*, 27, 1386-1402

Qu, C. F.; Song, J. M.; Li, N. (2014). Causes of jellyfish blooms and their influence on marine environment. *Ying Yong Sheng Tai Xue Bao*, *25*, 3701–3712.

Raghunathan, C. (2015). Diversity of Reef Associated Macrofauna of Rutland Island, Andaman and Nicobar Islands. *Records of the Zoological. Survey. India, Occasional Paper 370*, 1-152 (Published by the Director, Zool. Surv. India, Kolkata).

Rambaut, A. (2018). FigTree, Version 1.4.4. https://www.google.com/url?q=https://github.com/rambaut/figtree/releases&sa=D&source=editors&ust=1614339947350000&usg=AOvVaw3a6mHrF4JUUiZH4ome0tbm.

Rangan, J. K. (2010). Inventarisasi Gastropoda di lantai hutan mangrove desa Rap-rap kabupaten Minahasa Selatan Sulawesi Utara. *Jurnal Perikanan dan Kelautan VI (1),* 63-66.

Ritson-Williams, R. & Paul, V. J. (2007). Marine benthic Invertebrates use multimodal cues for defense against reef fish. *Marina Ecology Progress Series 340*, 29-39.

Rompis, B. R.; Langoy, M. L. D.; Papu, A. (2013). Diversitas Echinodermata di Pantai Meras Kecamatan Bunaken Sulawesi Utara (Diversity of Echinoderms on the Meras Beach, Bunaken District, North Sulawesi). *Jurnal Bios Logos 3(1)*. DOI: 10.35799/jbl.3.1.2013.3465.

Rudman, W. B. (2004 July 23) Mimicry. *[In] Sea Slug Forum.* Australian Museum, Sydney. Available from http://www.seaslugforum.net/find/mimicry

Rudman, W. B. (2004a July 27) Opisthobranch defence mechanisms. *[In] Sea Slug Forum.* Australian Museum, Sydney. Available in http://www.seaslugforum.net/find/defgen

Ruga, L.; Langoy, M. Kolondam B.; Papu, A. (2014). Identifikasi zooplankton di perairan Pulau Bunaken Manado. Jurnal MIPA 3(2):84-86. DOI: 10.35799/jm.3.2.2014.5856

Sasaki, H.; Hosokawa, T.; Sawada, M.; Ando, K. (1973). Isolation and structure of ascofuranone and ascofuranol, antibiotics with hypolipidemic activity. *Journal of Antibiotics*, 26(11), 676-680.

Schaduw, J. N. W. (2016). Kondisi ekologi mangrove pulau Bunaken Kota Manado provinsi Sulawesi Utara. *Jurnal LPPM Bidang Sains dan Teknologi 3(2)*, 64-74.

Schillo, D.; Wipfler, B.; Undap, N.; Böhringer, N.; Eisenbarth, J.-H.; Kaligis, F.; Bara, R.; Schäberle, T.F.; König, G.M.; Wägele, H. (2019). Description of a new *Moridilla* species from North Sulawesi, Indonesia (Mollusca: Nudibranchia: Aeolidioidea)—based on MicroCT, histological and molecular analyses, *Zootaxa, 4652(2)*, 265–295.

Schulte, G.R. (1982). Allochemistry of some Hawaiian dorid nudibranchs. *Dissertation Abstracts International, B 42(7)*, 2841.

Sea Slug Forum Home page. Available online: http://www.seaslugforum.net/ (accessed on 10 December 2019).

Setiawan, F.; Kusen, J. D.; Kaligis, G. J. F. (2013). Struktur komunitas ikan karang di perairan terumbu karang Taman Nasional Bunaken, Sulawesi Utara. *Jurnal Perikanan dan Kelautan Tropis, 9 (1),* 13. DOI: 10.35800/jpkt.9.1.2013.3447

Shannon P.; Markiel, A.; Ozier, O.; Baliga, N. S.; Wang, J. T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research, 13(11),* 2498–504.

Sharma, G., Magdoff-Fairchild, B. (1977). Natural Products of Marine Sponges. 7. The Constitution of Weakly Basic Guanidine Compounds, Dibromophakellin and Monobromophakellin. *Journal of Organic Chemistry, 42(25)*, 4118-4124.

Sim, D. C-M.; Wayan, M. I.; White, A. M.; Wayan, M. N.; Loh, J. J. M.; Cheney, K.; Garson, M. J. (2018). New sesquiterpenoid isonitriles from three species of phyllidid nudibranchs. *Fitoterapia*, *126*, 69–73.

Simpson, J. S.; Brust, A.; Garson, M. J. (2004). Biosynthetic pathways to dichloroimines; precursor incorporation studies on terpene metabolites in the tropical marine sponge *Stylotella aurantium*. *Organic and Biomolecular Chemistry, 2*, 949–956.

Smith, S. D. A.; Nimbs, M. J. (2017). Quantifying temporal variation in heterobranch (Mollusca: Gastropoda) sea slug assemblages: Tests of alternate models. *Moll Res., 37*, 140–147.

Smith, S. D. A.; Davis, T. R. (2019). Slugging it out for science: Volunteers provide valuable data on the diversity and distribution of heterobranch sea slugs. *Molluscan Research, 39*, 214–233, doi: 10.1080/13235818.2019.1594600.

Stoffels, B. E. M. W.; van der Meij, S. E.T.; Hoeksema, B.W.; van Alphen, J.; van Alen, T.; Meyers-Munoz, M. A.; de Voogd, N.J.; Tuti, Y.; & van der Velde, G. (2016). Phylogenetic relationships within the Phyllidiidae (Opisthobranchia, Nudibranchia). *ZooKeys, 605*, 1–35, doi:10.3897/zookeys.605.7136.

Sudarto, Lalu, X.C.; Kosen, J.D. Tjakrawidjaja, A. H.; Kusumah, R. V.; Sadhotomo, B.; Kadarusman; Pouyaud L.; Slembroujck, J.; Paradis E. (2010). Mitochondrial genomic divergence in coelacanths (*Latimeria*): slow rate of evolution or recent speciation? *Marine Biology, 157*, 2253–2262.

Tanaka, J.; Higa, T. (1999). Two new cytotoxic carbonimidic dichlorides from the Nudibranch *Reticulidia fungia*. *Journal of Natural Products, 62*, 1339–1340.

Tasdemir, D., Bugn, T. S., Mangalindan, G. C., Concepcion, G. P., Harper, M. K., Ireland, C. M. (2002). Cytotoxic Bromoindole Derivatives and Terpenes from the Philippine Marine Sponge *Smenospongia* sp. *Zeitschrift für Naturforschung, 57c*, 914-922.

Tibiriçá, Y.; Pola, M.; Cervera, J.L. (2017). Astonishing diversity revealed: An annotated and illustrated inventory of Nudipleura (Gastropoda: Heterobranchia) from Mozambique, *Zootaxa, 4359(1)* 1–133, https://doi.org/10.11646/zootaxa.4359.1.1.

Thompson, J. E., Walker, R. P., Wratten, S. J., Faulkner, J. D. (1981) A Chemical Defense Mechanism for the Nudibranch *Cadlina luteomarginata. Tetrahedron, 38(13),* 1865-1873.

Tito, K; Setiawan, A.; Cahyarini, S. Y.; Nugroho, S. C.; Zaky, A. R.; Saputra, A. J. (2015). Analisis pertumbuhan karang Porites di Pulau Siladen Utara, Bunaken, Sulawesi Utara. *Conference: Seminar Nasional Tahunan XII Hasil Penelitian Perikanan dan Kelautan, Universitas Gajah Mada.* Kelautan (KL–12)-217.

Trifinopoulos, J.; Nguyen, L-T.; von Haeseler, A.; Minh, B. Q. (2016). W-IQ-tree: a fast online pgylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research. 44*, 232-235. Doi: 10.1093/nar/gkw256.

Tonozuka, T. (2003). *Opisthobranchs of Bali and Indonesia*; Hankyu Communications Co. Ltd.: Tokyo, Japan, pp. 164.

Undap, N.; Papu, A.; Schillo, D.; Ijong, F.G.; Kaligis, F.; Lepar, M.; Hertzer, C.; Böhringer, N.; König, G.M.; Schäberle, T.F.; & Wägele. H. (2019). First survey of heterobranch

sea slugs (Mollusca, Gastropoda) from the island Sangihe, North Sulawesi, Indonesia. *Diversity, 11*, 1–30.

Usman, M. S.; Kusen, J. D.; Rimper, J. R. T. S. L. (2013). Struktur Komunitas Plankton di Perairan Pulau Bangka Kabupaten Minahasa Utara, *J. Pesisir dan Laut Tropis*, *2*, 51–57.

Valdés, Á & Ortea, J. (1996). Review of the family Phyllidiidae in the Atlantic Ocean (Nudibranchia, Doridoidea). *American Malacological Bulletin, 13(1/2),* 1-9.

Valdés, Á. & Gosliner, T. M. (1999). Phylogeny of the Radula-Less Dorids (Mollusca, Nudibranchia), with the description of a New Genus and a New Family. *Zoologica Scripta, 28(3–4),* 315–360.

Valdés, Á. (2001). Deep-water phyllidiid nudibranchs (Gastropoda: Phyllidiidae) from the tropical south-west Pacific Ocean. *In* P. Bouchet & B. A. Marshall (Eds), *Tropical deep-sea benthos, 22(185),* 331-368.

Valdés, Á. (2003). Preliminary Molecular Phylogeny of the Radula-Less Dorids (Gastropoda: Opisthobranchia), Based on 16S MtDNA Sequence Data. *Journal of Molluscan Studies, 69(1),* 75–80.

Van Soest, R. W. M.; Boury-Esnault, N.; Hooper, J. N. A.; Rützler, K.; de Voogd, N. J.; Alvarez, B.; Hajdu, E.; Pisera, A. B.; Manconi, R.; Schönberg, C.; Klautau, M.; Kelly, M.; Vacelet, J.; Dohrmann, M.; Díaz, M.-C.; Cardenas, P.; Carballo, J. L.; Ríos, P.; Downey, R.; Morrow, C. C. World Porifera Database. Pseudaxinyssa pitys de Laubenfels, 1954. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=193138 on 2019-10-17.

Venkataraman, K.; Raghunathan, C.; Raghuraman, R. and Dixit, S. (2015). *Fascinating Seaslugs & Flatworms of Indian Seas*: 1-149. Publication Division by the Director, Zoological Survey of India, New Alipore, Kalkota, India. Wägele H. (1985). The anatomy and histology of *Phyllidia pulitzeri* Pruvot-Fol, 1962, with remarks on the three Maditerranean species of *Phyllidia* (Nudibranchia, Doridacea). *The veliger 28(1)*, 63-79.

Wägele, H. (1987). The distribution of some Antartic nudibranchs (Opisthobranchia). *Jurnal of Mollusca Studies, 53,* 179-188.

Wägele, H. & Willan, R. C. (2000). Phylogeny of the Nudibranchia. *Zoological Journal of the Linnean Society, 130(1),* 83–181.

Wägele, H. (2004). Potential key characters in Opisthobranchia (Gastropoda, Mollusca) enhancing adaptive radiation. *Organisms, Diversity & Evolution 4,* 175–188.

Wägele H.; Ballesteros M.; Avila, C. (2006). Defensive glandular structures in opisthobranch molluscs - from histology to ecology. *Oceanography and Marine Biology: An Annual Review, 44,* 197-276.

Wägele, H.; Klussmann-Kolb, A.; Vonnemann, V. and Medina, M. 2008. Heterobranchia I: the Opisthobranchia. In *Phylogeny and Evolution of the Mollusca.* Edited by W. F. Ponder and D. R. Lindberg. Berkeley: University of California Press. pp 383-406.

Wägele, J-W. (2005). *Foundations of phylogenetic systematics*. Verlag Dr. Friedrich Pfeil, München.

Waisaley, R. S.; Kaligis, E.; Ompi, M.; Kumampung, D. R. H.; Sinjal C. A.: Rangan, J. (2019). Inventarisasi jenis Dekapoda di perairan pantai kelurahan Tongkaina, kota Manado. *Jurnal Pesisr dan Laut Tropis 7(2),* 71-78.

Wallraff, K. T. (2018). Investigation of the Hermaphrodite Reproductive System of Selected Phyllidiid Species (Nudibranchia, Heterobranchia, Gastropoda). *Bachelor Thesis*. Rheinischen Friedrich-Wilhelms-Universität Bonn.

Wang, M.; Carver, J. J.; Phelan, V. V.; Sanchez, L. M.; Garg, N.; Peng, Y.; Nguyen, D. D. (2016). Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Natural Biotechnology*, *34(8),* 828.

Wells, F.E.; Bryce, C.W. (1993). *Sea Slugs and Their Relatives of Western Australia;* Western Australian Museum: Perth, Australia, p. 184.

White, A. M.; Pierens, G. K.; Skinner-Adams, T.; Andrews, K. T.; Bernhardt, P. V.; Krenske, E. H.; Mollo, E.; Garson, M. J. (2015). Antimalarial isocyano and isothiocyanato sesquiterpenes with tri- and bicyclic skeletons from the nudibranch *Phyllidia ocellata. Journal of Natural Products*, 78, 1422-1427.

White, A. M.; Dao, K.; Vrubliauskas, D.; Könst, Z. A.; Pierens, G. K.; Mandi, A.; Andrews, K. T.; Skinner-Adams, T. S.; Clarke, M.́ E.; Narbutas, P. T.; Sim, D. C-M.; Cheney, K. L.; Kurtan, T.; Garson, M. J.; Vanderwal. (2017). Catalyst-Controlled Stereoselective Synthesis Secures the Structure of the Antimalarial Isocyanoterpene Pustulosaisonitrile-1. *Journal of Organic Chemistry, 82,* 13313-13323.

Whitten, T; Henderson, G. S.; Mustafa, M. (2002). *The ecology of Sulawesi vol. IV*. Periplus Eds Ltd., pp. 754.

Winters, A.E.; Wilson, N.G.; van den Berg, C.P.; How, M.J.; Endler, J. A.; Marshall, N.J.; White, A.M.; Garson, M.J.; Cheney, K.L. (2018). Toxicity and taste: Unequal chemical defences in a mimicry ring. *Proceedings of the Royal Society B: Biological Sciences*, *285* (1880), doi:10.1098/rspb.2018.0457.

Wollscheid-Lengeling, E.; Boore, J.; Brown, W.; & Wägele, H. (2001). The Phylogeny of Nudibranchia (Opisthobranchia, Gastropoda, Mollusca) Reconstructed by Three Molecular Markers. *Organisms Diversity & Evolution, 1(4),* 241–56.

WoRMS Editorial Board (2021). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 2020-01-17. Doi: 10.14284/170 (accessed on 17 January 2020).

Wratten, S. J.; Faulkner, D. J. (1977). Carbonimidic dichlorides from the marine sponge *Pseudaxinyssa pitys. Journal of the American Chemical Society, 99,* 7367–7368.

Wright, A. D. (2003). GC-MS and NMR analysis of *Phyllidiella pustulosa* and one of its dietary sources, the sponge *Phakellia carduus*. *Comparative Biochemistry and Physiology part A, 134*, 307–313.

Yajima, A.; Saitou, F.; Sekimoto, M.; Maetoku, S.; Nukada, T.; Yabuta, G. (2005). Synthesis of cordiaquinones J and K via *B*-alkyl Suzuki-Miyaura coupling as a key step and determination of the absolute configuration of natural products. *Tetrahedron, 61*, 9164–9172.

Yasman. (2003). Observation on the feeding of nudibranch *Phyllidia varicosa* Lamarck, 1801 on the sponge *Axinyssa* cf. *aculeata* Wilson, 1925 in coral reefs of Pramuka Island, Thousands Islands National Park, Indonesia, *Makara, Sains. 7(1)*, 15–21.

Yonow, N. (1986). Red Sea Phyllidiidae (Mollusca, Nudibranchia), with Descriptions of New Species. *Journal of Natural History, 20(6)*, 1401–1428.

Yonow, N. (1996). Systematic revision of the family Phyllidiidae in the Indian Ocean Province: Part 1 (Opisthobranchia: Nudibranchia: Doridoidea). *Journal of Conchology, 35(6)*, 483 - 516.

Yonow, N. (2001). Results from the Rumphius Biohistorical Expedition to Ambon (1990). Part 11. Doridacea of the families Chromodorididae and Hexabranchidae (Mollusca, Gastropoda, Opisthobranchia, Nudibranchia). *Zoologische Mededelingen*, *75*, 1–50.

Yonow, N. (2011). Results from the Rumphius Biohistorical Expedition to Ambon (1990). Part 15. The suborder Doridina (Mollusca, Gastropoda, Opisthobranchia, Nudibranchia). *Zoologische Mededelingen*, *85*, 905-956. http://www.zoologischemededelingen.nl/cgi/t/text/get-pdf?c=zoomed;idno=8504a17

Yonow, N. (2012). Opisthobranchs from the Western Indian Ocean, with descriptions of two new species and ten new records (Mollusca, Gastropoda). *ZooKeys, 197,* 1-129. https://zookeys.pensoft.net/articles.php?id=2811

Yonow, N. (2017). Results from the Rumphius Biohistorical Expedition to Ambon (1990). Part 16. Nudibranchia—Dendronotina, Arminina, Aeolidina, and Doridina (Mollusca: Gastropoda: Heterobranchia). *Archiv für Molluskenkunde, 146,* 135–172, doi:10.1127/arch.moll/146/135-172.

Yonow, N. (2018). Red Sea Opisthobranchia 5: new species and new records of chromodorids from the Red Sea (Heterobranchia, Nudibranchia, Chromodorididae). *ZooKeys 770*, 9–42. https://doi.org/10.3897/zookeys.770.26378.

Yonow, N.; Jensen, K.R. (2018). Results of the Rumphius Biohistorical Expedition to Ambon (1990). Part 17. The Cephalaspidea, Anaspidea, Pleurobranchida, and Sacoglossa (Mollusca: Gastropoda: Heterobranchia). *Archiv für Molluskenkunde*, *147*, 1–48, doi:10.1127/arch.moll/147/001-048.

Yonow, N. (2020). Red Sea Opisthobranchia 6: Phyllidiidae and their paradorid mimic: new species and new records (Heterobranchia, Nudibranchia, Doridina). *ZooKeys, 1006*, 1-34. https://zookeys.pensoft.net/article/59732/

Zdero, C.; Bohlmann, F.; Niemeyer, H. M. (1990). *Seco*-Labdanes and other constituents from *Ophryosporus floribundus*. *Phytochemistry, 29(10)*, 3247–32537.

Zhang, P.; Bao, B.; Dang, H. T.; Hong, J.; Lee, H. J.; Yoo, E. S.; Bae, K. S.; Jung, J. H. (2009). Anti-inflammatory sesquiterpenoids from a sponge-derived fungus *Acremonium* sp. *Journal of Natural Product, 72*, 270–275.

Zhang, J.; Kapli, P.; Pavlidis, P.; & Stamatakis. A. (2013). A General Species Delimitation Method with Applications to Phylogenetic Placements. *Bioinformatics, 29(22),* 2869–2876.

Appendix

1. Participation in other studies during my thesis

This study was funded by BMBF and DAAD within the project "Indonesian Opisthobranchs and Associated Microorganisms – From Biodiversity to Drug Lead Discovery (INDOBIO)", a joint collaboration between two countries. This project has involved Germany and Indonesian students. For further information about the project please go to following website of the DAAD: Indonesian Opisthobranchs and Associated Microorganisms – From Biodiversity to Drug Lead Discovery (INDOBIO).

During my PhD study, two papers have been published with me as first authors (chapter 3 and Chapter 4). I additionally have taken part in following studies and have co-authored the publications:

1. Eisenbarth J-H, Undap N, **Papu A**, Schillo D, Dialao J, Reumschüssel, S, Kaligis F, Bara R, Schäberle TF, König GM, Yonow N, Wägele H. 2018. Marine Heterobranchia (Gastropoda, Mollusca) in Bunaken National Park, North Sulawesi, Indonesia - a follow-up diversity study. Diversity 10, 127.

   My task here was to identify the phyllidiid taxa using morphological and molecular data. (barcoding). I validated or corrected preliminary identification, helped in writing the method part and summarized and translated local references.

2. Schillo D, Wipfler B, Undap N, **Papu A**, Böhringer N, Eisenbarth J-H, Kaligis F, Bara R, Schäberle T, König GM, Wägele H. 2019. Description of a new Moridilla species from North Sulawesi, Indonesia (Nudibranchia: Cladobranchia, Aeolidioidea) – based on MicroCT, histological and molecular analyses. Zootaxa 4652, 265-295.

   For this study, I collected facelinid specimens (Cladobranchia) at the Bangka Archipelago (BA), prepared and analysed facelinid sequences from BA, provided the metadata for the study. I helped in writing the method part.

3. Ompi M, Undap N, **Papu A,** Wägele H. 2019.Monitoring marine Heterobranchia in Lembeh Strait, North Sulawesi (Indonesia), in a changing environment. AACL Bioflux 12, 664-677

   In this study, my task was to identify the Phyllidiidae by barcoding and correct the identification, as well as write the text with regard to this group. In this study, Phyllidiidae were the major group represented.

4. Undap N, **Papu A**, Schillo D, Ijong FG, Kaligis F, Lepar M, Hertzer C, Böhringer N, König GM, Schäberle TF, & Wägele H. 2019. First survey of Heterobranch sea slugs (Mollusca, Gastropoda) from the Island Sangihe, North Sulawesi, Indonesia. Diversity, 11(9), 1–32.

   My task was again to identify the Phyllidiidae by barcoding and correct the identification, as well as write the text with regard to Phyllidiidae. The workload was higher than in the other studies, because the collection comprised many more phyllidiids, then other taxa.

5. Papu A, Raubold I, Simon J, Wägele H. (in preparation). Description of a new species of *Phyllidia* from Bunaken National Park Indonesia, by using integrative methodologies with a revision of the genus *Phyllidia*. (To be submitted to the Journal of European taxonomy).

6. In this manuscript that is still in the writing process, I started with the MicroCT and histological analyses, compiled many data from literature, but then handed it over to students because of lack of time. I am helping at the moment Prof. H. Wägele in supervising the students (one master and one bachelor student). With this manuscript, I intend to pursue and deepen my interests in the large Phyllidiidae group. With this project, where I learned all necessary tools for describing a species and gained a lot of expertise in this subject, I will be able to pass on my knowledge on taxonomic and phylogenetic analyses to students in my country and my university, UNSRAT, in Manado. This kind of scientific investigation is not so popular in my country, although it is the base for so many other disciplines, including sustainable use of marine ecosystems.

2. Curriculum Vitae

3. Supplementary material Chapter 4

Figure S1. ^{1}H NMR spectrum of compound **1** in MeOD-*d*$_4$.

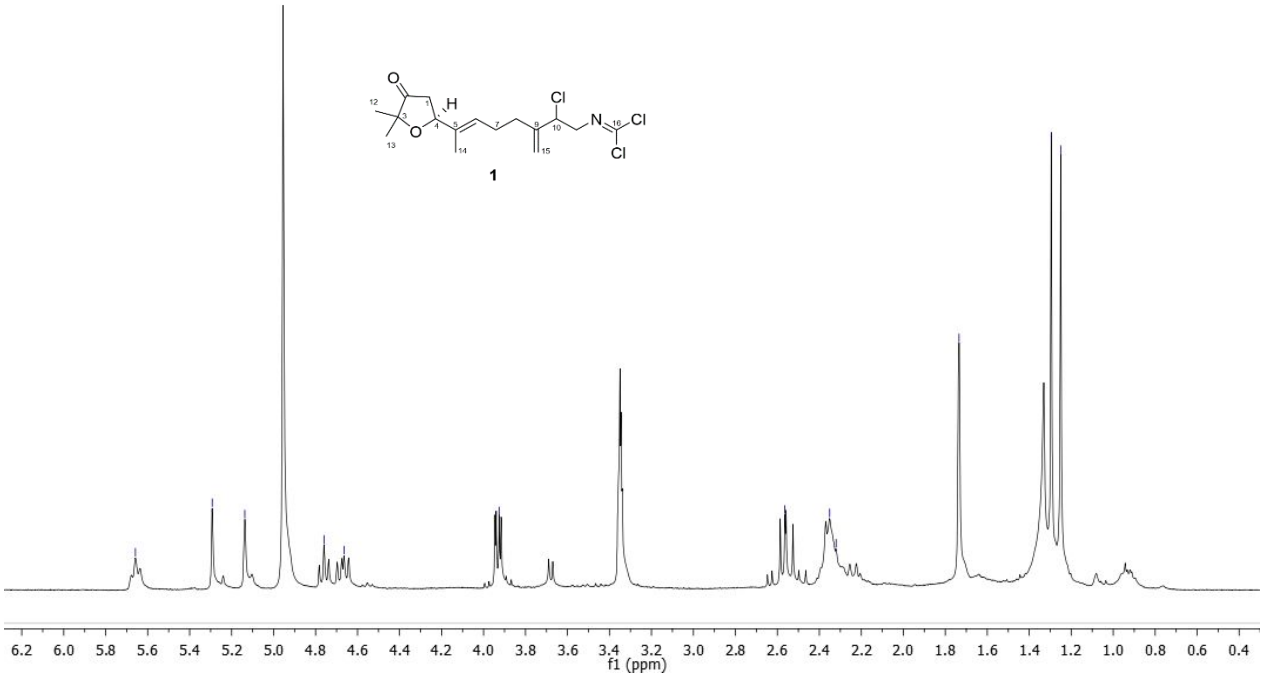


Figure S2. ^{13}C NMR spectrum of compound **1** in MeOD-*d*$_4$.

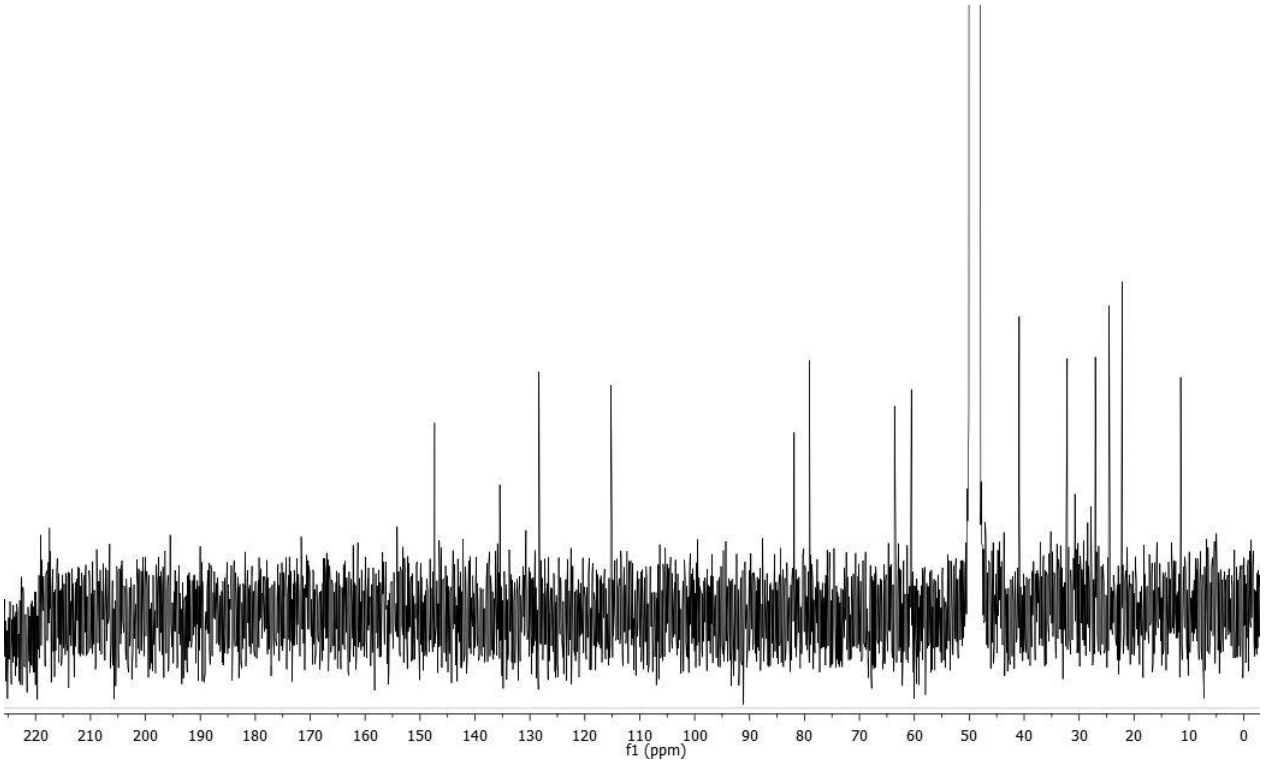


Figure S3. HR-ESIMS spectrum of compound **1**.

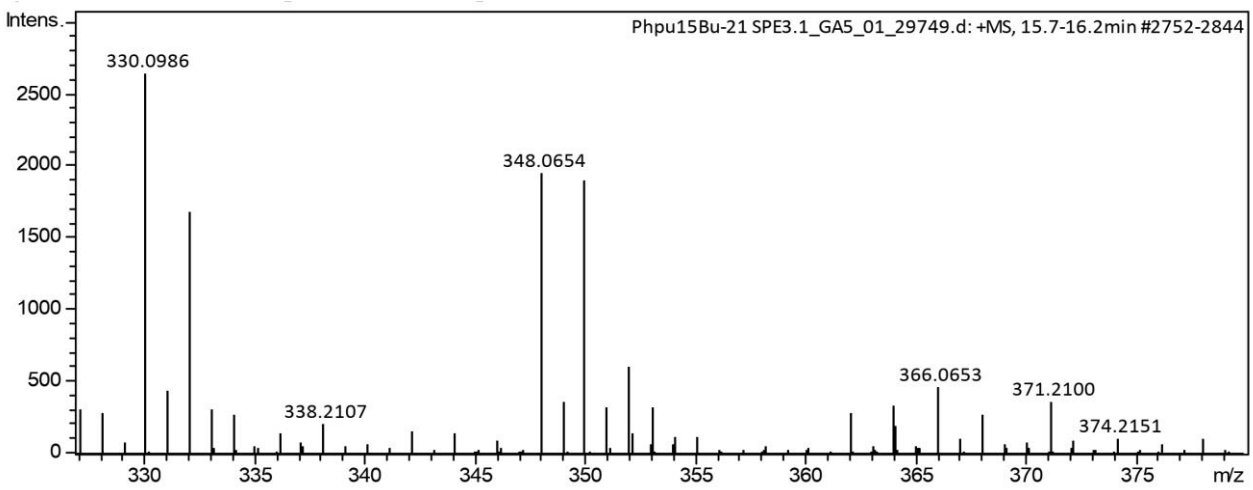


Figure S4. IR spectrum of compound **1**.

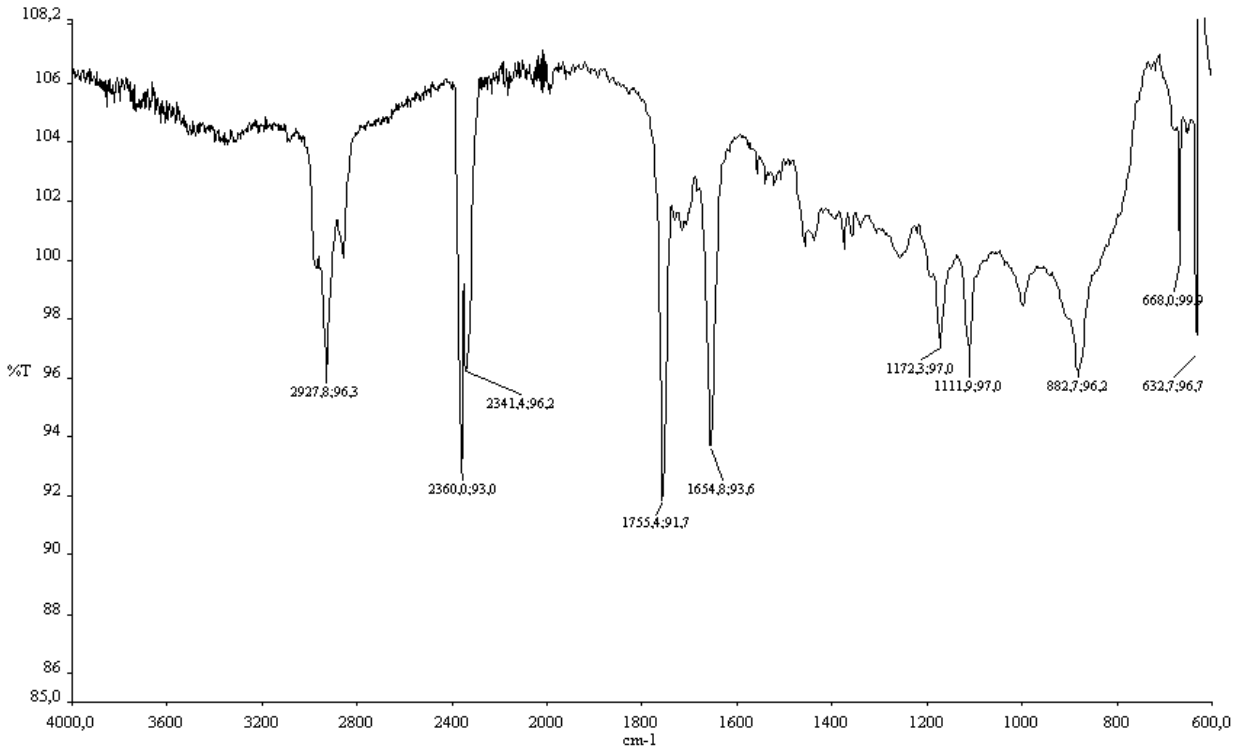


Figure S6. ^{1}H NMR spectrum of compound **2** in MeOD-*d*$_4$.

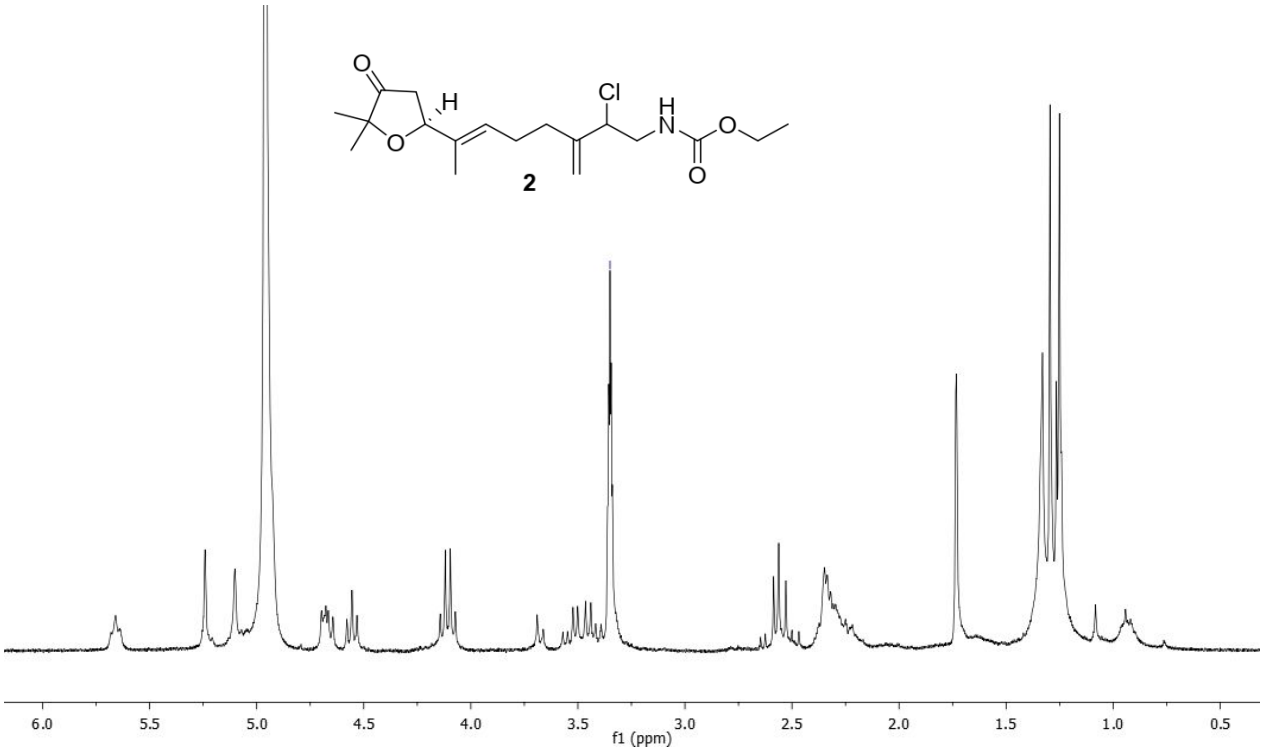


Figure S7. ^{13}C NMR spectrum of compound **2** in MeOD-*d*$_4$.

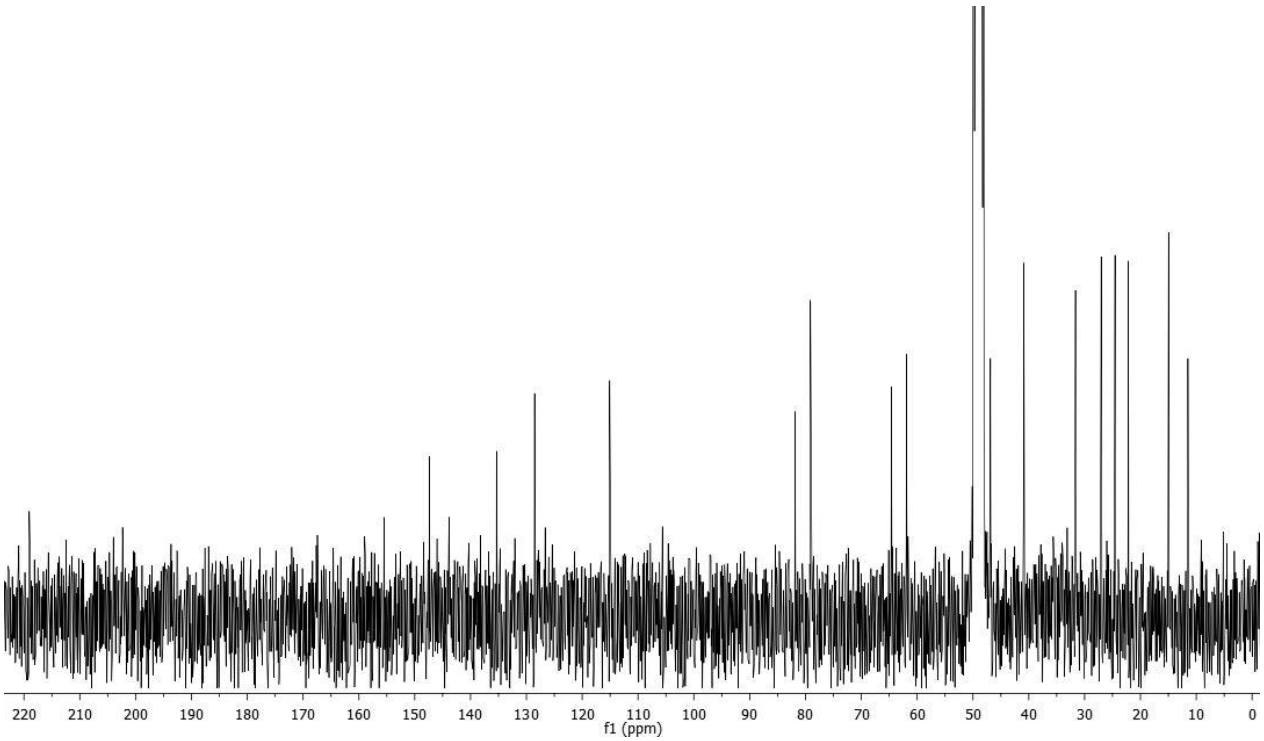


Figure S7. HR-ESIMS spectrum of compound **2**.

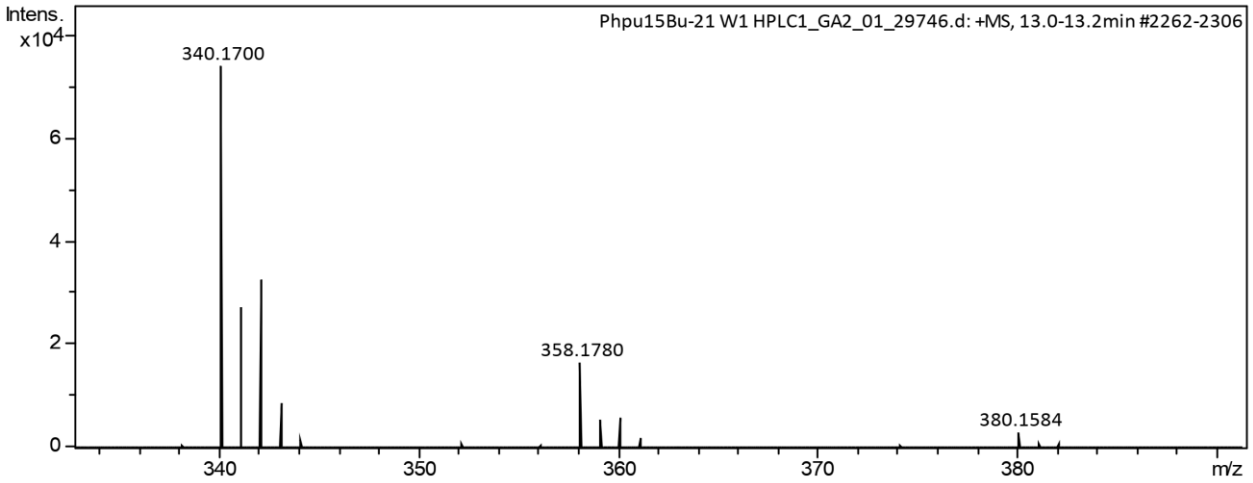


Figure S8. IR spectrum of compound **2**.

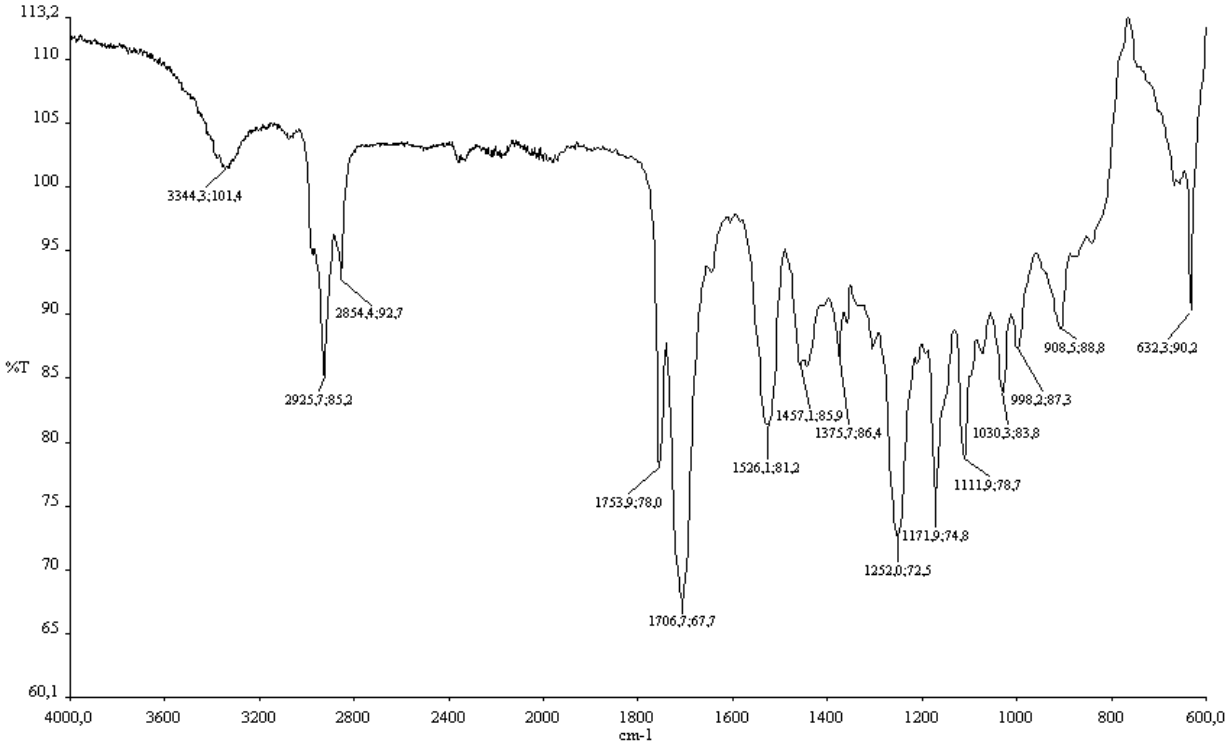


Figure S10. ECD spectrum of compound **2** in acetonitrile *c* 2.8 mmol/L.

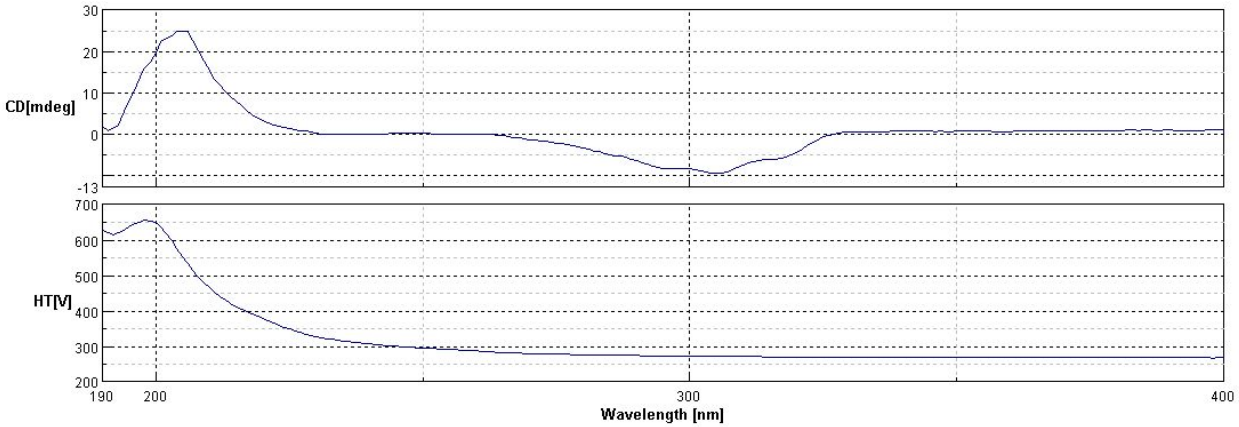


Figure S11. ^{1}H NMR spectrum of compound **3** in MeOD-d_4.

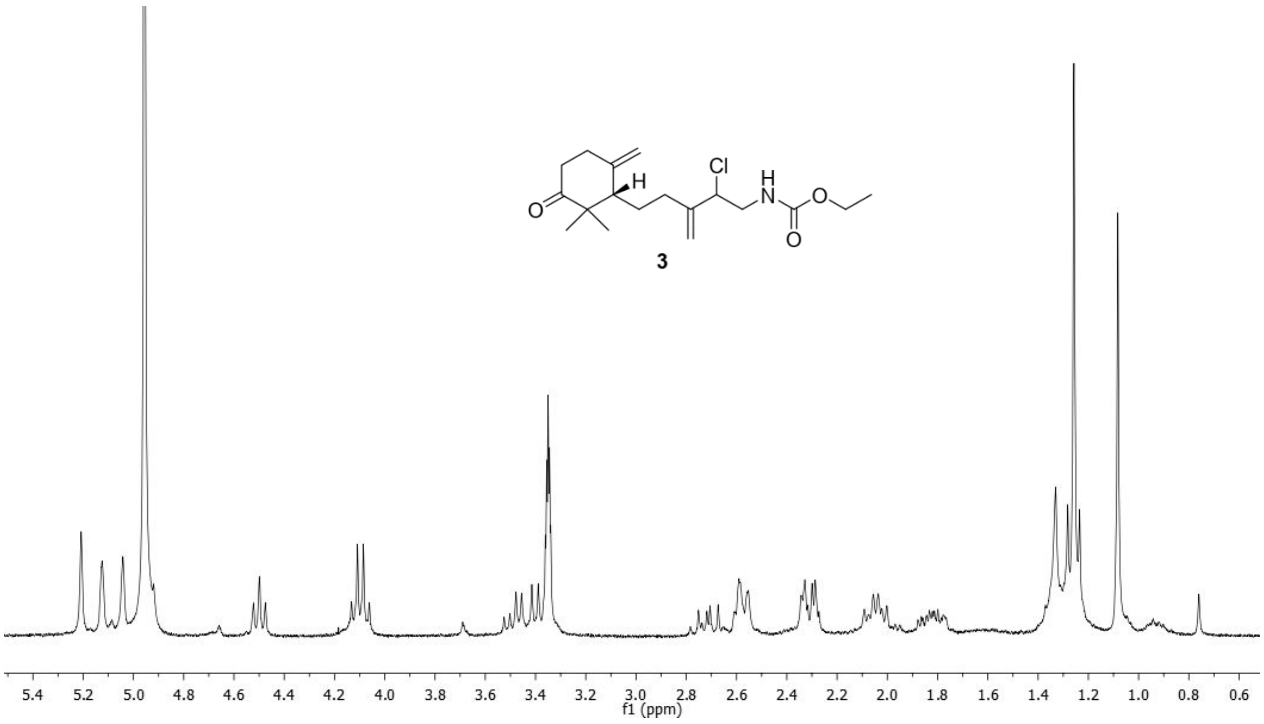


Figure S12. ^{13}C NMR spectrum of compound **3** in MeOD-d_4.

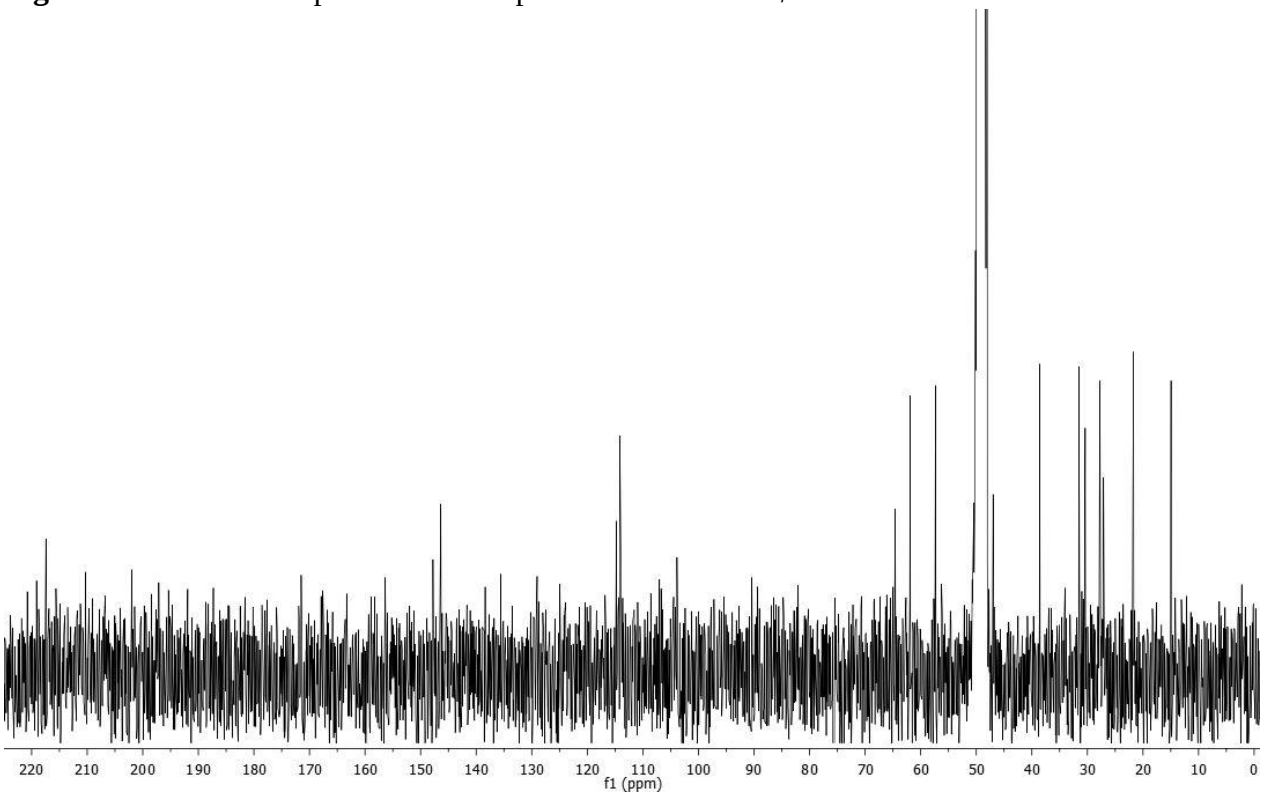


Figure S13. HR-ESIMS spectrum of compound **3**.

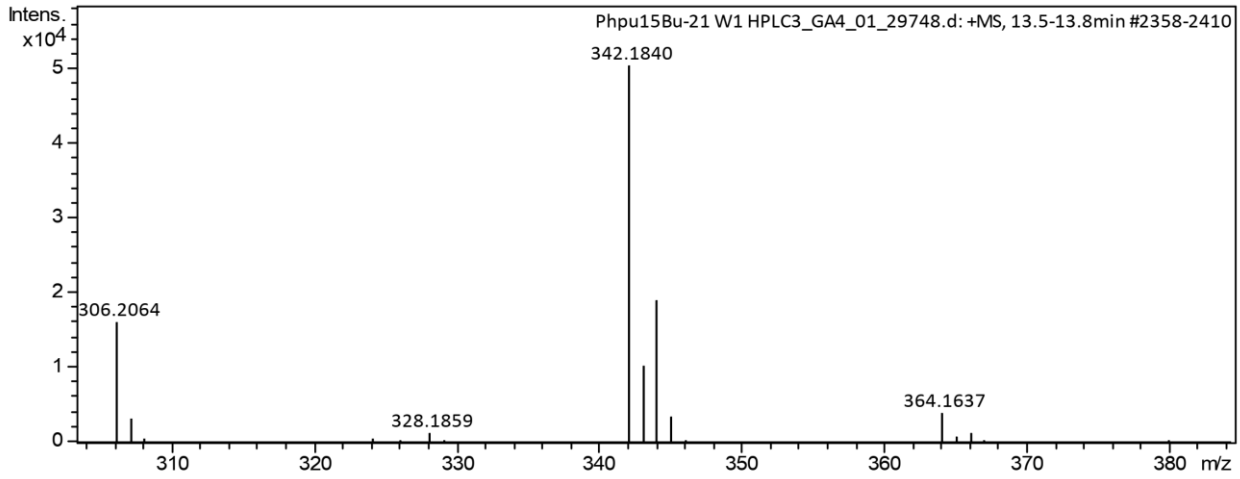


Figure S14. IR spectrum of compound **3**.

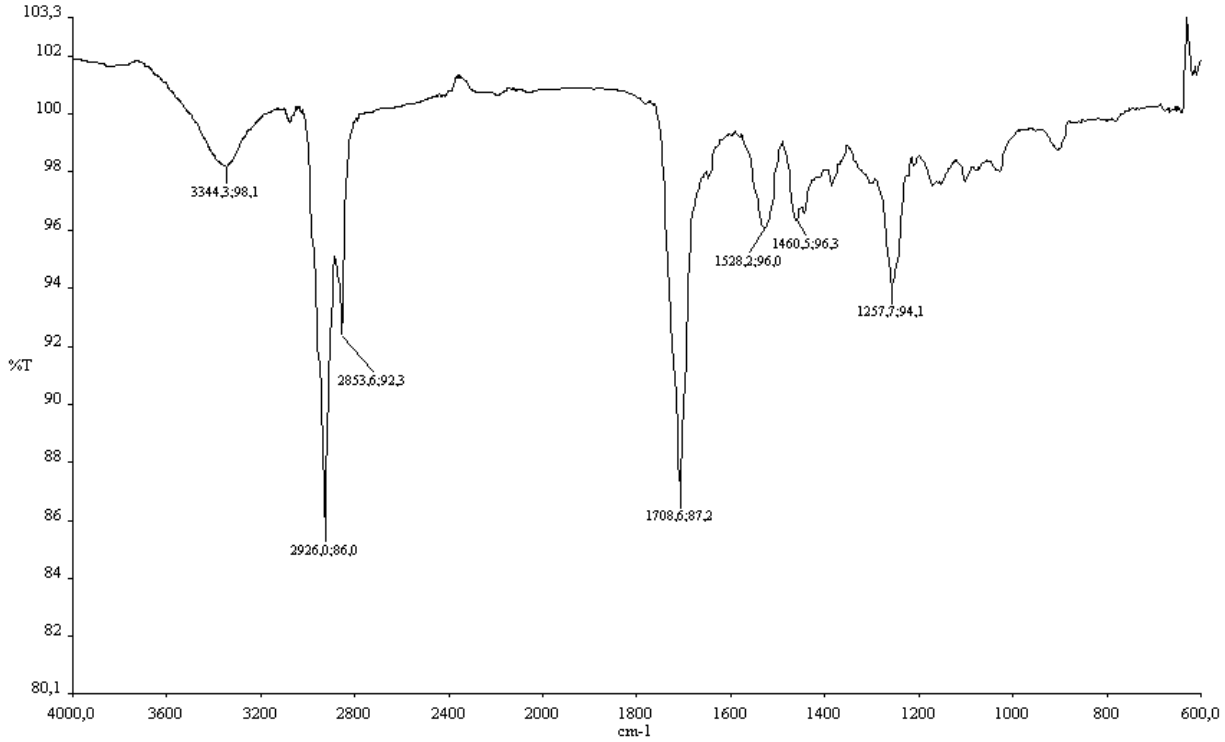


Figure S16. ECD spectrum of compound **3** in acetonitrile 2.9 mmol/L.

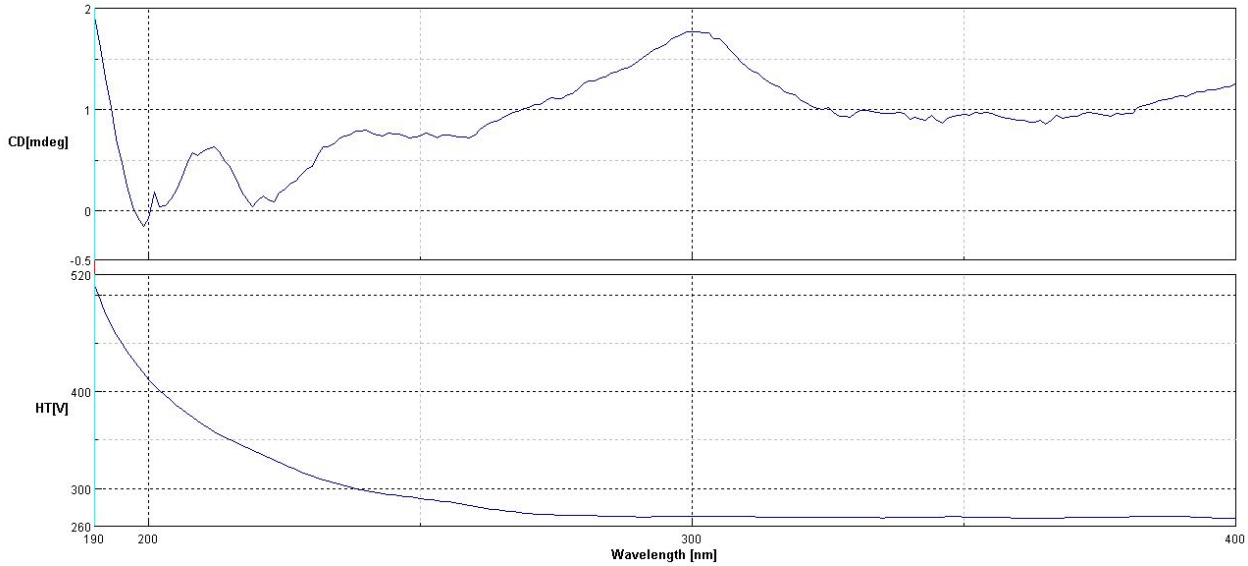


Figure S17. ^{1}H NMR spectrum of compound **4** in MeOD-*d*$_4$.

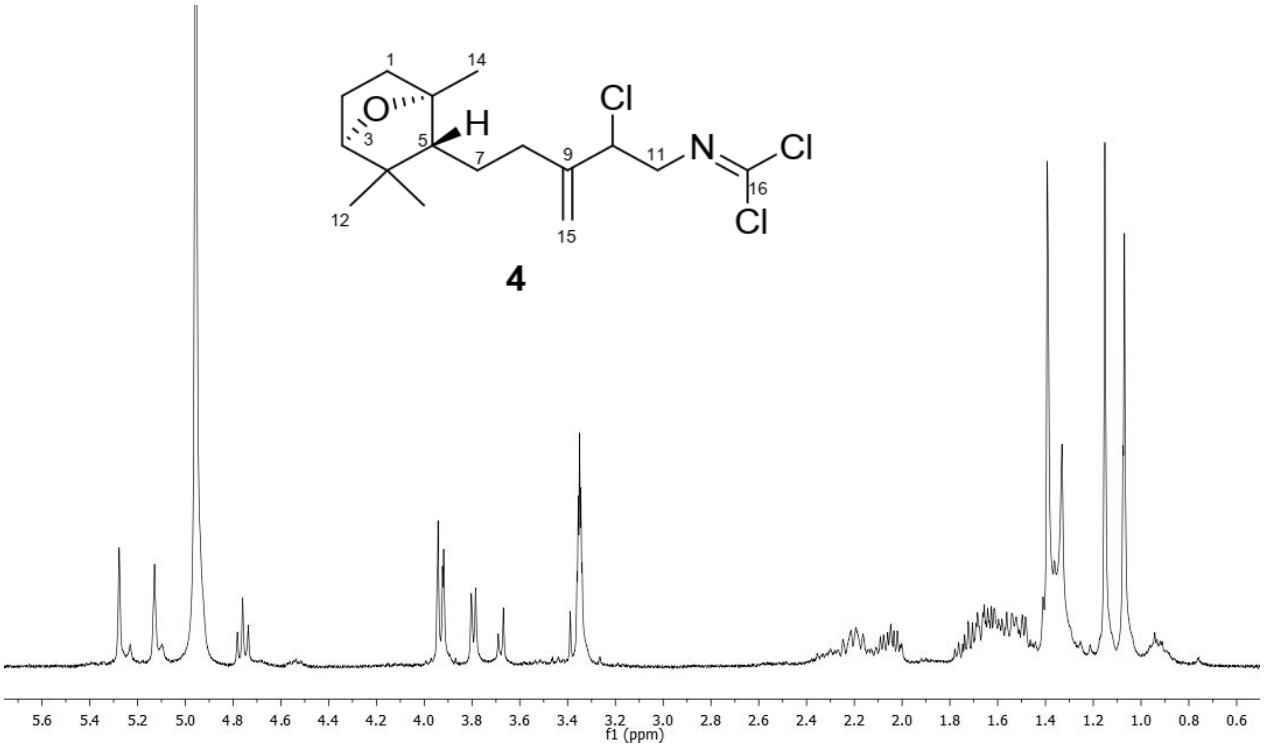


Figure S18. ^{13}C NMR spectrum of compound **4** in MeOD-*d*$_4$.

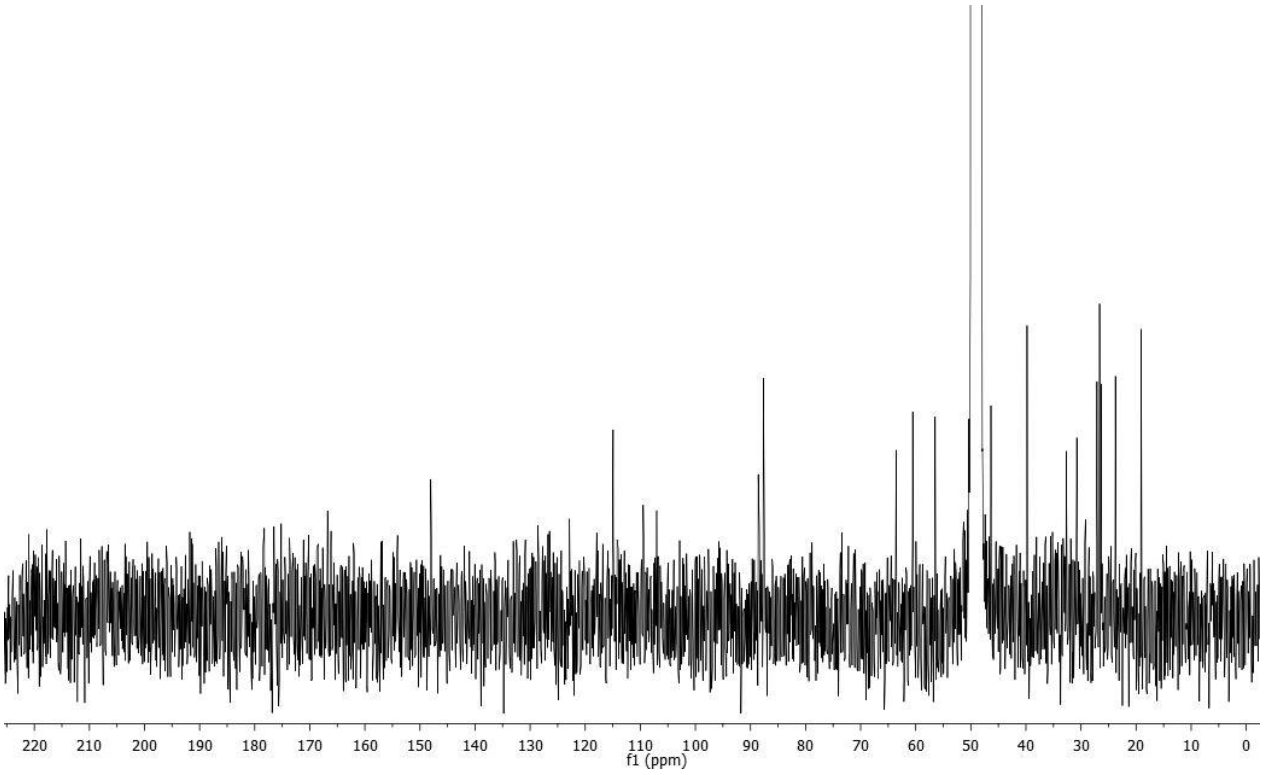


Figure S19. HR-ESIMS spectrum of compound **4**.

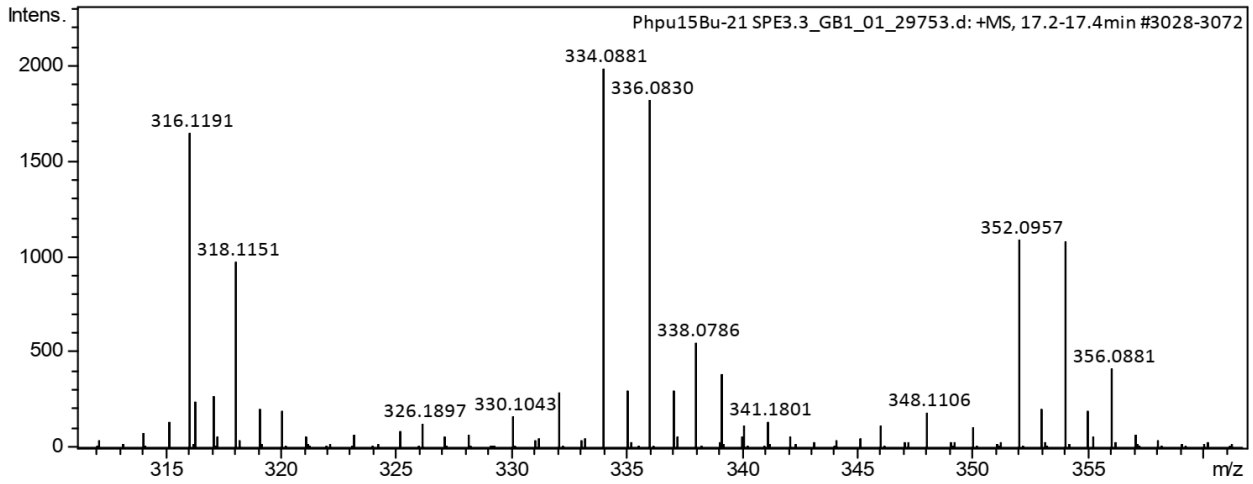


Figure S20. IR spectrum of compound **4**.

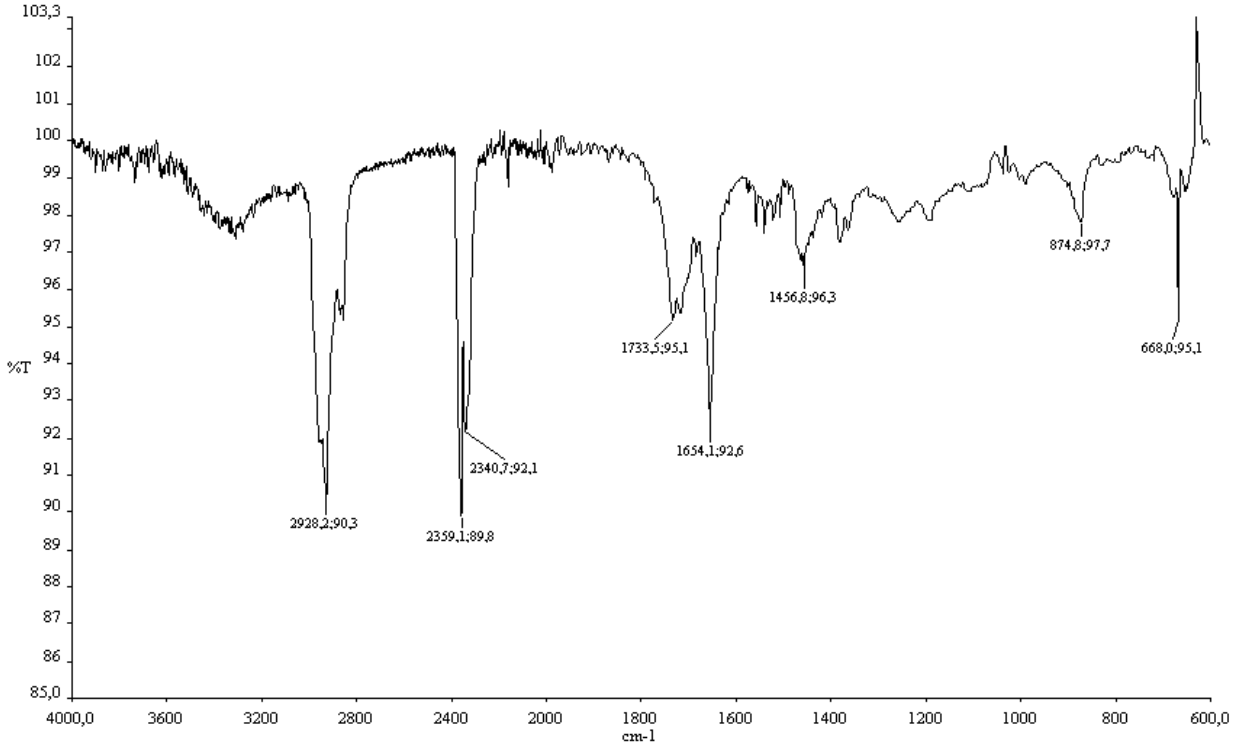


Figure S22. ECD spectrum of compound **4** in CH_3CN at 2.8 mmol/L.

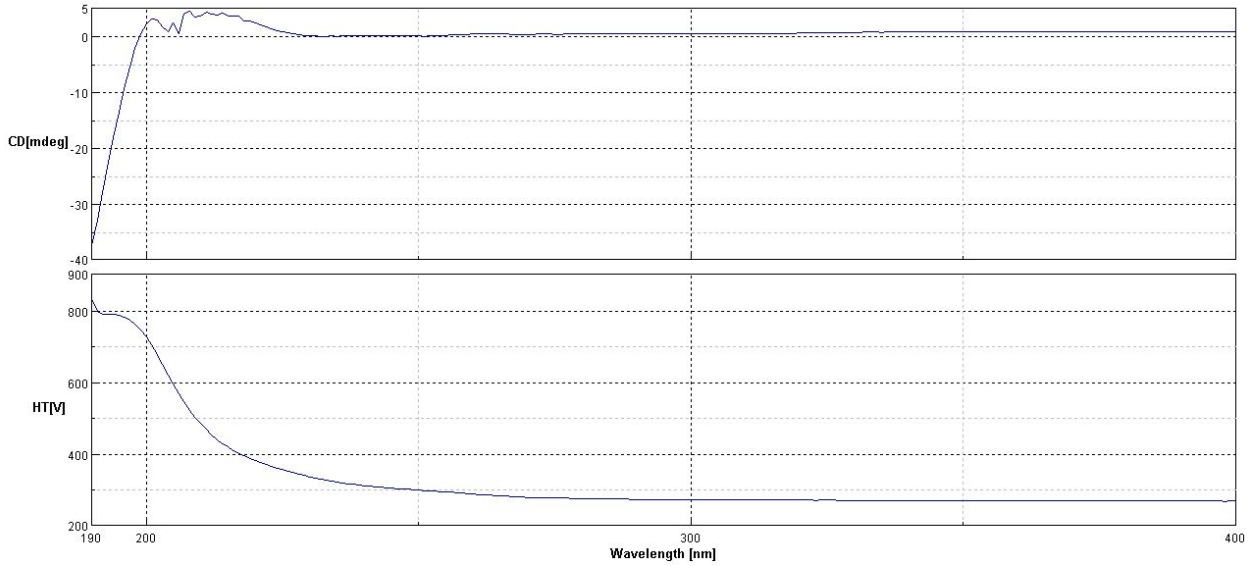


Figure S23. ^{1}H NMR spectrum of compound **5** (in mixture with **3**) in MeOD-*d*$_4$.

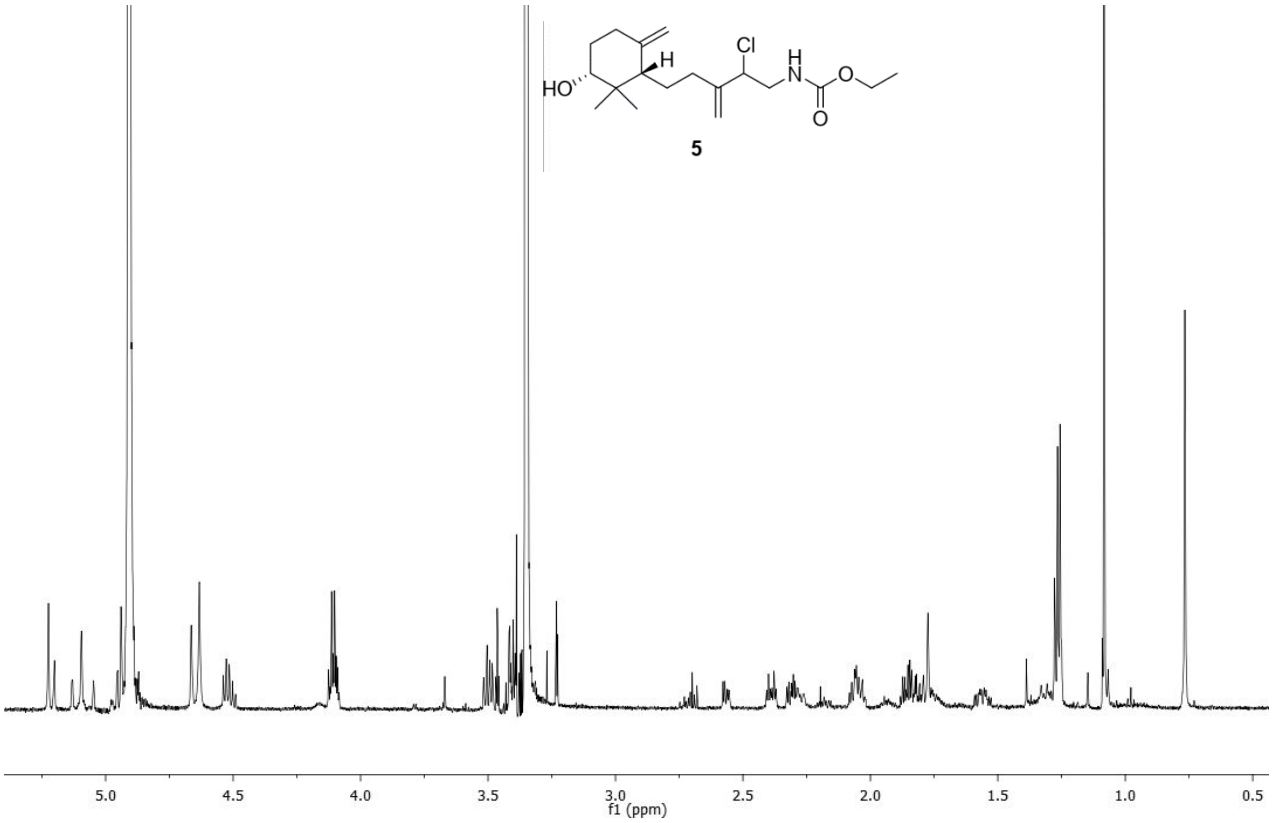


Figure S24. ^{13}C NMR spectrum of compound **5** (in mixture with **3**) in MeOD-d_4.

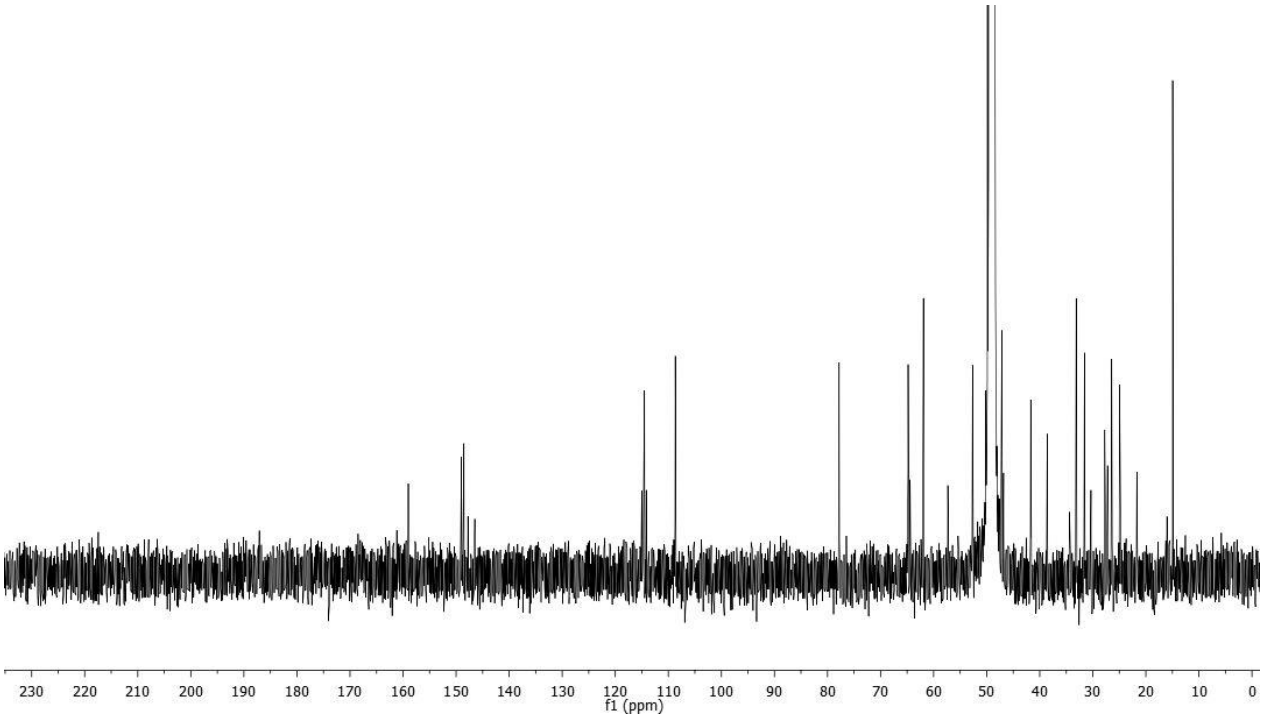


Figure S25. HR-ESIMS spectrum of compound **5**.

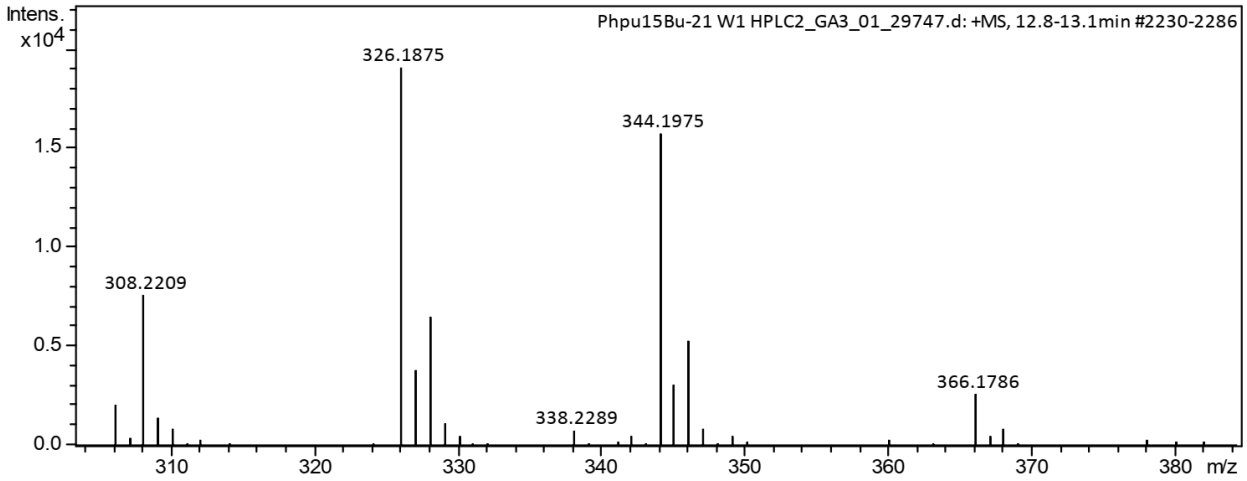


Figure S26. HPLC chromatogram (UV) of compound mixture **3** and **5**.

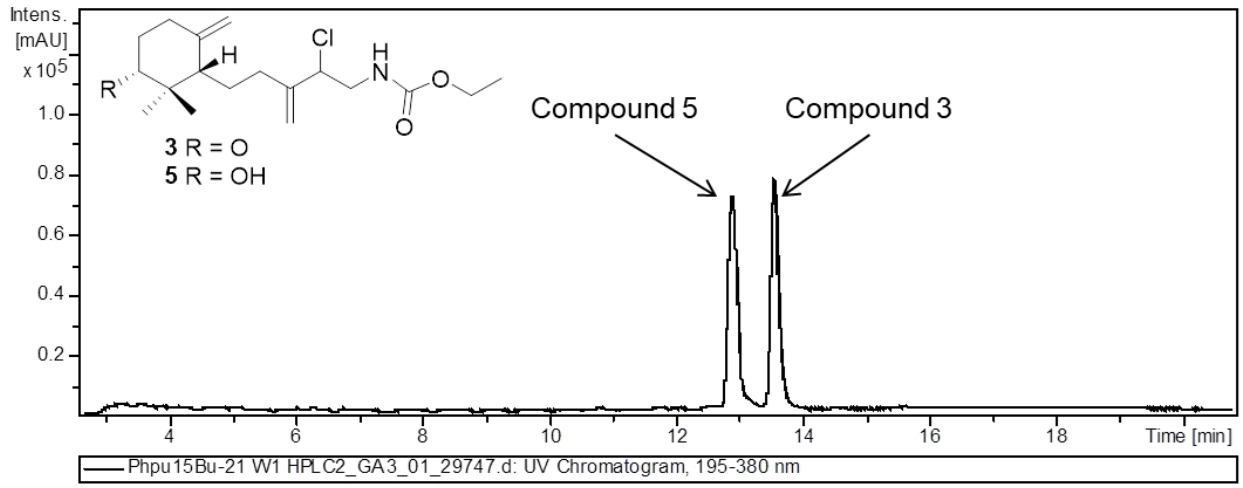


Figure S27. IR spectrum of compound **5** (in mixture with **3**).

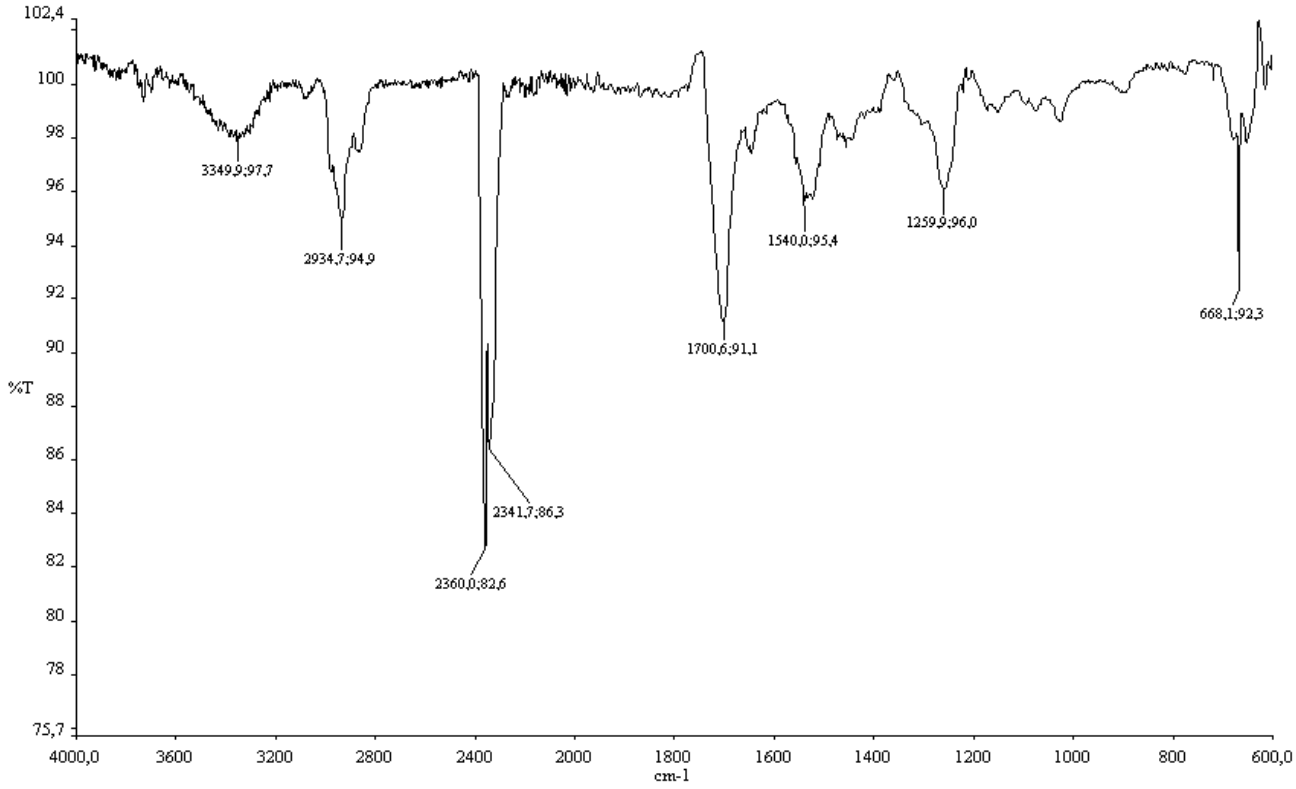


Figure S28. ^{1}H NMR spectrum of compound **6** (in mixture with **7**) in MeOD-*d*$_4$.

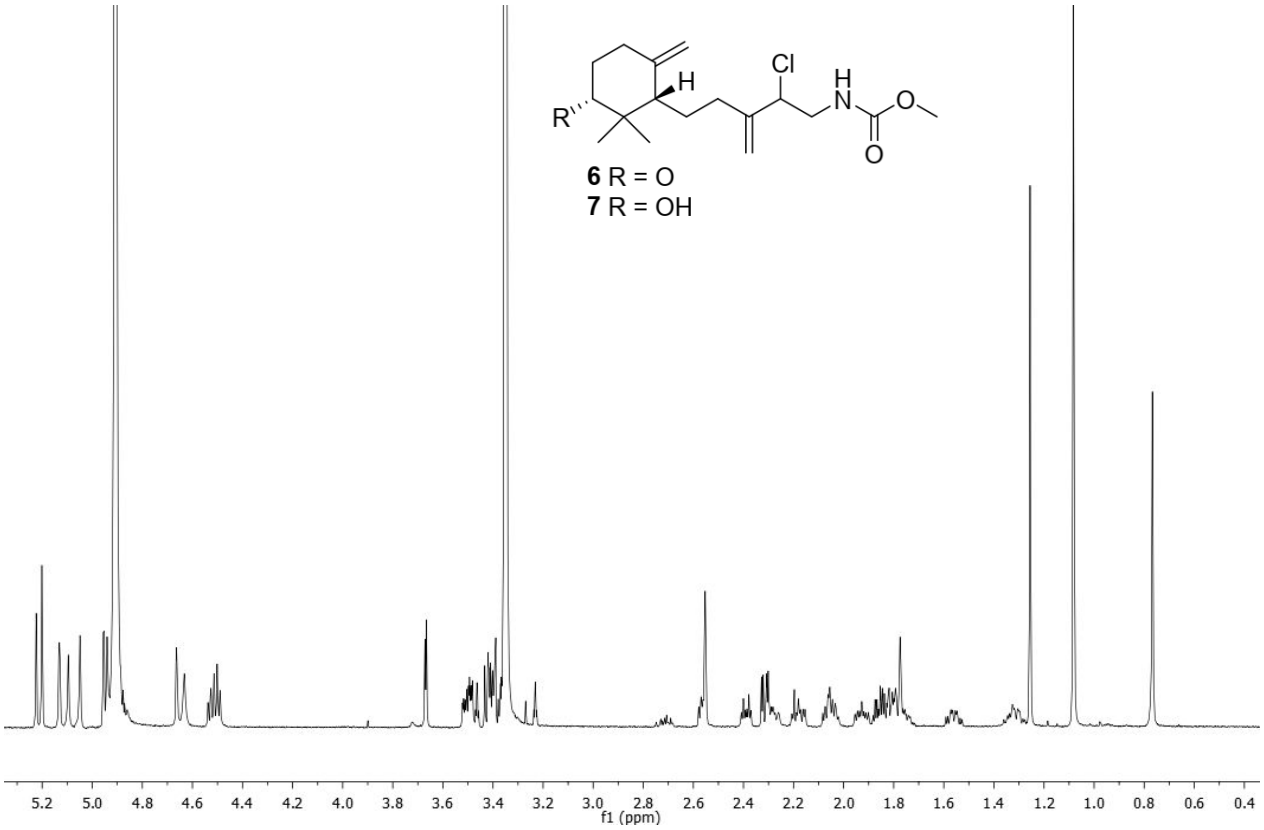


Figure S29. ^{13}C NMR spectrum of compound **6** (in mixture with **7**) in MeOD-*d*$_4$.

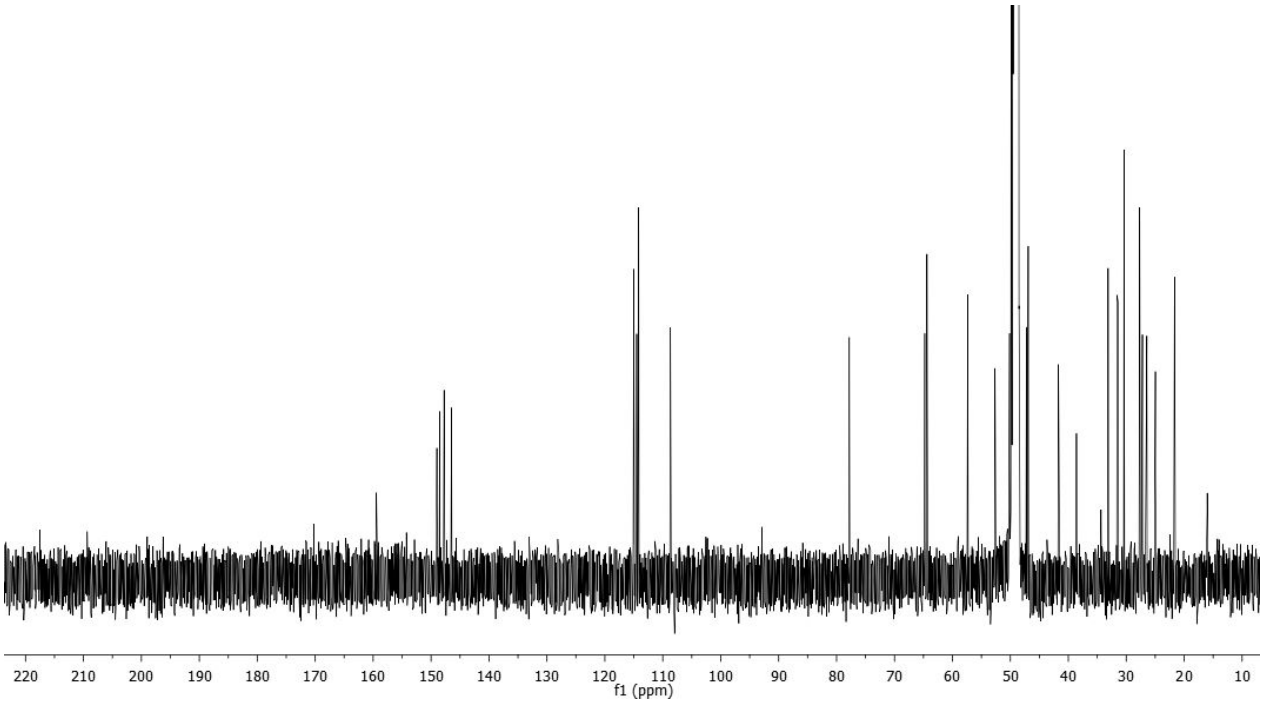


Figure S30. HPLC chromatogram (UV) of compound mixture **6** and **7**.

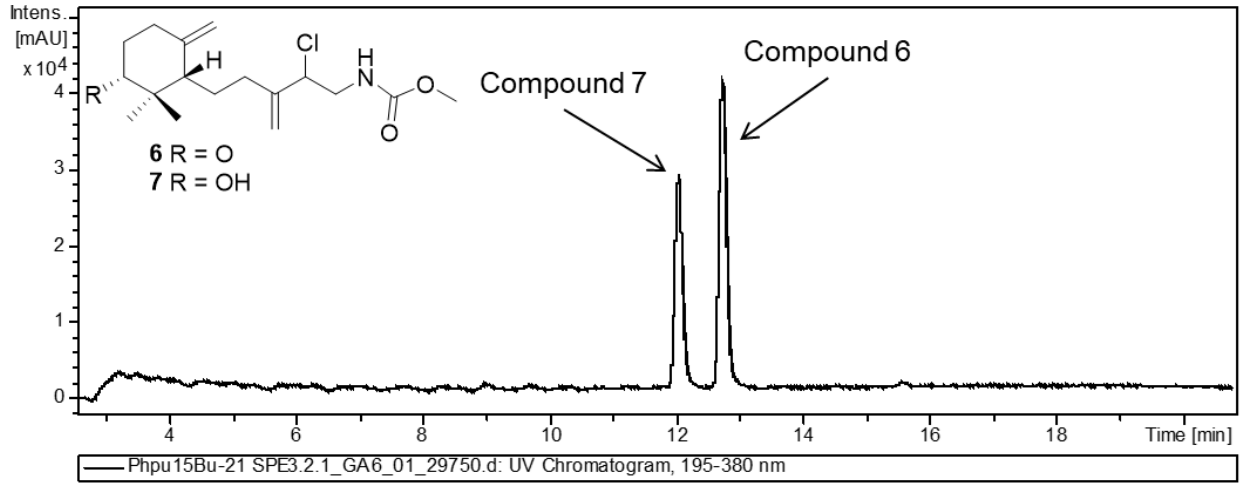


Figure S31. HR-ESIMS spectrum of compound **6**.

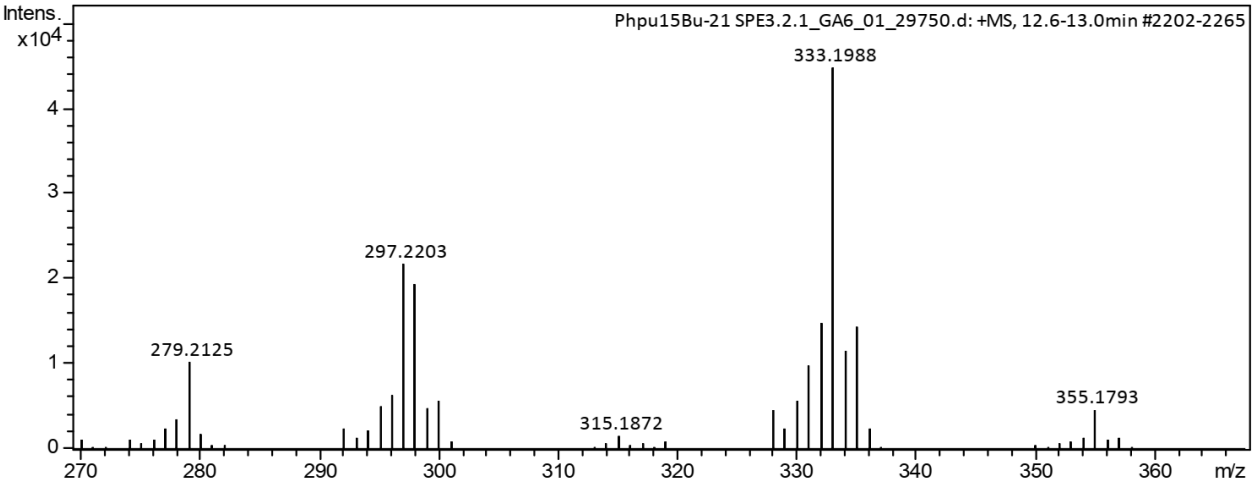


Figure S32. HR-ESIMS spectrum of compound **7**.

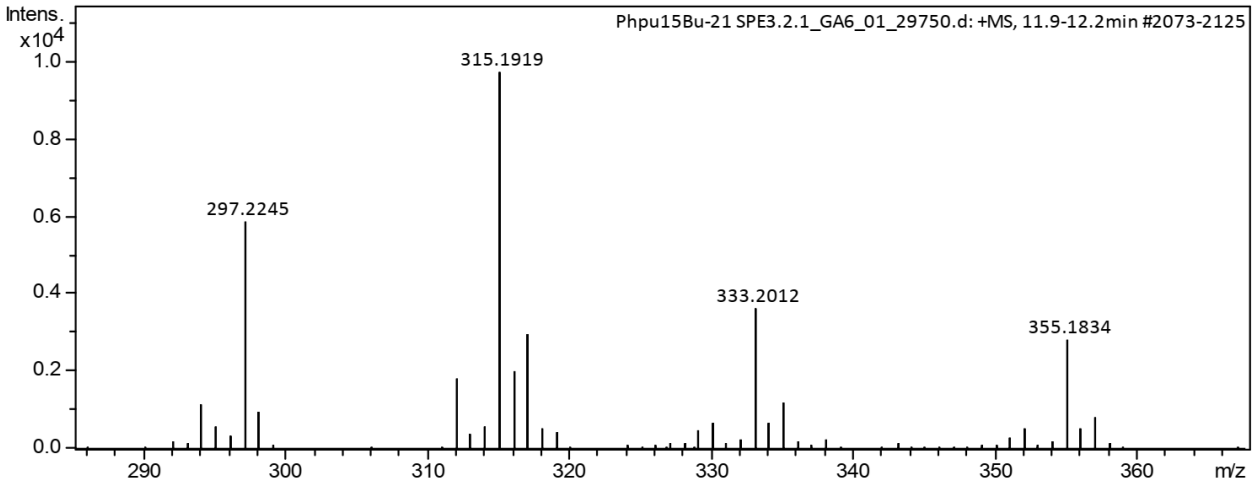


Figure S33. IR spectrum of compound mixture **6** and **7**.

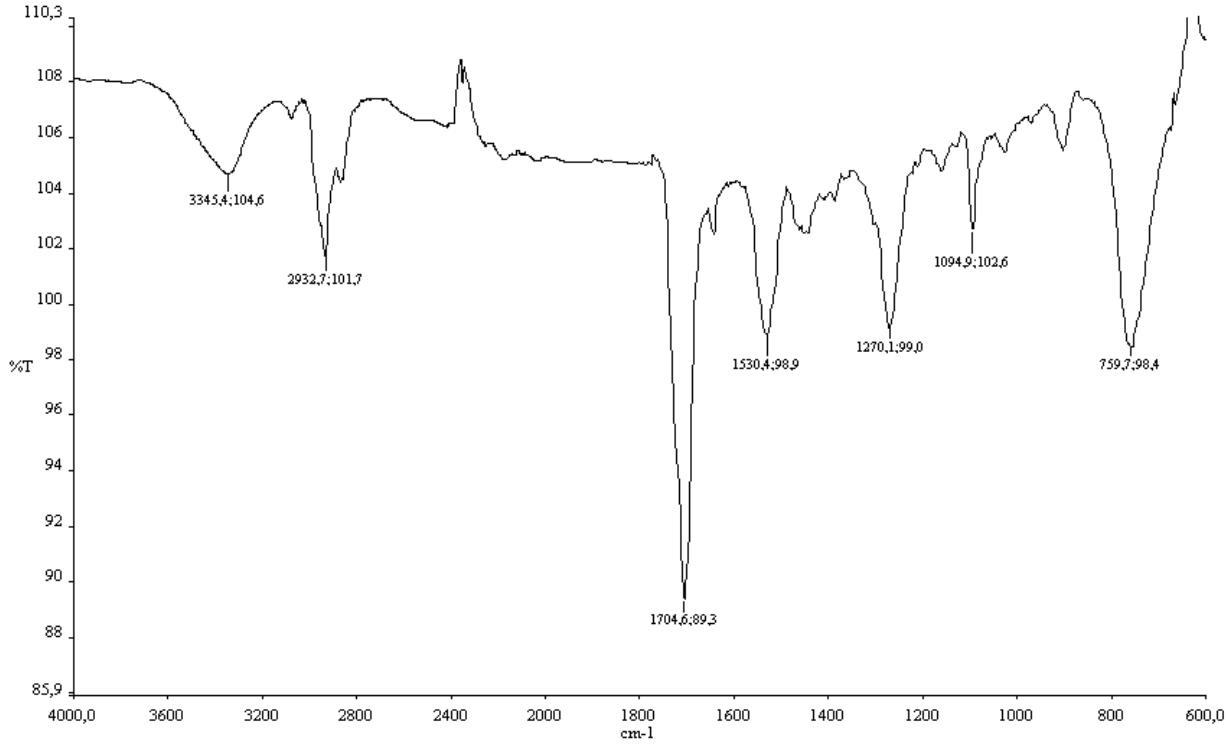


Figure S34. HPLC chromatogram (UV) of *P. pustulosa* (ID: Phpu15Bu-21) extract. Obtained in course of LC-HRMS analysis. Peaks containing compounds **8** and **9** are marked.

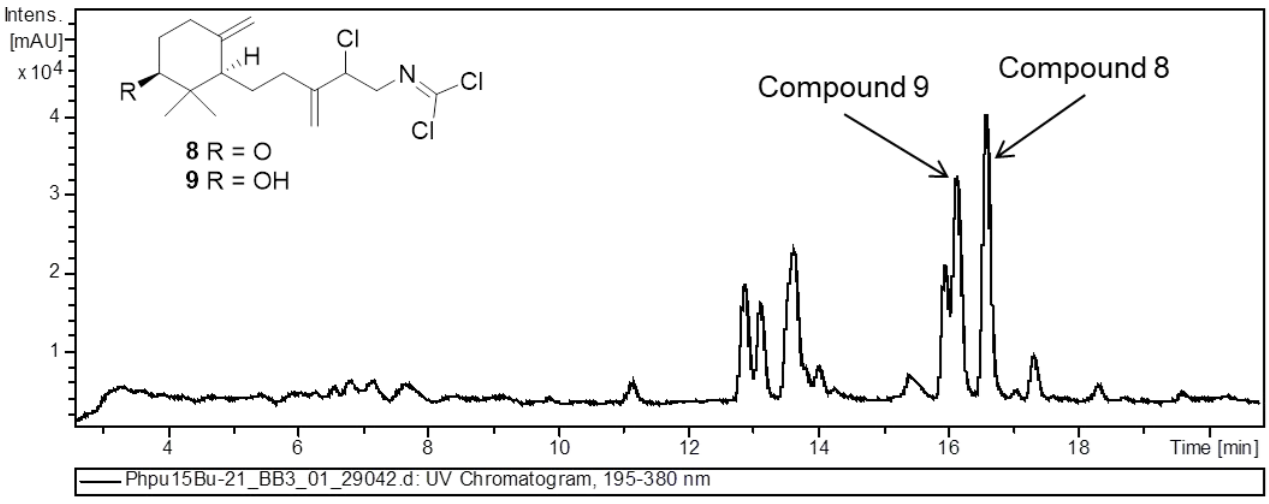


Figure S35. HR-ESIMS spectrum of compound **8**.

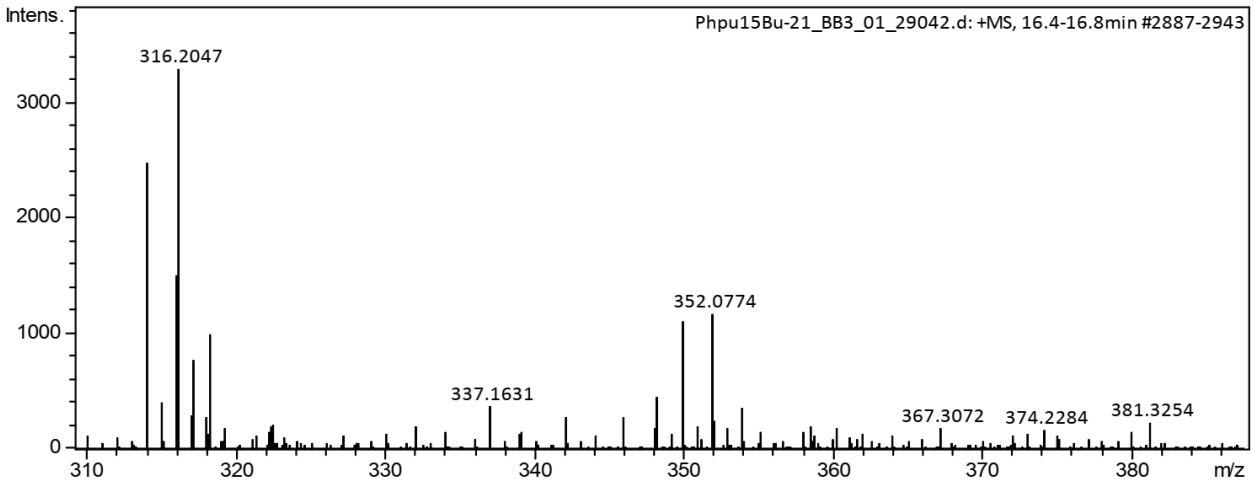
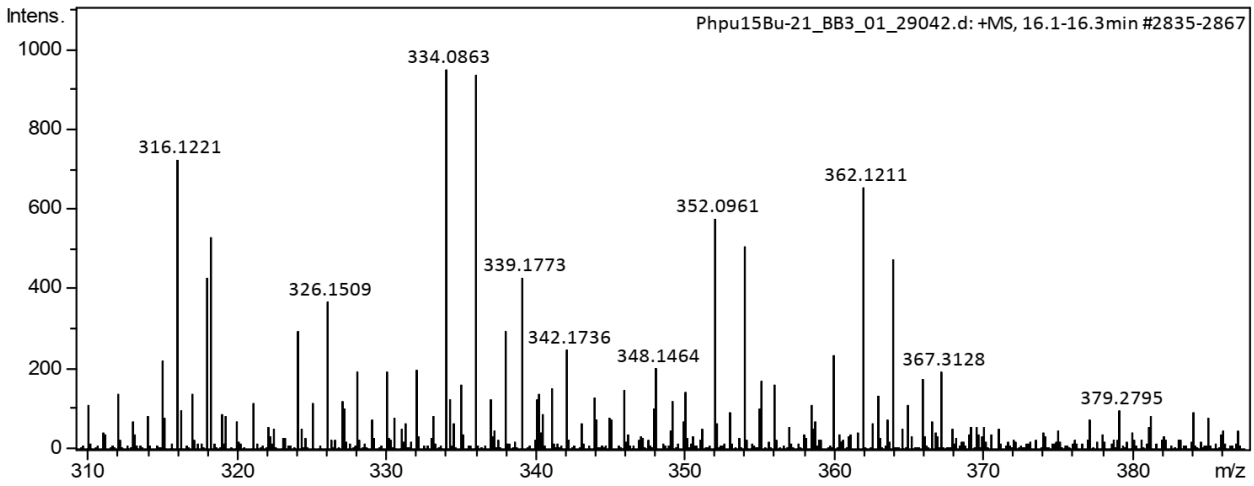
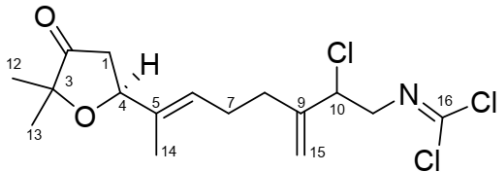


Figure S36. HR-ESIMS spectrum of compound **9**.



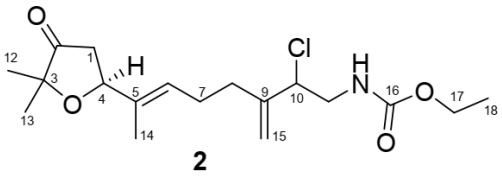


1

Table S1. NMR data of compound **1** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	40.9, CH_2	a 2.61, dd (6.5, 18.2)	H-1b, H-4	C-2, C-4, C-5	H-4, H_3-14
		b 2.51, dd (9.5, 18.2)	H-1a, H-4	C-2, C-4, C-5	H-4, H_3-14
2	219.1, C				
3	81.9, C				
4	79.1, CH	4.67, dd (6.5, 9.5)	H_2-1, H-6 (w)	C-5, C-6 (w)	H_2-1, H-6, H_3-13
5	135.5, C				
6	128.3, CH	5.66, t (6.0)	H_2-7, H_3-14		H-4, H_2-7, H_2-8
7	27.1, CH_2	ab 2.35, m	H-6, H_2-8	C-5 (w), C-6 (w), C-8	H-6, H-15b
8	32.2, CH_2	a 2.34, m	H_2-7, H-8b, H_2-15	C-7, C-9, C-15 (w)	H-6
		b 2.22, m	H_2-7, H-8a, H_2-15		H-6
9	147.4, C				
10	63.6, CH	4.76, brt (6.8)	H_2-11, H-15a	C-8, C-9, C-11, C-15	H_2-11, H-15a
11	60.5, CH_2	a 3.96, dd (6.3, 14.6)	H-10, H-11b	C-9, C-10, C-16	H_2-8, H-10, H-15a
		b 3.90, dd (7.3, 14.6)	H-10, H-11a	C-9, C-10, C-16	H_2-8, H-10, H-15a
12	24.6, CH_3	1.30, s		C-2, C-3, C-13	
13	22.2, CH_3	1.25, s		C-2, C-3, C-12	H-4
14	11.5, CH_3	1.73, s	H-6	C-4, C-5, C-6	H_2-1, H_2-7
15	115.2, CH_2	a 5.29, br s	H_2-8, H-10, H-15b	C-8, C-9, C-10	H-10
		b 5.14, br s	H_2-8, H-15a	C-8, C-10	H_2-7
16	127.3, C				

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

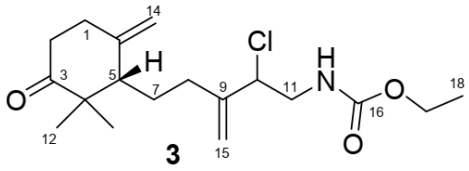


2

Table S2. NMR data of compound **2** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	40.9, CH_2	a 2.61, dd (6.5, 18.2)	H-1b, H-4	C-2, C-4, C-5	H-4
		b 2.51, dd (9.5, 18.2)	H-1a, H-4	C-2, C-4, C-5	H-4
2	219.1, C				
3	81.9, C				
4	79.1, CH	4.67, dd (6.5, 9.5)	H_2-1	C-6, C-14 (w)	H_2-1, H-6, H_3-13
5	135.3, C				
6	128.5, CH	5.66, brt (6.0)	H_2-7, H_3-14	C-4 (w), C-8	H-4, H_2-7, H_2-8
7	27.0, CH_2	ab 2.35, m	H-6, H_2-8	C-5 (w), C-6 (w), C-8	H-6, H-15b
8	31.6, CH_2	ab 2.27, m	H_2-7, H_2-15	C-7, C-9	H-6
9	147.4, C				
10	64.6, CH	4.55, t (7.0)	H_2-11, H-15a	C-8, C-9, C-11, C-15	H_2-11, H-15a
11	46.9, CH_2	a 3.54, dd (7.0, 14.5)	H-10, H-11b	C-9, C-10, C-16	H_2-8, H-10, H-15a
		b 3.43, dd (7.0, 14.5)	H-10, H-11a	C-9, C-10, C-16	H_2-8, H-10, H-15a
12	24.6, CH_3	1.30, s		C-2, C-3, C-13	
13	22.2, CH_3	1.25, s		C-2, C-3, C-12	H-4
14	11.5, CH_3	1.73, s	H-6	C-4, C-5, C-6	H_2-1, H_2-7
15	115.1, CH_2	a 5.24, br s	H_2-8, H-10, H-15b	C-8, C-9, C-10	H-10
		b 5.10, br s	H_2-8, H-15a	C-8, C-10	H_2-7
16	159.0, C				
17	61.9, CH_2	4.11, q (7.0)	H_3-18	C-16, C-18	
18	14.9, CH_3	1.26, t (7.0)	H_2-17	C-17	

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

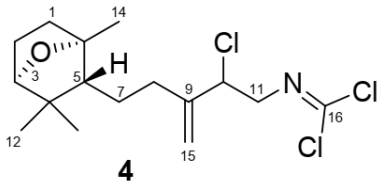


3

Table S3. NMR data of compound **3** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	31.5, CH_2	ab 2.59, m	H_2-2, H_2-14	C-2, C-5, C-6, C-14	H-14a
2	38.6, CH_2	a 2.70, m	H_2-1, H-2b	C-1, C-4, C-6	H_3-12
		b 2.31, m	H_2-1, H-2a	C-1, C-3, C-4	
3	217.5, C				
4	50.0, C				
5	57.3, CH	2.30, m	H_2-7, H-14b	C-4 (w), C-6, C-8, C-14	H_2-8, H_3-12, H-14b
6	146.4, C				
7	27.1, CH_2	a 1.82, m	H-5, H-7b, H_2-8	C-6 (w), C-8	H_3-13
		b 1.30, m	H-5, H-7a, H_2-8		H_2-1
8	30.4, CH_2	ab 2.04, m	H_2-7, H-15b	C-15 (w)	
9	147.8, C				
10	64.6, CH	4.50, br t (7.1)	H_2-11, H-15a	C-8, C-9, C-11, C-15	H_2-11, H-15a
11	46.9, CH_2	a 3.49, dd (6.8, 14.1)	H-10, H-11b	C-9, C-10, C-16	
		b 3.37, dd (7.3, 14.1)	H-10, H-11a		
12	27.7, CH_3	1.25, s		C-3, C-4, C-5, C-13	H-2a; H-5
13	21.7, CH_3	1.08, s		C-3, C-4, C-5, C-12	H-7a
14	114.1, CH_2	a 5.12, br s	H_2-1	C-1, C-5	H_2-1
		b 4.95, br s		C-1, C-5	H-5
15	114.8, CH_2	a 5.21, br s	H_2-8, H-10	C-8, C-9, C-10	H_2-11
		b 5.04, br s	H_2-8	C-8, C-10	H-7a, H_2-8
16	159,0 C				
17	61.9, CH_2	4.10, q (7.0)	H_3-18	C-16, C-18	
18	15.0, CH_3	1.26, t (7.0)	H_2-17	C-17	

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

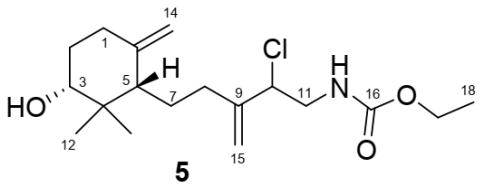


4

Table S4. NMR data of compound **4** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	39.8, CH_2	a 1.65, m	H_2-2	C-5	H-5
		b 1.49, m	H_2-2	C-2, C-5, C-6, C-14	
2	26.6, CH_2	a 2.04, m	H_2-1; H-2b	C-1, C-4, C-6	H-1a, H_3-12
		b 1.72, m	H_2-1, H-2a, H-3	C-1, C-3, C-4	H-1b, H-3
3	87.7, CH	3.79, d (5.5)	H-2b, H-2a (w)	C-1, C-2 (w), C-5, C-6	H-2b, H_3-13
4	46.4, C				
5	56.5, CH	1.38, t (8.2)	H_2-7	C-4 (w), C-6, C-7, C-8	H-1a
6	88.6, C				
7	27.1, CH_2	ab 1.58, m	H-5, H_2-8	C-4 (w), C-5, C-8	H_3-13, H_3-14
8	32.7, CH_2	ab 2.19, m	H_2-7, H-15b	C-15 (w)	
9	148.1, C				
10	63.6, CH	4.76, t (7.0)	H_2-11, H-15a	C-8 (w), C-9, C-11, C-15	H_2-11, H-15a
11	60.5, CH_2	a 3.96, m	H-10, H-11b	C-9, C-10, C-16	
		b 3.92, m	H-10, H-11a	C-9, C-10, C-16	
12	26.3, CH_3	1.15, s		C-3, C-4, C-5, C-13	H-2a
13	23.7, CH_3	1.07, s		C-3, C-4, C-5, C-12	H-3, H_2-7
14	19.1, CH_3	1.39, s		C-1, C-5, C-6	H_2-8
15	114.9, CH_2	a 5.28, br s	H_2-8, H-10, H-15b	C-8, C-9, C-10	H-10
		b 5.13, br s	H_2-8, H-15a	C-8, C-10 (w)	
16	127.2, C				

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

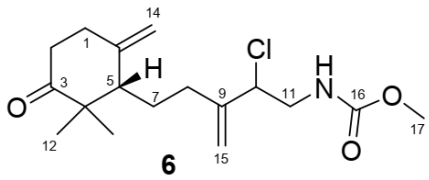


5

Table S5. NMR data of compound **5** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	34.4, CH_2	a 2.39, dt (13.0, 4.4)	H-1b, H_2-2, H_2-14	C-2, C-3, C-5, C-6, C-14	
		b 2.06, m	H-1a, H_2-2, H-14a	C-2, C-3, C-5, C-6, C-14	
2	33.2, CH_2	a 1.86, m	H_2-1, H-2b, H-3	C-1, C-3, C-4, C-6	
		b 1.56, m	H_2-1, H-2a, H-3		
3	77.8, CH	3.40, m	H_2-2	C-1, C-2, C-4, C-5, C-12, C-13	
4	41.7, C				
5	52.7, CH	1.77, br d (10.5)	H_2-7, H_2-14	C-4, C-6 (w)	
6	149.0, C				
7	24.9, CH_2	a 1.83, m	H-5, H-7b, H_2-8	C-6 (w), C-8	
		b 1.78, m	H-5, H-7a, H_2-8	C-4 (w)	
8	31.6, CH_2	a 2.28, m	H_2-7, H-8b, H-15b		
		b 2.04, m	H_2-7, H-8a, H-15b		
9	148.5, C				
10	64.8, CH	4.53, br t (7.0)	H_2-11, H-15a	C-9 (w), C-11 (w)	H-11a, H-11b
11	47.1, CH_2	a 3.50, dd (6.4, 14.3)	H-10, H-11b	C-9, C-16	H-10
		b 3.41, m	H-10	C-9, C-10, C-16	H-10
12	26.4, CH_3	1.08 s		C-3, C-4, C-5, C-13	
13	16.0, CH_3	0.77 s		C-3, C-4, C-5, C-12	H-7a
14	108.7, CH_2	a 4.94, br s	H_2-1, H-5	C-1, C-5	
		b 4.66, br s	H-1b, H-5	C-1, C-5	H-5
15	114.5, CH_2	a 5.22, br s	H-8a, H-10	C-8, C-9, C-10	
		b 5.09, br s	H_2-8	C-8, C-10	
16	159.0, C				
17	61.9, CH_2	4.11, q (7.0)	H_3-18	C-16, C-18	H_3-18
18	14.9, CH_3	1.27, t (7.0)	H_2-17	C-17	

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

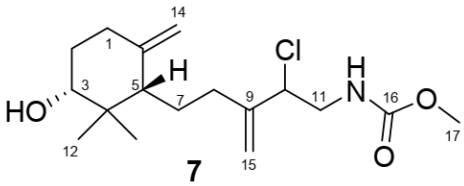


6

Table S6. NMR data of compound **6** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (*J* in Hz)	COSY	HMBC	NOESY
1	31.4, CH_2	ab 2.56, m	H_2-2, H-14a	C-2, C-3, C-5, C-6, C-14	H-14a
2	38.6, CH_2	a 2.71, m	H_2-1; H-2b		
		b 2.30, m	H_2-1, H-2a		
3	217.5, C				
4	50.2, C				
5	57.4, CH	2.31, dd (4.0, 12.1)	H_2-7	C-1, C-3, C-4, C-6, C-7, C-8, C-13, C-14	H-8b, H_3-12, H-14b
6	146.5, C				
7	27.2, CH_2	a 1.81, m	H-5, H-7b, H_2-8	C-5, C-6, C-8	H_3-13
		b 1.31, m	H-5, H-7a, H_2-8		
8	30.4, CH_2	a 2.18, ddd (4.5, 11.2, 16.0)	H_2-7, H-8b	C-7, C-9, C-10, C-15	
		b 1.93, ddd (6.0, 10.6, 16.0)	H_2-7, H-8a		
9	147.7, C				
10	64.4, CH	4.50, t (7.0)	H_2-11	C-8, C-9, C-11, C-15	
11	46.9, CH_2	a 3.49, dd (7.0, 14.0)	H-10, H-11b	C-9, C-10, C-16	
		b 3.41, m	H-10, H-11a		
12	27.6, CH_3	1.26, s		C-3, C-4, C-5, C-13	H-5
13	21.6, CH_3	1.08, s		C-3, C-4, C-5, C-12	H-7a
14	114.1, CH_2	a 5.13, br s	H_2-1	C-1, C-5	
		b 4.95, br s		C-1, C-5	H-5
15	115.0, CH_2	a 5.20, br s		C-8, C-9, C-10	
		b 5.05, br s		C-8, C-10	
16	159.4 C				
17	52.6, CH_3	3.66, s		C-16	

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.



7

Table S7. NMR data of compound **7** in MeOH-d_4 ($\delta_{H/C}$ 3.35/49.0).

a	δ_C *b*	δ_H (mult, *J* in Hz)	COSY	HMBC	NOESY
1	34.3, CH_2	a 2.39, dt (13.0, 4.7)	H-1b, H_2-2, H-14a	C-2, C-3, C-5, C-6, C-14	
		b 2.06, m	H-1a, H-14a	C-2, C-6, C-14	
2	33.1, CH_2	a 1.86, m	H_2-1, H-2b, H-3	C-1, C-3, C-4, C-6	
		b 1.56, m	H_2-1, H-2a, H-3	C-3	
3	77.8, CH	3.40, m	H_2-2	C-1, C-2, C-4, C-5, C-12, C-13	H_2-2, H-5
4	41.7, C				
5	52.7, CH	1.78, br s	H_2-7, H-14b	C-4, C-6, C-8, C-13, C-14	H-14b
6	149.0, C				
7	25.0, CH_2	a 1.83, m	H-5, H-7b, H_2-8	C-8	H_3-13
		b 1.76, m	H-5, H-7a, H_2-8	C-5	
8	31.6, CH_2	a 2.28, m	H_2-7, H-8b,H-15b	C-9	H-5
		b 2.05, m	H_2-7, H-8b,H-15b		
9	148.5, C				
10	64.8, CH	4.53, br t (7.0)	H_2-11	C-8, C-9, C-11, C-15	
11	47.1, CH_2	a 3.50, dd (6.5, 14.0)	H-10, H-11b	C-9, C-10, C-16	
		b 3.41, m	H-10, H-11a	C-9, C-10, C-16	
12	26.4, CH_3	1.08 s		C-3, C-4, C-5, C-13	H-2b, H-7a
13	16.0, CH_3	0.77 s		C-3, C-4, C-5, C-12	
14	108.7, CH_2	a 4.94, br s	H_2-1	C-1, C-5	H-1a
		b 4.66, br s	H-5	C-1, C-5	H-7b, H-8a
15	114.5, CH_2	a 5.22, br s	H_2-8	C-8, C-9, C-10	H-10
		b 5.10, br s	H_2-8	C-8	H_2-8
16	159.4, C				
17	52.6, CH_3	3.67, s		C-16	

a All assignments are based on extensive 1D and 2D NMR measurements (COSY, HSQC, HMBC).
b Multiplicities determined by DEPT.

4. Supplementary material Chapter 5

Table 1. DNA sequences of specimens included in the analyses.

Identifier/ voucher number	DNA number	16S	CO1	Species name in NCBI	Year	Locality	Coordinates	Station	Species name in my tree
PhvaNN3	NU336			*Phyllidia varicosa*	-	North Sulawesi	-	-	*Phyllidia varicosa*
Phva17Ba2	AP16			*Phyllidia varicosa*	2017	Bangka	1°41'23.01″N; 125°09'55.26″E	Tanjung Kusu	*Phyllidia varicosa*
Phva16Bu3	NU318			*Phyllidia varicosa*	2016	Sangihe	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia varicosa*
Phva17Ba1	AP15			*Phyllidia varicosa*	2017	Bangka	1°44'07.74″N; 125°09'05,59″E	Tanjung Husi	*Phyllidia varicosa*
Phva17Bu1	AP25			*Phyllidia varicosa*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia varicosa*
Phpic16Sa7	NU128			*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
18TMPhpi1	RB021			*Phyllidia varicosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia varicosa*
18TMPhva1	RB008			*Phyllidia varicosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia varicosa*
Phva15Bu8	DS270			*Phyllidia varicosa*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pantai Parigi	*Phyllidia varicosa*
Phva15Bu9	DS271			*Phyllidia varicosa*	2015	Bunaken	1°38′46.1″N; 124°42′48″E	Manado tua / Battu lohang	*Phyllidia varicosa*
18TMPhva5	RB007			*Phyllidia varicosa*	2018	Minahasa	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia varicosa*
Phva16Bu5	NU320			*Phyllidia varicosa*	2016	Sangihe	1°43′11.7″N; 124°43′33.7″E	Mantehage 2	*Phyllidia varicosa*
Phva15Bu7	DS269			*Phyllidia varicosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidia varicosa*
Phva15Bu10	DS272			*Phyllidia varicosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia varicosa*
Phva15Bu2	DS264			*Phyllidia varicosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia varicosa*
18TMPhva2	RB009			*Phyllidia varicosa*	2018	Minahasa	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia varicosa*
18TMPhva4	RB006			*Phyllidia varicosa*	2018	Minahasa	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia varicosa*

Phva15Bu3	DS265		*Phyllidia varicosa*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia varicosa*
Phva15Bu5	DS267		*Phyllidia varicosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidia varicosa*
Phva16Bu4	NU319		*Phyllidia varicosa*	2016	Sangihe	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia varicosa*
Phva16Bu1	NU316		*Phyllidia varicosa*	2016	Sangihe	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidia varicosa*
Phva15Bu4	DS266		*Phyllidia varicosa*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia varicosa*
Phva17M1	AP32		*Phyllidia varicosa*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidia varicosa*
Phva15Bu6	DS268		*Phyllidia varicosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidia varicosa*
PhvaNN2	NU335		*Phyllidia varicosa*	-	North Sulawesi	-	-	*Phyllidia varicosa*
Phva18Ba2	AP095		*Phyllidia varicosa*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia varicosa*
Phva18Ba15	AP108		*Phyllidia varicosa*	2018	Bangka	1°48'41.3″N; 125°06'45.1″E	Pearl Farm	*Phyllidia varicosa*
Phel18Ko2	AP078		*Phyllidia varicosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidia varicosa*
Phva18Ba10	AP103		*Phyllidia varicosa*	2018	Bangka	1°44'07.74″N; 125°09'05.59″E	Tanjung Husi	*Phyllidia varicosa*
Phva18Bu3	AP128		*Phyllidia varicosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia varicosa*
Phva18Ba9	AP102		*Phyllidia varicosa*	2018	Bangka	1°44'07.74″N; 125°09'05,59″E	Tanjung Husi	*Phyllidia varicosa*
Phva18Ba14	AP107		*Phyllidia varicosa*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia varicosa*
Phva18Ba8	AP101		*Phyllidia varicosa*	2018	Bangka	1°50'45.44″N; 125°5'55.26″E	Kinabuhutan	*Phyllidia varicosa*
Phel18Bu6	AP123		*Phyllidia varicosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia varicosa*
Phva18Ba11	AP104		*Phyllidia varicosa*	2018	Bangka	1°44'07.74″N; 125°09'05,59″E	Tanjung Husi	*Phyllidia varicosa*
Phva18Ba1	AP094		*Phyllidia varicosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidia varicosa*
Phva18Ba4	AP097		*Phyllidia varicosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia varicosa*
Phva18Ba5	AP098		*Phyllidia varicosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia varicosa*
Phva18Ba16	AP109		*Phyllidia varicosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidia varicosa*
Phva18Ba17	AP110		*Phyllidia varicosa*	2018	Bangka	1°46'07.8″N; 125°10'34.6″E	Areng Kambing	*Phyllidia varicosa*
Phva18Po1	AP113		*Phyllidia varicosa*	2018	Pomalaa	4°10'11.30″S; 121°35'8.63″E	Pomalaa Harbour	*Phyllidia varicosa*
Phva18Bu1	AP126		*Phyllidia varicosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia varicosa*
Phva18Ba7	AP100		*Phyllidia varicosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia varicosa*
Phva18Bl1	AP111	-	*Phyllidia varicosa*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidia varicosa*
Phva18Ba13	AP106	-	*Phyllidia varicosa*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia varicosa*

Phel18Ko3	AP079	*Phyllidia varicosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidia varicosa*
Phva18Ba3	AP096	*Phyllidia varicosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia varicosa*
Phva18Ba6	AP099	*Phyllidia varicosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia varicosa*
Phva18Bu2	AP127	*Phyllidia varicosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia varicosa*
Phva18Ko1	AP112	*Phyllidia varicosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidia varicosa*
Phel15Bu1	DS198	*Phyllidia varicosa*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado tua	*Phyllidia varicosa*
18TMPhel4	RB002	*Phyllidia elegans*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia elegans*
Phel15Bu10a	DS208	*Phyllidia elegans*	2015	Bunaken	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidia elegans*
18TMPhel3	RB012	*Phyllidia elegans*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia elegans*
18TMPhel1	RB010	*Phyllidia elegans*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia elegans*
Phel15Bu6	DS203	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phpi17M1	AP41	*Phyllidia elegans*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidia elegans*
18TMPhel2	RB011	*Phyllidia elegans*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia elegans*
Phel17Tu2	AP28	*Phyllidia elegans*	2017	North Sulawesi	0°58'13.4"N; 124°53'03.0"E	Tumbak	*Phyllidia elegans*
Phel15Bu4	DS201	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phel15Bu5	DS202	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phel17Bu1	AP19	*Phyllidia elegans*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia elegans*
Phel18Ba2	AP075	*Phyllidia elegans*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia elegans*
Phel15Bu3	DS200	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phsp616Sa1	NU235	*Phyllidia elegans*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia elegans*
Phel15Bu10	DS207	*Phyllidia elegans*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia elegans*
Phel17 Tu1	AP27	*Phyllidia elegans*	2017	Tumbak	0°58'13.4"N; 124°53'03.0"E	Tumbak	*Phyllidia elegans*
Phel15Bu7	DS204	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phel15Bu12	DS209	*Phyllidia elegans*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidia elegans*

Phel15Bu2	DS199	*Phyllidia elegans*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia elegans*
Phel15Bu8	DS205	*Phyllidia elegans*	2015	Bunaken	1°36'48.4″N; 124°44'22.8″E	Johnson	*Phyllidia elegans*
Phel15Bu9	DS206	*Phyllidia elegans*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia elegans*
Phaly16Bu1	NU298	*Phyllidia elegans*	2016	Bunaken	1°38'12.6″N; 124°46'3.4″E	Mikes Point	*Phyllidia elegans*
Phel18Ba1	AP074	*Phyllidia elegans*	2018	Bangka	1°50'45.44″N; 125°5'55.26″E	Kinabuhutan	*Phyllidia elegans*
Phel17 Tu3	AP29	*Phyllidia elegans*	2017	Tumbak	0°58'13.4"N; 124°53'03.0"E	Tumbak	*Phyllidia elegans*
Phel18Bu3	AP120	*Phyllidia elegans*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia elegans*
Phel18Bu4	AP121	*Phyllidia elegans*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia elegans*
Phel18Bu5	AP122	*Phyllidia elegans*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia elegans*
Phel18Bu1	AP118	*Phyllidia elegans*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia elegans*
Phel18Sm1	AP080	*Phyllidia elegans*	2018	Samalona	5°07'20.7″S; 119°20'30.7″E	Mercusuar	*Phyllidia elegans*
Phel18Po1	AP081	*Phyllidia elegans*	2018	Pomalaa	4°10'11.30″S; 121°35'8.63″E	Pomalaa Harbour	*Phyllidia elegans*
Phel18Ko1	AP077	*Phyllidia elegans*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidia elegans*
Phel18Ba3	AP076	*Phyllidia elegans*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia elegans*
Phel18Bu2	AP119	*Phyllidia elegans*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia elegans*
Phco15Bu16	DS192	*Phyllidia coelestis*	2015	Bunaken	1°38′46.1″N; 124°42′48″E	Manado tua / Battu lohang	*Phyllidia coelestis*
Phco15Bu14	DS191	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pantai Parigi	*Phyllidia coelestis*
Phco15Bu8	DS185	*Phyllidia coelestis*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia coelestis*
Phco17Bu1	AP17	*Phyllidia coelestis*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia coelestis*
Phco15Bu11	DS188	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pantai Parigi	*Phyllidia coelestis*
Phco15Bu19	DS194	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia coelestis*
Phco15Bu5	DS182	*Phyllidia coelestis*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia coelestis*
Phco17Bu2	AP18	*Phyllidia coelestis*	2017	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia coelestis*
Phco15Bu3	DS180	*Phyllidia coelestis*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia coelestis*
Phco15Bu7	DS184	*Phyllidia coelestis*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia coelestis*
Phco15Bu4	DS181	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia coelestis*
Phco15Bu13	DS189	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pantai Parigi	*Phyllidia coelestis*

Phco15Bu20	DS195	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia coelestis*
Phva15Bu1	DS263	*Phyllidia coelestis*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia coelestis*
Phco17M1	AP30	*Phyllidia coelestis*	2015	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidia coelestis*
Phco16Bu3	NU302	*Phyllidia coelestis*	2016	Bunaken	1°37'41.7″N; 124°45'60″E	Sachiko	*Phyllidia coelestis*
Phco15Bu9	DS186	*Phyllidia coelestis*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidia coelestis*
Phco15Bu23	DS197	*Phyllidia coelestis*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidia coelestis*
18TMPhco2	RB004	*Phyllidia coelestis*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia coelestis*
Phco15Bu2	DS179	*Phyllidia coelestis*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia coelestis*
Phpic16Sa2	NU123	*Phyllidia coelestis*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidia coelestis*
Phco15Bu22	DS196	*Phyllidia coelestis*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidia coelestis*
18TMPhco1	RB003	*Phyllidia coelestis*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia coelestis*
Phco15Bu10	DS187	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pantai Parigi	*Phyllidia coelestis*
Phco18Po1	AP089	*Phyllidia coelestis*	2018	Pomalaa	4°13'48.97″S; 121°34'19.33″E	Latumbi	*Phyllidia coelestis*
PhpiNN1	NU334	*Phyllidia coelestis*	-	North Sulawesi	-	-	*Phyllidia coelestis*
Phco16Bu2	NU301	*Phyllidia coelestis*	2016	Bunaken	1°37'41.7″N; 124°45'60″E	Sachiko	*Phyllidia coelestis*
Phco15Bu18	DS193	*Phyllidia coelestis*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia coelestis*
Phco15Bu14a	DS190	*Phyllidia coelestis*	2015	Bunaken	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidia coelestis*
Phco16Bu1	NU300	*Phyllidia coelestis*	2016	Bunaken	1°36'19.2″N; 124°46'01.5E	Lekuan 3	*Phyllidia coelestis*
Phco15Bu6	DS183	*Phyllidia coelestis*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidia coelestis*
Phco17Ba1	AP03	*Phyllidia coelestis*	2017	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia coelestis*
Phco18Ba1	AP082	*Phyllidia coelestis*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidia coelestis*
Phco18Ba6a	AP088	*Phyllidia coelestis*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia coelestis*
Phco18Ba2	AP083	*Phyllidia coelestis*	2018	Bangka	1°44'27.4″N; 125°08'35.1″E	Busa Bora Timur	*Phyllidia coelestis*
Phco18Ba3	AP084	*Phyllidia coelestis*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia coelestis*
Phco18Ba4	AP085	*Phyllidia coelestis*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidia coelestis*
Phco18Ba5	AP086	*Phyllidia coelestis*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia coelestis*
Phco18Ba6	AP087	*Phyllidia coelestis*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia coelestis*

Phco18Bu2	AP125	*Phyllidia coelestis*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidia coelestis*
Phco18Bu1	AP124	*Phyllidia coelestis*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidia coelestis*
Phva16Sa1	NU240	*Phyllidia picta*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidia picta*
18TMPhva3	RB005	*Phyllidia picta*	2018	North Sulawesi	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia picta*
Phsp18Ba1	AP062	*Phyllidia picta*	2018	Bangka	1°44'27.4″N; 125°08'35.1″E	Busa Bora Timur	*Phyllidia picta*
Phva16Sa6	NU245	*Phyllidia picta*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia picta*
Phpi17Ba1	AP04	*Phyllidia picta*	2017	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia picta*
Phpi18Bl2	AP091	*Phyllidia picta*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidia picta*
Phpi18Bl3	AP092	*Phyllidia picta*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidia picta*
Phpi18Ba1	AP090	*Phyllidia picta*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidia picta*
Phsp18Bu1	AP117	*Phyllidia picta*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia picta*
Phex15Bu2	DS211	*Phyllidia picta*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidia picta*
Phsp18Ba5	AP066	*Phyllidia picta*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidia picta*
Phsp18Ba3	AP064	*Phyllidia picta*	2018	Bangka	1°40'51.06″N; 125°8'22.45″E	Sempini 2	*Phyllidia picta*
Phsp17M1	AP40	*Phyllidia picta*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidia picta*
Phsp18Ba2	AP063	*Phyllidia picta*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidia picta*
Phsp18Ba4	AP065	*Phyllidia picta*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidia picta*
Phco16Sa8	NU101	*Phyllidia picta*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia picta*
Phpi18Po1	AP093	*Phyllidia picta*	2018	Pomalaa	4°16'13.77″S; 121°31'34.70″E	Watu Kilat	*Phyllidia picta*
Phex17Ba1	AP01	*Phyllidia exquisita*	2017	Bangka	1°44'35.11″N; 125°09'43.50″E	Sahaung I	*Phyllidia exquisita*
Phex18Bl2	AP115	*Phyllidia exquisita*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidia exquisita*
Phex18Bl1	AP116	*Phyllidia exquisita*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidia exquisita*
Phex15Bu1	DS210	*Phyllidia* sp. 3	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia* sp. 3
Phal15Bu1	DS214	*Phyllidia* sp. 3	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidia* sp. 3
Phva16Sa14	NU253	*Phyllidia haegeli*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia haegeli*
Phva16Sa19	NU258	*Phyllidia haegeli*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia haegeli*
Phpic16Sa4	NU125	*Phyllidia haegeli*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia haegeli*
Phva16Bu2	NU317	*Phyllidia haegeli*	2016	Bunaken	1°37'41.7″N; 124°45'60″E	Sachiko	*Phyllidia haegeli*

Phco15Bu1	DS178	*Phyllidia haegeli*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia haegeli*
Phva16Sa51	NU290	*Phyllidia haegeli*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia haegeli*
Phex18Ba1	AP114	*Phyllidia haegeli*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia haegeli*
Phsp15Bu1	DS260	*Phyllidia* sp. 9	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia haegeli*
Phasp15Bu1	DS177	*Phyllidia* sp. 9	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidia haegeli*
Phoc18Ba7	AP073	*Phyllidia* cf. *babai*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidia* cf. *babai*
Phoc16Sa3	NU117	*Phyllidia* cf. *babai*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia* cf. *babai*
Phoc18Ba5	AP071	*Phyllidia ocellata*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidia ocellata*
Phoc18Ba1	AP067	*Phyllidia ocellata*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidia ocellata*
Phoc18Ba4	AP070	*Phyllidia ocellata*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia ocellata*
Phoc18Ba2	AP068	*Phyllidia ocellata*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidia ocellata*
Phoc18Ba6	AP072	*Phyllidia ocellata*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidia ocellata*
Phoc18Ba3	AP069	*Phyllidia ocellata*	2018	Bangka	1°44'27.4″N; 125°08'35.1″E	Busa Bora Timur	*Phyllidia ocellata*
Phoc15Bu1	DS213	*Phyllidia ocellata*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidia ocellata*
18TMPhoc1	RB001	*Phyllidia ocellata*	2018	North Sulawesi	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidia ocellata*
Phma16Sa1	NU114	*Phyllidia ocellata*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia ocellata*
Phoc17Ba1	AP02	*Phyllidia ocellata*	2017	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidia ocellata*
Phsp17Bu1	AP24	*Phyllidia* sp. a	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidia* sp. a
Phpu18Bu16	AP196	*Phyllidiella pustulosa*	2018	Bunaken	1°35'46.4″N; 124°46'3.4″E	Lekuan I	*Phyllidiella pustulosa*
Phpu18Ko6	AP172	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella pustulosa*
Phpu18Ba17	AP147	*Phyllidiella pustulosa*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidiella pustulosa*
Phpu18Bu9	AP189	*Phyllidiella pustulosa*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella pustulosa*
Phpu18Bu13	AP193	*Phyllidiella pustulosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidiella pustulosa*
Phpu18Bu14	AP194	*Phyllidiella pustulosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidiella pustulosa*
Phpu18Ba6	AP136	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu18Ba18	AP148	*Phyllidiella pustulosa*	2018	Bangka	1°48'41.3″N; 125°06'45.1″E	Pearl Farm	*Phyllidiella pustulosa*
Phpu18Ba14	AP144	*Phyllidiella pustulosa*	2018	Bangka	1°50'45.44″N; 125°5'55.26″E	Kinabuhutan	*Phyllidiella pustulosa*

Phpu18Ko12	AP178	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella pustulosa*
Phpu15Bu44	DS258	*Phyllidiella pustulosa*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella pustulosa*
Phpu15Bu13	DS231	*Phyllidiella pustulosa*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado tua	*Phyllidiella pustulosa*
Phpu16Bu6	NU310	*Phyllidiella pustulosa*	2016	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella pustulosa*
Phpu17M6	AP39	*Phyllidiella pustulosa*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella pustulosa*
PhpuNN1	NU330	*Phyllidiella pustulosa*	-	North Sulawesi	-	-	*Phyllidiella pustulosa*
Phpu18Ba24	AP154	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu18Bu11	AP191	*Phyllidiella pustulosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidiella pustulosa*
Phpu18Bu12	AP192	*Phyllidiella pustulosa*	2018	Bunaken	1°36'38.4″N; 124°46'57.5″E	Pangalisang	*Phyllidiella pustulosa*
Phpu18Bu7	AP187	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella pustulosa*
Phpu18Bu1	AP181	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella pustulosa*
Phpu18Ko3	AP169	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella pustulosa*
Phpu18Ko5	AP171	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella pustulosa*
Phpu18Ba2	AP132	*Phyllidiella pustulosa*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiella pustulosa*
Phpu18Ba25	AP156	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu18Ba5	AP135	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu18Bl3	AP164	*Phyllidiella pustulosa*	2018	Barrang Lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella pustulosa*
Phpu18Ba4	AP134	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu18Ba12a	AP142	*Phyllidiella pustulosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidiella pustulosa*
Phpu18Ba7	AP137	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu17Ba1	AP05	*Phyllidiella pustulosa*	2017	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidiella pustulosa*
Phpu17Bu2	AP21	*Phyllidiella pustulosa*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella pustulosa*
Phpu17Ba8	AP11	*Phyllidiella pustulosa*	2017	Bangka	1°44'35.11″N; 125°09'43.50″E	Sahaung I	*Phyllidiella pustulosa*
Phpu17Tu1	AP37	*Phyllidiella pustulosa*	2017	Tumbak	0°58'13.4"N; 124°53'03.0"E	Tumbak	*Phyllidiella pustulosa*
Phpu17Ba2	AP06	*Phyllidiella pustulosa*	2017	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidiella pustulosa*
Phpu15Bu4	DS222	*Phyllidiella pustulosa*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiella pustulosa*
Phpu16Bu2	NU306	*Phyllidiella pustulosa*	2016	Bunaken	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidiella pustulosa*

Phpu15Bu40	DS254	*Phyllidiella pustulosa*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella pustulosa*
Phpu18Sm2	AP180	*Phyllidiella pustulosa*	2018	Samalona	5°07'20.7″S; 119°20'30.7″E	Mercusuar	*Phyllidiella pustulosa*
Phpu18Ba24a	AP155	*Phyllidiella pustulosa*	2018	Bangka	1°40'52.34″N; 125°9'10.55″E	Efrata	*Phyllidiella pustulosa*
Phpu16Bu9	NU313	*Phyllidiella pustulosa*	2016	Bunaken	1°38'12.6″N; 124°46'3.4″E	Mikes Point	*Phyllidiella pustulosa*
Phpu15Bu42	DS256	*Phyllidiella pustulosa*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella pustulosa*
Phpu15Bu9	DS227	*Phyllidiella pustulosa*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella pustulosa*
Phpu16Sa78a	NU213	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella pustulosa*
Phpu15Bu8	DS226	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella pustulosa*
Phpu15Bu23	DS240	*Phyllidiella pustulosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella pustulosa*
Phpu16Bu5	NU309	*Phyllidiella pustulosa*	2016	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella pustulosa*
Phpu15Bu33	DS249	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella pustulosa*
Phli16Sa3	NU108	*Phyllidiella pustulosa*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella* cf. *pustulosa*
Phpu18Ba22	AP152	*Phyllidiella pustulosa*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidiella* cf. *pustulosa*
Phpu18Ko1	AP167	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Ko2	AP168	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Ko2a	AP220	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Ko8	AP174	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Ko7	AP173	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Ko10	AP176	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phli18Ba4	AP202	*Phyllidiella pustulosa*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidiella* cf. *pustulosa*
Phpu18Ko11	AP177	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* cf. *pustulosa*
Phpu18Sm1	AP179	*Phyllidiella pustulosa*	2018	Samalona	5°07'20.7″S; 119°20'30.7″E	Mercusuar	*Phyllidiella* cf. *pustulosa*
Phpu18Ba3	AP133	*Phyllidiella pustulosa*	2018	Bangka	1°40'51.06″N; 125°8'22.45″E	Sempini 2	*Phyllidiella* cf. *pustulosa*

Phpu15Bu20	DS237	*Phyllidiella nigra*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella nigra*
Phpu17M5	AP36	*Phyllidiella nigra*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella nigra*
Phpu15Bu43	DS257	*Phyllidiella nigra*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella nigra*
Phpu16Sa64	NU198	*Phyllidiella nigra*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella nigra*
Phpu15Bu29	DS246	*Phyllidiella nigra*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella nigra*
Phpu15Bu10	DS228	*Phyllidiella nigra*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella nigra*
Phpu15Bu7	DS225	*Phyllidiella nigra*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella nigra*
Phpu17Ba9	AP12	*Phyllidiella nigra*	2017	Bangka	1°44'35.11″N; 125°09'43.50″E	Sahaung I	*Phyllidiella nigra*
Phpu15Bu6	DS224	*Phyllidiella nigra*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiella nigra*
Phpu15Bu18	DS235	*Phyllidiella nigra*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella nigra*
18TMPhni1	RB013	*Phyllidiella nigra*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella nigra*
Phpu15Bu19	DS236	*Phyllidiella nigra*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella nigra*
Phpu17Tu2	AP38	*Phyllidiella nigra*	2017	Tumbak	0°58'13.4"N; 124°53'03.0"E	Tumbak	*Phyllidiella nigra*
Phpu17M3	AP34	*Phyllidiella nigra*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella nigra*
Phpu16Bu1	NU303	*Phyllidiella nigra*	2016	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella nigra*
Phpu17Bu1	AP20	*Phyllidiella nigra*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella nigra*
Phpu16Bu4	NU308	*Phyllidiella nigra*	2016	Bunaken	1°38'12.6″N; 124°46'3.4″E	Mikes Point	*Phyllidiella nigra*
Phpu16Bu7	NU311	*Phyllidiella nigra*	2016	Bunaken	1°38′46.1″N; 124°42′48″E	Tanjung kopi (Manado tua)	*Phyllidiella nigra*
Phpu18Ba19	AP149	*Phyllidiella nigra*	2018	Bangka	1°44'39.8″N; 125°08'24.4″E	Busa Bora Kampung	*Phyllidiella nigra*
Phpu18Ba20	AP150	*Phyllidiella nigra*	2018	Bangka	1°44'39.8″N; 125°08'24.4″E	Busa Bora Kampung	*Phyllidiella nigra*
Phpu18Ba26	AP157	*Phyllidiella nigra*	2018	Bangka	1°46'07.8″N; 125°10'34.6″E	Areng Kambing	*Phyllidiella nigra*
Phpu18Ba27	AP158	*Phyllidiella nigra*	2018	Bangka	1°46'07.8″N; 125°10'34.6″E	Areng Kambing	*Phyllidiella nigra*
Phpu18Bu8	AP188	*Phyllidiella nigra*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella nigra*
Phpu18Ko9	AP175	*Phyllidiella nigra*	2018	Kodingareng keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella nigra*
Phni18Po1	AP198	*Phyllidiella nigra*	2018	Pomalaa	4°10'11.30″S; 121°35'8.63″E	Pomalaa Harbour	*Phyllidiella nigra*

Phpu17Bu4	AP23	*Phyllidiella zeylanica*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella zeylanica* aucct.
Phpu16Sa12	NU147	*Phyllidiella zeylanica*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* aucct.
Phpu15Bu3	DS221	*Phyllidiella zeylanica*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella zeylanica* aucct.
Phpu15Bu11	DS229	*Phyllidiella zeylanica*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella zeylanica* aucct.
Phpu15Bu25	DS242	*Phyllidiella zeylanica*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiella zeylanica* aucct.
PhpuNN2	NU332	*Phyllidiella zeylanica*	-	North Sulawesi	-	-	*Phyllidiella zeylanica* aucct.
Phpu17Ba7	AP10	*Phyllidiella zeylanica*	2017	Bangka	1°41'23.01″N; 125°09'55.26″E	Tanjung Kusu	*Phyllidiella zeylanica* aucct.
Phpi15Bu1	DS215	*Phyllidiella zeylanica*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella zeylanica* aucct.
Phpu15Bu12	DS230	*Phyllidiella zeylanica*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado Tua	*Phyllidiella zeylanica* aucct.
Phpu15Bu41	DS255	*Phyllidiella zeylanica*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella zeylanica* aucct.
18TMPhyfi1	RB023	*Phyllidiella zeylanica*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella zeylanica* aucct.
Phli18Bl1	AP203	*Phyllidiella zeylanica*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella zeylanica* aucct.
Phze18Bu1	AP208	*Phyllidiella zeylanica*	2018	Bunaken	1°35'35.9″N; 124°46'44.1″E	Muka Kampung	*Phyllidiella zeylanica* aucct.
Phpu18Bl4	AP165	*Phyllidiella zeylanica*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella zeylanica* aucct.
Phpu18Bl1	AP162	*Phyllidiella zeylanica*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella zeylanica* aucct.
Phli18Bu1	AP205	*Phyllidiella zeylanica*	2018	Bunaken	1°35'35.9″N; 124°46'44.1″E	Muka Kampung	*Phyllidiella zeylanica* aucct.
Phan16Bu1	NU299	*Phyllidiella zeylanica*	2016	Sangihe	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidiella zeylanica* aucct.
Phpu16Bu10	NU304	*Phyllidiella zeylanica*	2016	Bunaken	1°44′55″N; 124°43′33.5″E	Mantehage 1	*Phyllidiella zeylanica* aucct.
Phpu17Bu3	AP22	*Phyllidiella pustulosa*	2017	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. a
Phpu16Sa77a	NU211	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. a
Phpu17M1	AP31	*Phyllidiella pustulosa*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella* sp. a
Phpu17M2	AP33	*Phyllidiella pustulosa*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella* sp. a
Phpu18Bu3	AP183	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. a
Phpu18Bu4	AP184	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. a
Phpu18Po2	AP160	*Phyllidiella pustulosa*	2018	Pomalaa	4°13'48.97″S; 121°34'19.33″E	Latumbi	*Phyllidiella* sp. a
Phpu18Ba8	AP138	*Phyllidiella pustulosa*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidiella* sp. a
Phpu17M4	AP35	*Phyllidiella pustulosa*	2017	Moinit	1°11'15.5"N; 124°29'18.9"E	Moinit	*Phyllidiella* sp. a
Phpu18Ba15	AP145	*Phyllidiella pustulosa*	2018	Bangka	1°44'07.74″N; 125°09'05,59″E	Tanjung Husi	*Phyllidiella* sp. a

Phpu18Bu2	AP182	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. a
Phpu18Ba9	AP139	*Phyllidiella pustulosa*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidiella* sp. a
Phpu18Ba23	AP153	*Phyllidiella pustulosa*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidiella* sp. a
Phpu15Bu15	DS233	*Phyllidiella pustulosa*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado Tua	*Phyllidiella* sp. a
Phpu18Ba10	AP140	*Phyllidiella pustulosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidiella* sp. a
18TMPhpu2	RB020	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. a
Phpi15Bu5	DS219	*Phyllidiella rudmani*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiella rudmani*
Phpi15Bu2	DS216	*Phyllidiella rudmani*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella rudmani*
Phpu18Bu5	AP185	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. b
Phpu16Sa17	NU152	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa37	NU172	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. c subclade 1
Phpu16Sa22	NU157	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa51	NU185	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 1
Phli16Sa8	NU113	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* sp. c subclade 1
Phpu16Sa16	NU151	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa41	NU176	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 1
18TMPhni**2**	RB014	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. c subclade 1
Phpu15Bu30	DS247	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella* sp. c subclade 1
18TMPhni5	RB017	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. c subclade 1
18TMPhni4	RB016	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. c subclade 1
Phpu15Bu32	DS248	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella* sp. c subclade 1
18TMPhni6	RB018	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. c subclade 1
Phpu18Bu15	AP195	*Phyllidiella pustulosa*	2018	Bunaken	1°35'35.9″N; 124°46'44.1″E	Muka Kampung	*Phyllidiella* sp. c subclade 1
Phli18Po1	AP204	*Phyllidiella pustulosa*	2018	Pomalaa	4°13'48.97″S; 121°34'19.33″E	Latumbi	*Phyllidiella* sp. c subclade 1
Phpu18Bu10	AP190	*Phyllidiella pustulosa*	2018	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella* sp. c subclade 1

Phli18Ba3	AP201	*Phyllidiella pustulosa*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiella* sp. c subclade 1
Phpu18Po4	AP161	*Phyllidiella pustulosa*	2018	Pomalaa	4°16'13.77″S; 121°31'34.70″E	Watu Kilat	*Phyllidiella* sp. c subclade 1
Phpu18Ba11	AP141	*Phyllidiella pustulosa*	2018	Bangka	1°50'45.44″N; 125°5'55.26″E	Kinabuhutan	*Phyllidiella* sp. c subclade 1
Phpu18Ba21	AP151	*Phyllidiella pustulosa*	2018	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidiella* sp. c subclade 1
Phpu15Bu2	DS220	*Phyllidiella pustulosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella* sp. c subclade 2
Phan15Bu1	DS174	*Phyllidiella pustulosa*	2015	Bunaken	1°38′46.1″N; 124°42′48″E	Manado tua / Battu lohang	*Phyllidiella* sp. c subclade 2
Phan15Bu2	DS175	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella* sp. c subclade 2
Phpu15Bu34	DS250	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella* sp. c subclade 2
Phpu15Bu27	DS244	*Phyllidiella pustulosa*	2015	Bunaken	1°38′46.1″N; 124°42′48″E	Manado tua / Battu lohang	*Phyllidiella* sp. c subclade 2
Phli18Ba2	AP200	*Phyllidiella pustulosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidiella* sp. c subclade 2
Phpu18Ba13	AP143	*Phyllidiella pustulosa*	2018	Bangka	1°50'45.44″N; 125°5'55.26″E	Kinabuhutan	*Phyllidiella* sp. c subclade 2
Phpu18Ba12	AP129	*Phyllidiella pustulosa*	2018	Bangka	1°53'38.32″N; 125°05'53.82″E	Talisei	*Phyllidiella* sp. c subclade 2
Phan15Bu3	DS176	*Phyllidiella pustulosa*	2015	Bunaken	1°37′41.7″N; 124°45′57″E	Pasir Panjang	*Phyllidiella* sp. c subclade 2
Phpu16Sa11	NU146	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 2
Phpu15Bu17	DS234	*Phyllidiella pustulosa*	2015	Bunaken	1°37'55.7″N; 124°48'3.6″E	Siladen	*Phyllidiella* sp. c subclade 2
Phpu15Bu26	DS243	*Phyllidiella pustulosa*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiella* sp. c subclade 2
Phpu18Ko4	AP170	*Phyllidiella pustulosa*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella* sp. c subclade 2
Phli18Ba1	AP199	*Phyllidiella pustulosa*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiella* sp. c subclade 2
Phpu16Sa49	NU183	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 2
Phpu18Bu6	AP186	*Phyllidiella pustulosa*	2018	Bunaken	1°36'50″N; 124°46'3.4″E	Panorama	*Phyllidiella* sp. c subclade 2
Phpu17Ba4	AP08	*Phyllidiella pustulosa*	2017	Bangka	1°46'22.20″N; 125°10'57.38″E	Batu Belah	*Phyllidiella albonigra*
Phpu15Bu5	DS223	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella albonigra*
Phpu16Bu3	NU307	*Phyllidiella pustulosa*	2016	Bunaken	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidiella albonigra*
Phpu16Sa32	NU167	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella albonigra*
Phpu16Bu8	NU312	*Phyllidiella pustulosa*	2016	Bunaken	1°38'12.6″N; 124°46'3.4″E	Mikes Point	*Phyllidiella albonigra*
Phpu15Bu22	DS239	*Phyllidiella pustulosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella albonigra*
Phpu15Bu21	DS238	*Phyllidiella pustulosa*	2015	Bunaken	1°36'48.4″N; 124°44'22.8″E	Johnson	*Phyllidiella albonigra*

Phpu15Bu24	DS241	*Phyllidiella pustulosa*	2015	Bunaken	1°36'42.4″N; 124°46'4.7″E	Cela Cela	*Phyllidiella albonigra*
18TMPhpu1	RB019	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. d
Phpu16Bu11	NU305	*Phyllidiella pustulosa*	2016	Bunaken	1°44′55″N; 124°43′33.5″E	Mantehage 1	*Phyllidiella* sp. d
Phsh16Sa1	NU231	*Phyllidiella pustulosa*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella* sp. d
18TMPhni**3**	RB015	*Phyllidiella pustulosa*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiella* sp. d
Phpu18Ba1	AP131	*Phyllidiella pustulosa*	2018	Bangka	1°45'03.61″N; 125°07'58,85″E	Coral Eye	*Phyllidiella* sp. d
Phpu18Bl5	AP166	*Phyllidiella pustulosa*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella* sp. d
Phpu18Po3	AP130	*Phyllidiella pustulosa*	2018	Pomalaa	4°16'13.77″S; 121°31'34.70″E	Watu Kilat	*Phyllidiella* sp. d
Phpu18Ba16	AP146	*Phyllidiella pustulosa*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidiella* sp. d
Phpu18Po1	AP159	*Phyllidiella pustulosa*	2018	Pomalaa	4°10'11.30″S; 121°35'8.63″E	Pomalaa Harbour	*Phyllidiella* sp. d
Phpu15Bu28	DS245	*Phyllidiella pustulosa*	2015	Bunaken	1°38′46.1″N; 124°42′48″E	Manado tua / Battu lohang	*Phyllidiella* sp. complex e
Phpu18Bl2	AP163	*Phyllidiella pustulosa*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiella* sp. complex e
Phpu15Bu39	DS253	*Phyllidiella pustulosa*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiella* sp. complex e
Phru18Ko1	AP206	*Phyllidiella hageni*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella hageni*
Phha18Ko1	AP207	*Phyllidiella hageni*	2018	Kodingareng Keke	5°06'15.6″S; 119°17'13.9″E	VW	*Phyllidiella hageni*
Phllsp15Bu1	DS212	*Phyllidiella hageni*	2015	Bunaken	1°36'60″N; 124°45'11.5″E	Alung Banua	*Phyllidiella hageni*
18TMPhni7	RB022	*Phyllidiopsis krempfi*	2018	Tanjung Mandolang	1°26'38.1″N; 124°44'29.2″E	Tanjung Mandolang	*Phyllidiopsis krempfi*
Phsh15Bu1	DS259	*Phyllidiopsis pipeki*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiopsis krempfi*
Phskr18Bl1	AP211	*Phyllidiopsis krempfi*	2018	Barrang lompo	5°3'1″S; 119°19'50″E	Dermaga Unhas	*Phyllidiopsis krempfi*
Phskr18Ba1	AP209	*Phyllidiopsis pipeki*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiopsis krempfi*
Phspi18Ba1	AP213	*Phyllidiopsis pipeki*	2018	Bangka	1°41'16,71″N; 125°09'42.79″E	Batu Mandi	*Phyllidiopsis krempfi*
Phpi15Bu3	DS217	*Phyllidiopsis burni*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiopsis burni*
Phpu16Sa81	NU217	*Phyllidiopsis burni*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis burni*
Phssp.a18Ba1	AP212	*Phyllidiopsis burni*	2018	Bangka	1°40'21.67″N; 125°8'6.60″E	Yellow Coco	*Phyllidiopsis burni*

Phpu16Sa43	NU178	-	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis* sp. a
18LePhni1	RB026		*Phyllidiopsis krempfi*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiopsis* sp. a
Phpu16Sa59	NU193	-	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis* sp. a
Phpu16Sa42	NU 177	-	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis* sp. a
Phpu16Sa45	NU179	-	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis* sp. a
Phskr18Ba2	AP210	-	*Phyllidiopsis krempfi*	2018	Bangka	1°46'07.8″N; 125°10'34.6″E	Areng Kambing	*Phyllidiopsis* sp. a
Phsan18Ba1	AP214		*Phyllidiopsis annae*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiopsis annae*
Phsan18Ba2	AP215		*Phyllidiopsis annae*	2018	Bangka	1°41'4.83″N; 125°8'32.65″E	Sempini	*Phyllidiopsis annae*
Phsan18Ba3	AP216		*Phyllidiopsis annae*	2018	Bangka	1°44'39.8″N; 125°08'24.4″E	Busa Bora Kampung	*Phyllidiopsis annae*
Phsan18Ba4	AP217		*Phyllidiopsis annae*	2018	Bangka	1°44'39.8″N; 125°08'24.4″E	Busa Bora Kampung	*Phyllidiopsis annae*
Phsph15Bu1	DS261		*Phyllidiopsis sphingis*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado Tua	*Phyllidiopsis sphingis*
Phst17Ba2	AP14		*Phyllidiopsis xishaensis*	2017	Bangka	1°41'16,71″N; 125°09'42.79″E	Batu Mandi	*Phyllidiopsis xishaensis*
Phst16Bu2	NU315		*Phyllidiopsis xishaensis*	2016	Bunaken	1°39'7.1″N; 124°41'49.6″E	Tanjung kopi (Manado tua)	*Phyllidiopsis xishaensis*
Phsst15Bu1	DS262		*Phyllidiopsis xishaensis*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiopsis xishaensis*
Phst17Ba1	AP13		*Phyllidiopsis xishaensis*	2017	Bangka	1°44'07.74″N; 125°09'05,59″E	Tanjung Husi	*Phyllidiopsis xishaensis*
Phst16Bu1	NU314		*Phyllidiopsis xishaensis*	2016	Bunaken	1°36'04.4″N; 124°45'54.4″E	Lekuan 2	*Phyllidiopsis xishaensis*
Phsxi18Ba1	AP218		*Phyllidiopsis xishaensis*	2018	Bangka	1°48'41.3″N; 125°06'45.1″E	Pearl Farm	*Phyllidiopsis xishaensis*
Phsxi18Bu1	AP219		*Phyllidiopsis xishaensis*	2018	Bunaken	1°35'35.9″N; 124°46'44.1″E	Muka Kampung	*Phyllidiopsis xishaensis*
Phsp16Sa1	NU233		*Phyllidiopsis shireenae*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiopsis shireenae*
Phpi15Bu4	DS218		*Phyllidiopsis shireenae*	2015	Bunaken	1°37′50.6″N; 124°45′48″E	Mamaling	*Phyllidiopsis shireenae*

Table 2. DNA sequences extracted from GenBank NCBI

Ident/voucher number	DNA number	16S	CO1	Species name in NCBI	year	Locality	coordinate	station	Species name in my tree
Phva16Sa21	NU260	MN243734	MN248563	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa26	NU265	MN243733	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa8	NU247	MN243735	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa58	NU297	MN243732	-	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa39	NU278	MN243744	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa7	NU246	MN243747	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa25	NU264	MN243746	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa29	NU268	MN243745	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa4	NU243	MN243778	MN248554	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa22	NU261	MN243777	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa12	NU251	MN243781	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa49	NU288	MN243743	-	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa3	NU242	MN243779	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa9	NU248	MN243783	MN248572	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa50	NU289	MN243742	MN248559	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa45	NU284	MN243741	MN248564	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa33	NU272	MN243767	MN248574	*Phyllidia varicosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia varicosa*
Phva16Sa32	NU271	MN243768	-	*Phyllidia varicosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia varicosa*
Phva16Sa28	NU267	MN243748	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa2	NU241	MN243776	-	*Phyllidia varicosa*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidia varicosa*
Phva16Sa36	NU275	MN243772	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa47	NU286	MN243737	MN248558	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa57	NU296	MN243736	-	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa30	NU269	MN243740	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa41	NU280	MN243739	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa46	NU285	MN243738	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*

Phva16Sa43	NU282	MN243764	MN248565	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa18	NU257	MN243780	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa52	NU291	MN243765	MN248560	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa44	NU283	MN243766	MN248561	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa56	NU295	MN243769	MN248566	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa31	NU270	MN243770	MN248567	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa27	NU266	MN243771	MN248573	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa42	NU281	MN243749	MN248562	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa10	NU249	MN243750	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa23	NU262	MN243773	-	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa16	NU255	MN243775	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa15	NU254	MN243782	MN248555	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa55	NU294	MN243751	-	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa34	NU273	MN243756	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa48	NU287	MN243753	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa17	NU256	MN243759	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa11	NU250	MN243761	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa38	NU277	MN243763	-	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa54	NU293	MN243752	-	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
Phva16Sa24	NU263	MN243757	MN248568	*Phyllidia varicosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia varicosa*
Phva16Sa37	NU276	MN243755	MN248569	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa13	NU252	MN243760	MN248571	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa5	NU244	MN243774	-	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa40	NU279	MN243754	MN248557	*Phyllidia varicosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia varicosa*
Phva16Sa20	NU259	MN243758	MN248556	*Phyllidia varicosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia varicosa*
Phva16Sa53	NU292	MN243762	MN248570	*Phyllidia varicosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia varicosa*
18LePhva1	RB043	MN508641	MN507469	*Phyllidia varicosa*	2018	Lembeh	1°27'40.9"N; 125°13'37"E	Nudifalls	*Phyllidia varicosa*
18LePhva4	RB050	MN508642	MN507468	*Phyllidia varicosa*	2018	Lembeh	1°27'40.9"N; 125°13'37"E	Nudifalls	*Phyllidia varicosa*
Phva18LS3	MO37	MK911031	MK852558	*Phyllidia varicosa*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidia varicosa*

Phva18LS1	MO26	MK911030	-	*Phyllidia varicosa*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidia varicosa*
18LePhva5	RB051	MN508643	MN507470	*Phyllidia varicosa*	2018	Lembeh	1°27'40.9"N; 125°13'37"E	Nudifalls	*Phyllidia varicosa*
18LePhva3	RB045	MN508644	MN507471	*Phyllidia varicosa*	2018	Lembeh	1°27'40.9"N 125°13'37"E	Nudifalls	*Phyllidia varicosa*
336489		-	KX235931	*Phyllidia varicosa*	2009	TER16	0°51'50.4"N; 127°20'36.7" E	Tanjung Pasir Putih	*Phyllidia varicosa*
336568		-	KX235942	*Phyllidia varicosa*	2007	RAJ38	0°34'05.0"S; E130°38'31.5" E	Northeast Mansuar	*Phyllidia varicosa*
336569		-	KX235941	*Phyllidia varicosa*	2007	RAJ38	0°34'05.0" S; E130°38'31.5" E	Northeast Mansuar	*Phyllidia varicosa*
336570		-	KX235943	*Phyllidia varicosa*	2007	RAJ29	0°49'42.5" S; E130°42'42.0" E	North Batanta, West Telok Gegenlol	*Phyllidia varicosa*
336571		-	KX235938	*Phyllidia varicosa*	2007	RAJ25 South Gam	0°30'51.5"S; E130°34'11.5" E	Eastern entrance Besir Bay, Cape Besir	*Phyllidia varicosa*
336572		-	KX235940	*Phyllidia varicosa*	2007	RAJ25 South Gam	0°30'51.5"S; E130°34'11.5" E	Eastern entrance Besir Bay, Cape Besir	*Phyllidia varicosa*
336604		-	KX235932	*Phyllidia varicosa*	2009	TER25	0°46'55.3"N; E127°23'19.9" E	East side Ternate Harbour (outside)	*Phyllidia varicosa*
336609		-	KX235933	*Phyllidia varicosa*	2009	TER26	0°53'20.5"N; E127°27'34.2" E	Pasir Lamo (West side)	*Phyllidia varicosa*
336612		-	KX235934	*Phyllidia varicosa*	2009	TER26	0°53'20.5"N; E127°27'34.2" E	Pasir Lamo (West side)	*Phyllidia varicosa*
336617		-	KX235935	*Phyllidia varicosa*	2009	TER27	0°54'44.5"N; E127°29'09.9" E	Tanjung Ratemu (South of river)	*Phyllidia varicosa*
336621		-	KX235936	*Phyllidia varicosa*	2009	TER28	0°01'51.9"S; E127°14'01.8" E	Pulau Popaco E	*Phyllidia varicosa*
336637		-	KX235937	*Phyllidia varicosa*	2009	TER36	0°50'47.8"N; E127°37'48.7" E	Teluk Dodinga East; North of Pulau Jere	*Phyllidia varicosa*
336647		-	KX235939	*Phyllidia varicosa*	2009	TER39	0°51'09.1"N; 127°35'19.5" E	Teluk Dodinga, Karang Galiasa Kecil West	*Phyllidia varicosa*
Ali_126		-	KP873170	*Phyllidia varicosa*		Pahang, Malaysia	-	Pantai Balok	*Phyllidia varicosa*
Ali_125		-	KP873169	*Phyllidia varicosa*		Terengganu, Malaysia	-	Bidong Island	*Phyllidia varicosa*
AF430364		AF430364	-	*Phyllidia varicosa*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidia varicosa*

KJ001306		-	KJ001306	*Phyllidia varicosa*	(2014)	Queensland, Australia	14°40'S; 145°28'E	Lizard Island	*Phyllidia varicosa*
18LePhsp1	RB041	MN508640	MN507478	*Phyllidia elegans*	2018	Lembeh	1°28'56.4"N; 125°14'19"E	Nudi retreat	*Phyllidia elegans*
336475		-	KX073972	*Phyllidia elegans*	2009	TER12	0°50'05.1"N; 127°23'10.0"E	Tanjung Tabam	*Phyllidia elegans*
336478		-	KX073973	*Phyllidia elegans*	2009	TER13	0°54'42.7" N; 127°18'32.9"E	Pulau Maka	*Phyllidia elegans*
336488		-	KX073974	*Phyllidia elegans*	2009	TER16	0°51'50.4"; 127°20'36.7"E	Tanjung Pasir Putih	*Phyllidia elegans*
336514		-	KX073975	*Phyllidia elegans*	2009	TER24	0°48'49.1"N; 127°23'21.6"E	Dufadufa / Benteng Toloko	*Phyllidia elegans*
336515		-	KX073976	*Phyllidia elegans*	2009	TER24	0°48'49.1"N; 127°23'21.6"E	Dufadufa / Benteng Toloko	*Phyllidia elegans*
336554		-	KX073985	*Phyllidia elegans*	2007	RAJ43	0°25'45.2"S; 130°33'37.3"E	Passage	*Phyllidia elegans*
336555		-	KX073990	*Phyllidia elegans*	2007	RAJ14	0°34'15.2"S; 130°39'33.7"E	Akber Reef	*Phyllidia elegans*
336556		-	KX073988	*Phyllidia elegans*	2007	RAJ43	0°25'45.2"S; 130°33'37.3"E	Passage	*Phyllidia elegans*
336557		-	KX073987	*Phyllidia elegans*	2007	RAJ43	0°25'45.2"S; 130°33'37.3"E	Passage	*Phyllidia elegans*
336558		-	KX073984	*Phyllidia elegans*	2007	RAJ40	0°33'58.1"S; 130°39'46.2"E	Southwest Pulau Kri	*Phyllidia elegans*
336559		-	KX073991	*Phyllidia elegans*	2007	RAJ37	0°31'08.2"S; 130°38'28.0"E	South Gam, shoal near mangroves	*Phyllidia elegans*
336560		-	KX073983	*Phyllidia elegans*	2007	RAJ40	0°33'58.1"S; 130°39'46.2"E	Southwest Pulau Kri	*Phyllidia elegans*
336561		-	KX073986	*Phyllidia elegans*	2007	RAJ43	0°25'45.2"S; 130°33'37.3"E	Passage	*Phyllidia elegans*
336562		-	KX073989	*Phyllidia elegans*	2007	RAJ14	0°34'15.2"S; 130°39'33.7"E	Akber Reef	*Phyllidia elegans*
336628		-	KX073977	*Phyllidia elegans*	2009	TER32	0°01'17.3"S; 127°14'17.2"E	Pulau Gura Ici, East	*Phyllidia elegans*
336629		-	KX073978	*Phyllidia elegans*	2009	TER32	0°01'17.3"S; 127°14'17.2"E	Pulau Gura Ici, East	*Phyllidia elegans*
336631		-	KX073979	*Phyllidia elegans*	2009	TER34	0°42'49.8"N; 127°28'45.4"E	Pulau Pilongga, North	*Phyllidia elegans*
336632		-	KX073980	*Phyllidia elegans*	2009	TER34	0°42'49.8"N; 127°28'45.4"E	Pulau Pilongga, North	*Phyllidia elegans*
336633		-	KX073981	*Phyllidia elegans*	2009	TER34	0°42'49.8"N; 127°28'45.4"E	Pulau Pilongga, North	*Phyllidia elegans*
336649		-	KX073982	*Phyllidia elegans*	2009	TER40	0°46'25.3"N; 127°32'22.0"E	Teluk Dodinga; Karang Ngeli West	*Phyllidia elegans*

-		AJ225201	AJ223276	*Phyllidia elegans*	1999	Papua New Guinea	-	Tab Island	*Phyllidia elegans*
AF430362		AF430362	-	*Phyllidia elegans*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidia elegans*
Phco16Sa1	NU094	MN172238	MN234119	*Phyllidia coelestis*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia coelestis*
Phco16Sa2	NU095	MN172237	MN234113	*Phyllidia coelestis*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia coelestis*
Phco16Sa4	NU097	MN172235	MN234118	*Phyllidia coelestis*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia coelestis*
Phco16Sa10	NU103	MN172231	MN234112	*Phyllidia coelestis*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia coelestis*
Phco16Sa9	NU102	MN172232	MN234114	*Phyllidia coelestis*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia coelestis*
Phco16Sa3	NU096	MN172236	MN234115	*Phyllidia coelestis*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia coelestis*
Phco16Sa7	NU100	MN172233	MN234116	*Phyllidia coelestis*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia coelestis*
Phco16Sa5	NU098	MN172234	MN234112	*Phyllidia coelestis*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidia coelestis*
Phco18LS1	MO21	MK852557	MK911039	*Phyllidia coelestis*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidia coelestis*
336573		-	KX235921	*Phyllidia coelestis*	2007	RAJ44	0°25'44.3"S; 130°33'56.8"E	Eastern entrance of passage	*Phyllidia coelestis*
336574		-	KX235922	*Phyllidia coelestis*	2007	RAJ13	0°26'31.1"S; 130°41'08.0"E	Wallace Lake	*Phyllidia coelestis*
AF430361		AF430361	-	*Phyllidia coelestis*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidia coelestis*
-		KJ018917	KJ001305	*Phyllidia coelestis*	(2014)	Queensland, Australia	14°40'S; 145°28'E	Lizard Island	*Phyllidia coelestis*
-		KJ018916	KJ001304	*Phyllidia picta*	(2014)	Queensland, Australia	14°40'S; 145°28'E	Lizard Island	*Phyllidia coelestis*
CASIZ190982		MF958279	MF958412.1	*Phyllidia coelestis*		Kranket Island,	-	Madang, Papua New Guinea	*Phyllidia coelestis*
Phsp616Sa3	NU237	MN217677	MN248550	*Phyllidia picta*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia picta*
Phpic16Sa13	NU134	MN217676	MN248548	*Phyllidia picta*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia picta*
Phpic16Sa6	NU127	MN217675	MN248546	*Phyllidia picta*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia picta*
Phpic16Sa1	NU122	MN217674	MN248545	*Phyllidia picta*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidia picta*
Phpic16Sa11	NU132	MN217679	MN248549	*Phyllidia picta*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*
Phpic16Sa12	NU133	MN217678	MN248547	*Phyllidia picta*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*

Phspec116Sa2	NU239	MN217670	MN248541	*Phyllidia picta*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*
Phpic16Sa9	NU130	MN217669	MN248539	*Phyllidia picta*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*
Phpic16Sa8	NU129	MN217671	MN248540	*Phyllidia picta*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidia picta*
Phsp316Sa1	NU234	MN217673	MN265389	*Phyllidia* spec.	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*
Phpic16Sa10	NU131	MN217672	MN248542	*Phyllidia picta*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia picta*
Phpic16Sa14	NU135	MN217681	MN248544	*Phyllidia picta*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia picta*
Phpic16Sa5	NU126	MN217680	MN248543	*Phyllidia picta*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia picta*
18LePhpi1	RB042	MN508645	MN507477	*Phyllidia picta*	2018	Lembeh	1°27'40.9"N 125°13'37"E	Nudifalls	*Phyllidia picta*
336565		-	KX235927	*Phyllidia picta*	2007	RAJ37	0°31'08.2"S; 130°38'28.0"E	South Gam, Shoal near mangroves	*Phyllidia picta*
336566		-	KX235929	*Phyllidia picta*	2007	RAJ43	0°25'45.2"S; 130°33'37.3"E	Passage	*Phyllidia picta*
336567		-	KX235928	*Phyllidia picta*	2007	RAJ29	0°49'42.5"S; 130°42'42.0"E	North Batanta, West Telok Gegenlol	*Phyllidia picta*
AF430358		AF430358	-	*Phyllidia rueppelii*	1995	Egypt	-	Hurghada	*Phyllidia rueppelii*
336484		-	KX235923	*Phyllidia exquisita*	2009	TER14	0°54'38.3"N; 127°29'20.7"E	Tanjung Ngafauda	*Phyllidia exquisita*
AF430363		AF430363	-	*Phyllidia ocellata*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidia* sp.3
336619		-	KX235930	*Phyllidia* sp.	2009	TER28	0°01'51.9"S; 127°14'01.8"E	Pulau Popaco, East	*Phyllidia haegeli*
336575		-	KX235920	*Phyllidia* cf. *babai*	2007	RAJ37	0°31'08.2"S; 130°38'28.0"E	South Gam, shoal near mangroves	*Phyllidia* cf. *babai*
336614		-	KX235919	*Phyllidia* cf. *babai*	2009	TER27	0°54'44.5"N; 127°29'09.9"E	Tanjung Ratemu (South of river)	*Phyllidia* cf. *babai*
Phoc16Sa5	NU119	MN173893	MN173893	*Phyllidia ocellata*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia ocellata*
Phoc16Sa4	NU118	MN173894	MN173894	*Phyllidia ocellata*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia ocellata*
Phoc16Sa6	NU120	-	MN173892	*Phyllidia ocellata*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidia ocellata*
Phoc16Sa7	NU121	MN173891	MN173891	*Phyllidia ocellata*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidia ocellata*
Phoc16Sa1	NU115	MN173896	MN173896	*Phyllidia ocellata*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia ocellata*
Phoc16Sa2	NU116	MN173895	MN173895	*Phyllidia ocellata*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidia ocellata*
336494			KX235924	*Phyllidia ocellata*	2009	TER19	0°44'56.6"N; 127°23'13.5"E	Southwest of Tobala	*Phyllidia ocellata*

336563			KX235926	*Phyllidia ocellata*	2007	RAJ11	0°28'29.9"S; 130°41'54.8"E	Southeast Gam, Friwen Wonda	*Phyllidia ocellata*
336564			KX235925	*Phyllidia ocellata*	2007	RAJ11	0°28'29.9"S; 130°41'54.8"E	Southeast Gam, Friwen Wonda	*Phyllidia ocellata*
KJ001307			KJ001307	*Phyllidia ocellata*	(2014)	Queensland, Australia	26°40'S; 153°7'E	Mooloolaba	*Phyllidia ocellata*
18LePhoc3	RB046	MN508636	-	*Phyllidia ocellata*	2018	Lembeh	1°27'40.9"N 125°13'37"E	Nudifalls	*Phyllidia ocellata*
Phoc18LS2	MO29	MK911025	MK911037	*Phyllidia ocellata*	2018	Lembeh	1°29.093'N; 125°14.459'E	Nudi Retreat	*Phyllidia ocellata*
Phoc18LS3	MO22	MK911026	MK911033	*Phyllidia ocellata*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidia ocellata*
18LePhoc2	RB039	MN508635	MN507475	*Phyllidia ocellata*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidia ocellata*
Phoc18LS1	MO28	MK911024	MK911038	*Phyllidia ocellata*	2018	Lembeh	1°27.674'N; 125°13.602'E	Nudi Falls	*Phyllidia ocellata*
Phoc18LS4	MO23	MK911027	MK911034	*Phyllidia ocellata*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidia ocellata*
18LePhoc1	RB038	MN508638	MN507476	*Phyllidia ocellata*	2018	Lembeh	1°28'56.4"N; 125°14'19"E	Nudi Retreat	*Phyllidia ocellata*
18LePhoc4	RB047	MN508639	MN507473	*Phyllidia ocellata*	2018	Lembeh	1°27'40.9"N; 125°13'37"E	Nudi Falls	*Phyllidia ocellata*
18LePhoc5	RB052	MN508637	MN507474	*Phyllidia ocellata*	2018	Lembeh	1°27'40.9"N; 125°13'37"E	Nudi Falls	*Phyllidia ocellata*
18LePhel1	RB040	MN508634	MN507472	*Phyllidia ocellata*	2018	Lembeh	1°28'56.4"N; 125°14'19"E	Nudi Retreat	*Phyllidia ocellata*
Phoc18LS5	MO15	MK911028	MK911035	*Phyllidia ocellata*	2018	Lembeh	1°27.674"N; 125°13.602'E	Nudi Falls	*Phyllidia ocellata*
336464			KX235918	*Phyllidia babai*	2009	TER08	0°45'23.4"N; 127°24'26.5"E	Tanjung Ebamadu	*Phyllidia babai*
336640			KX235990	*Reticulidia fungia*	2009	TER36	0°50'47.8"N; 127°37'48.7"E	East Teluk Dodinga; North of Pulau Jere	*Reticulidia fungia*
AF430371		AF430371	-	*Reticulidia halgerda*		Lifou, New Caledonia	-	Baie du Santal	*Reticulidia halgerda*
AF430370		AF430370	-	*Reticulidia fungia*		Lifou, New Caledonia	-	Baie du Santal	*Reticulidia fungia*
CZ186491		MF958282	MF958414	*Reticulidia halgerda*		Philippines	-	Maricaban Island, Luzon	*Reticulidia halgerda*
336455		-	KX235991	*Reticulidia halgerda*	2009	TER06	0°47'15.0" N; 127°23'25.0"E	Kampung Cina / Tapak 2	*Reticulidia halgerda*
Phpu16Sa5	NU140	MN243996	MN248608	*Phyllidiella pustulosa*	2016	Sangihe	3°36'28.00″N; 125°29'38.00″E	Ship Wreck	*Phyllidiella pustulosa*
Phpu16Sa73	NU207	MN243998	-	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella pustulosa*

Phpu16Sa38	NU173	MN243997	MN248609	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella pustulosa*
Phpu16Sa86	NU222	MN244003	MN248611	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella pustulosa*
Phpu16Sa68	NU202	-	MN248610	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella pustulosa*
Phpu16Sa95	NU230	MN244004	MN248612	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella pustulosa*
Phpu16Sa6	NU141	MN244006	MN248602	*Phyllidiella pustulosa*	2016	Sangihe	3°36'28.00″N; 125°29'38.00″E	Shipwreck	*Phyllidiella pustulosa*
Phpu16Sa3	NU138	MN243991	MN248601	*Phyllidiella pustulosa*	2016	Sangihe	3°36'28.00″N; 125°29'38.00″E	Shipwreck	*Phyllidiella pustulosa*
Phpu16Sa94	NU229	-	MN248603	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella pustulosa*
Phpu16Sa77b	NU212	MN243993	MN248604	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella pustulosa*
Phpu16Sa4	NU139	MN243992	MN248606	*Phyllidiella pustulosa*	2016	Sangihe	3°36'28.00″N; 125°29'38.00″E	Shipwreck	*Phyllidiella pustulosa*
Phpu16Sa62	NU196	MN243995	MN248607	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella pustulosa*
Phpu16Sa61	NU195	MN244006	MN248613	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella pustulosa*
Phpu16Sa52	NU186	MN244002	MN248621	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella pustulosa*
Phpu16Sa30	NU165	MN244000	MN248620	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella pustulosa*
Phpu16Sa46	NU180	MN244001	MN248622	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella pustulosa*
Phpu16Sa8	NU143	MN243999	-	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella pustulosa*
Phpu16Sa56		MN248605	MN243994	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella pustulosa*
336436		-	KX235953	*Phyllidiella pustulosa*	2009	TER02	0°45'29.7″N; 127°20'59.2"E	Off Danau Laguna	*Phyllidiella pustulosa*
336460		-	KX235954	*Phyllidiella pustulosa*	2009	TER07	0°45'09.1″N; 127°23'31.3"E	Desa Tahua	*Phyllidiella pustulosa*
336461		-	KX235955	*Phyllidiella pustulosa*	2009	TER07	0°45'09.1″N; 127°23'31.3"E	Idem	*Phyllidiella pustulosa*
336470		-	KX235956	*Phyllidiella pustulosa*	2009	TER10	0°44'32.0″N; 127°21'50.9"E	Northwest side of Maitara	*Phyllidiella pustulosa*
336474		-	KX235957	*Phyllidiella pustulosa*	2009	TER12	0°50'05.1″N; 127°23'10.0"E	Tanjung Tabam	*Phyllidiella pustulosa*
336495		-	KX235958	*Phyllidiella pustulosa*	2009	TER21	0°54'24.7″N; 127°29'17.7"E	Tanjung Ratemu (South of river)	*Phyllidiella pustulosa*
336508		-	KX235959	*Phyllidiella pustulosa*	2009	TER24	0°48'49.1″N; 127°23'21.6"E	Dufadufa / Benteng Toloko	*Phyllidiella pustulosa*
336510		-	KX235960	*Phyllidiella pustulosa*	2009	TER24	0°48'49.1″N; 127°23'21.6"E	Idem	*Phyllidiella pustulosa*
336578		-	KX235965	*Phyllidiella pustulosa*	2007	RAJ32	0°30'45.2"S; 130°35'00.1"E	South Gam, Southeast Besir Bay	*Phyllidiella pustulosa*
336579		-	KX235971	*Phyllidiella pustulosa*	2007	RAJ35	0°48'58.3"S; 130°59'16.6"E	South Gam, Besir Bay	*Phyllidiella pustulosa*

336580		-	KX235967	*Phyllidiella pustulosa*	2007	RAJ40	0°33'58.1"S; 130°39'46.2"E	Southwest Pulau Kri	*Phyllidiella pustulosa*
336581		-	KX235963	*Phyllidiella pustulosa*	2007	RAJ35	0°48'58.3"S; 130°59'16.6"E	South Gam, Besir Bay	*Phyllidiella pustulosa*
336582		-	KX235968	*Phyllidiella pustulosa*	2007	RAJ40	0°33'58.1"S; 130°39'46.2"E	Southwest Pulau Kri	*Phyllidiella pustulosa*
336583		-	KX235964	*Phyllidiella pustulosa*	2007	RAJ25 South Gam	0°30'51.5"S; 130°34'11.5"E	East entrance Besir Bay, Cape Besir	*Phyllidiella pustulosa*
336584		-	KX235961	*Phyllidiella pustulosa*	2007	RAJ48	0°29'20.6"S; 130°30'04.9"E	West Pulau Yeben Kecil	*Phyllidiella pustulosa*
336585		-	KX235969	*Phyllidiella pustulosa*	2007	RAJ41	0°27'48.1"S; 130°41'14.6"E	Southeast Gam, Desa Besir	*Phyllidiella pustulosa*
336586		-	KX235966	*Phyllidiella pustulosa*	2007	RAJ41	0°27'48.1"S; 130°41'14.6"E	Idem	*Phyllidiella pustulosa*
336587		-	KX235962	*Phyllidiella pustulosa*	2007	RAJ25 South Gam	0°30'51.5"S; 130°34'11.5"E	East entrance Besir Bay, Cape Besir	*Phyllidiella pustulosa*
336588		-	KX235970	*Phyllidiella pustulosa*	2007	RAJ48	0°29'20.6" S, 130°30'04.9" E	West Pulau Yeben Kecil	*Phyllidiella pustulosa*
Ali_123		KP873167	-	*Phyllidiella pustulosa*	(2015)	Terengganu, Malaysia	-	Bidong Island	*Phyllidiella pustulosa*
Phpu15Bu14	DS232	MT483964	MT478669	*Phyllidiella pustulosa*	2015	Bunaken	1°36'59.l″N; 124°41'38″E	Manado tua	*Phyllidiella pustulosa*
18LePhni5	RB029	MN508656	MN507492	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella pustulosa*
Phli18LS2	MO19	MK911023	MK911032	*Phyllidiella pustulosa*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidiella pustulosa*
18LePhpu2	RB031	MN508655	MN507495	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella pustulosa*
18LePhpu1	RB030	MN508657	MN507493	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella pustulosa*
18LePhpu4	RB033	MN508658	MN507494	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella pustulosa*
Phpu18LS1	MO16	MK911029	MK911036	*Phyllidiella pustulosa*	2018	Lembeh	1°27.674'N; 125°13.602'E	Nudi Falls	*Phyllidiella pustulosa*
AF430365		AF430365	-	*Phyllidiella lizae*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidiella pustulosa*
Phpu16Sa85	NU221	MN243990	MN248618	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* cf. *pustulosa*
Phpu16Sa75	NU209	MN243987	MN248617	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* cf. *pustulosa*
Phpu16Sa79	NU215	MN243988	MN248619	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* cf. *pustulosa*
Phpu16Sa76	NU210	MN243989	MN248615	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* cf. *pustulosa*
Phpu16Sa33	NU168	MN243985	MN248614	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* cf. *pustulosa*

Phpu16Sa71	NU205	MN243986	MN248616	*Phyllidiella* cf. *pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* cf. *pustulosa*
18LePhni4	RB049	MN508654	MN507491	*Phyllidiella* cf. *pustulosa*	2018	Lembeh	1°27'40.9"N 125°13'37"E	Nudi Falls	*Phyllidiella* cf. *pustulosa*
Phli18LS1	MO18	MK911022	-	*Phyllidiella* cf. *pustulosa*	2018	Lembeh	1°26.733'N; 125°12.428'E	Makawidey	*Phyllidiella* cf. *pustulosa*
336434		-	KX235946	*Phyllidiella nigra*	2009	TER02	0°45'29.7″N; 127°20'59.2"E	Off Danau Laguna	*Phyllidiella nigra*
336471		-	KX235947	*Phyllidiella nigra*	2009	TER10	0°44'32.0″N; 127°21'50.9"E	Maitara Northwest	*Phyllidiella nigra*
336472		-	KX235948	*Phyllidiella nigra*	2009	TER10	0°44'32.0″N; 127°21'50.9"E	Idem	*Phyllidiella nigra*
336501		-	KX235949	*Phyllidiella nigra*	2009	TER22	0°52'03.6″N; 127°19'33.1"E	Sulamadaha I	*Phyllidiella nigra*
336505		-	KX235950	*Phyllidiella nigra*	2009	TER23	0°52'02.0″N; 127°19'45.8"E	Sulamadaha II	*Phyllidiella nigra*
336576		-	KX235952	*Phyllidiella nigra*	2007	RAJ26 South Gam	0°30'59.3"S; 130°33'48.7"E	Eastern entrance Besir Bay, Pulau Bun	*Phyllidiella nigra*
336577		-	KX235951	*Phyllidiella nigra*	2007	RAJ32	0°30'45.2"S; 130°35'00.1"E	South Gam, Southeast Besir Bay	*Phyllidiella nigra*
CASIZ 186196A		MF958280	-	*Phyllidiella nigra*		Philippines	-	Maricaban Strait, Batangas Prov. Luzon	*Phyllidiella nigra*
Phli16Sa7	NU112	MN244013	MN248633	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* sp. a
Phpu16Sa60	NU194	MN244014	MN248634	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* sp. a
Phpu16Sa69	NU203	MN244016	MN248635	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* sp. a
Phpu16Sa2	NU137	MN244015	MN248636	*Phyllidiella pustulosa*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella* sp. a
Phpu16Sa34	NU169	MN244017	MN248637	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. a
Phpu16Sa70	NU204	MN244018	MN248638	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* sp. a
KJ001310		-	KJ001310	*Phyllidiella pustulosa*	(2014)	Queensland, Australia	14°40'S; 145°28'E	Lizard Island	*Phyllidiella zeylanica* auctt.
Phpu16Sa91	NU226	MN243984	MN248631	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella zeylanica* auctt.
Phpu16Sa90	NU225	-	MN248630	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella zeylanica* auctt.
Phpu16Sa40	NU175	MN243981	MN248628	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella zeylanica* auctt.
Phpu16Sa87	NU223	MN243982	MN248629	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella zeylanica* auctt.
Phpu16Sa18	NU153	MN243983	MN248632	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* auctt.
Phpu16Sa9	NU144	MN243980	MN248627	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* auctt.
Phpu16Sa24	NU159	MN243978	MN248625	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* auctt.
Phpu16Sa1	NU136	MN243977	MN248624	*Phyllidiella pustulosa*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella zeylanica* auctt.

Phpu16Sa26	NU161	MN243979	MN248626	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* auctt.
Phli16Sa4	NU109	MN243976	MN248623	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella zeylanica* auctt.
AF430366		AF430366	-	*Phyllidiella pustulosa*	2000	Lifou, New Caledonia	20°51'22.1"S; 167°05'04.2"E	Baie du Santal,	*Phyllidiella zeylanica* auctt.
AF249232		AF249232	-	*Phyllidiella pustulosa*	(1999)	Great Barrier Reef	-	Australia	*Phyllidiella zeylanica* auctt.
-		KJ018918	KJ001309	*Phyllidiella lizae*	(2014)	Queensland, Australia	14°40'S; 145°28'E	Lizard Island	*Phyllidiella rudmani*
336589			KX235945	*Phyllidiella rudmani*	2007	RAJ11	0°28'29.9″S; 130°41'54.8"E	Southeast Gam, Friwen Wonda	*Phyllidiella rudmani*
Phpu16Sa25	NU160	MN244008	MN248595	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. b
Phpu16Sa7	NU142	MN244007	MN248594	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. b
Phpu16Sa27	NU162	MN244009	MN248596	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. b
Phpu16Sa36	NU171	MN244011	MN248597	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. b
Phpu16Sa39	NU174	MN244010	MN248598	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. b
Phpu16Sa84	NU220	MN244012	MN248599	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* sp. b
18LePhpu8	RB036	MN508659	MN507490	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. b
Phpu16Sa31	NU166	MN243963	MN248587	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. c subclade 1
Phpu16Sa23	NU158	MN243962	MN248586	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa15	NU150	MN243960	MN248585	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa92	NU227	MN243967	MN248592	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* sp. c subclade 1
Phpu16Sa13	NU148	MN243969	MN248581	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa14	NU149		MN248580	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa53	NU187	MN243968	MN248584	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 1
Phpu16Sa28	NU163	MN243970	MN248591	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. c subclade 1
Phpu16Sa20	NU155	-	MN248590	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu15Bu35	DS251	MT483977	MT483977	*Phyllidiella pustulosa*	2015	Bunaken	1°37′07″N; 124°45′32″E	Air Slobar	*Phyllidiella* sp. c subclade 1
18LePhpu5	RB034	MN508652	MN507488	*Phyllidiella lizae*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. c subclade 1
18LePhpu9	RB037	MN508653	MN507489	*Phyllidiella lizae*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. c subclade 1
Phpu16Sa17	NU152	MN243961	MN248588	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1

Phpu16Sa37	NU172	MN243964	MN248589	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. c subclade 1
Phpu16Sa22	NU157	MN243965	MN248582	*Phyllidiella pustulosa*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 1
Phpu16Sa51	NU185	MN243966	MN248583	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 1
Phli16Sa8	NU113	MN243959	MN248579	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella* sp. c subclade 1
18LePhpu**7**	RB054	MN508651	MN507487	*Phyllidiella annulata*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. c subclade 2
Phli16Sa5	NU110	MN243972	MN248576	*Phyllidiella lizae*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiella* sp. c subclade 2
Phli16Sa1	NU106	MN243971	MN248575	*Phyllidiella lizae*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella* sp. c subclade 2
Phli16Sa2	NU107	MN243973	MN248577	*Phyllidiella lizae*	2016	Sangihe	3°22'01.94″N; 125°34'26.67″E	Mendaku	*Phyllidiella* sp. c subclade 2
Phli16Sa6	NU111	MN243974	MN248578	*Phyllidiella lizae*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 2
18LePhpu3	RB032	MN508650	MN507486	*Phyllidiella annulata*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. c subclade 2
18LePhni2	RB027	MN508649	MN507485	*Phyllidiella annulata*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. c subclade 2
Phpu16Sa48	NU182	MN243975	MN248593	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. c subclade 2
CASIZ 181247		MF958281	MF958413	*Ceratophyllidia sp.*		Philippines	-	Beatrice	*Ceratophyllidia sp.*
Phpu16Sa80	NU216	MN244019	MN248600	*Phyllidiella pustulosa*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiella albonigra*
Phpu16Sa74	NU208	MN243956	MN248642	*Phyllidiella pustulosa*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiella* sp. d
Phpu16Sa35	NU170	MN243957	MN248640	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella* sp. Complex e
Phpu16Sa50	NU184	MN243958	MN248641	*Phyllidiella pustulosa*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiella* sp. Complex
18LePhpu**6**	RB035	MN508660	MN507484	*Phyllidiella pustulosa*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiella* sp. Complex
Phpu16Sa29	NU164	MN243955	MN248639	*Phyllidiella pustulosa*	2016	Sangihe	3°34'49.92″N; 125°34'34.93″E	Talengen Bay	*Phyllidiella hageni*
336590		-	KX235944	*Phyllidiopsis krempfi*	2007	Raja Ampat RAJ46	0°29'13.0″S; 130°40'23.6"E	Yenweres Bay	*Phyllidiella hageni*
Phpu16Sa58	NU192	MN244074	MN248650	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*
Phfi16Sa2	NU105	MN244068	MN248644	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis krempfi*
Phpu16Sa67	NU201	MN244069	MN248645	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*
Phpu16Sa72	NU206	MN244070	MN248646	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*
Phpu16Sa88	NU224	MN244075	MN248648	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis krempfi*
Phpu16Sa66	NU200	MN244073	MN248647	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*
Phfi16Sa1	NU104	MN244067	MN248643	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis krempfi*
Phpu16Sa65	NU199	MN244072	MN248649	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*

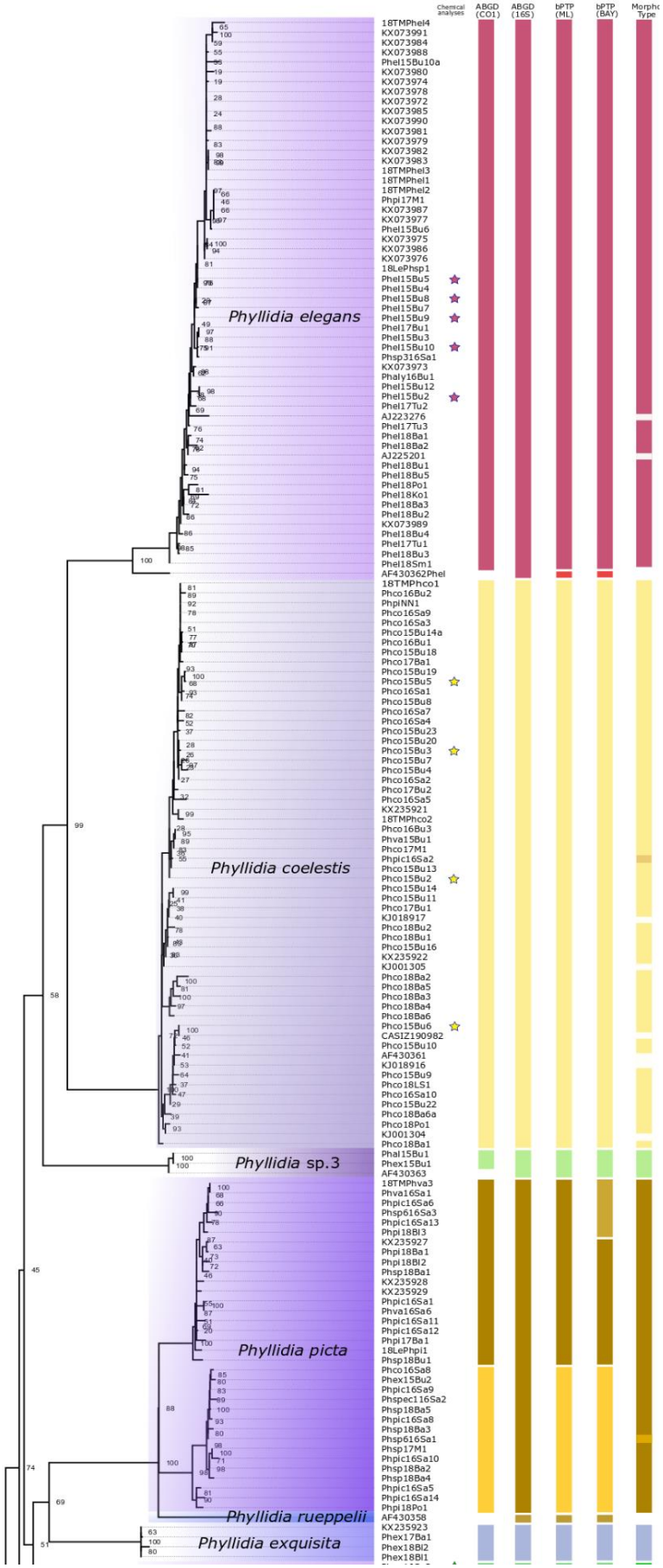
Phpu16Sa57	NU191	MN244071	MN248651	*Phyllidiopsis krempfi*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis krempfi*
336512		-	KX235976	*Phyllidiopsis krempfi*	2009	TER24	0°48'49.1"N; 127°23'21.6"E	Dufadufa / Benteng Toloko, Ternate	*Phyllidiopsis krempfi*
336466		-	KX235974	*Phyllidiopsis krempfi*	2009	TER08	0°45'23.4"N; 127°24'26.5"E	Tanjung Ebamadu	*Phyllidiopsis krempfi*
336453		-	KX235972	*Phyllidiopsis krempfi*	2009	TER06	0°47'15.0" N; 127°23'25.0"E	Kampung Cina / Tapak 2 Ternate	*Phyllidiopsis krempfi*
336595		-	KX235984	*Phyllidiopsis krempfi*	2007	RAJ40	0°33'58.1"S; 130°39'46.2"E	Southwest Pulau Kri	*Phyllidiopsis krempfi*
336594		-	KX235979	*Phyllidiopsis krempfi*	2007	RAJ15	0°33'42.8"S; 130°39'40.4"E	Southwest Pulau Kri, Kuburan	*Phyllidiopsis krempfi*
336596		-	KX235983	*Phyllidiopsis krempfi*	2007	RAJ49	0°32'53.5" S; 130°29'51.1"E	Northwest Pulau Mansuar, Lalosi reef	*Phyllidiopsis krempfi*
336462		-	KX235973	*Phyllidiopsis krempfi*	2009	TER08	0°45'23.4"N; 127°24'26.5"E	Tanjung Ebamadu	*Phyllidiopsis krempfi*
336469		-	KX235975	*Phyllidiopsis krempfi*	2009	TER09	0°43'47.6"N; 127°21'44.7"E	West Maitara	*Phyllidiopsis krempfi*
Ali_124		-	KP873168	*Phyllidiopsis krempfi*		Terengganu	-	Bidong Island	*Phyllidiopsis krempfi*
336599		-	KX235982	*Phyllidiopsis krempfi*	2007	RAJ12	0°33'13.2" S; 130°41'16.9"E	East Kri, Sorido Wall	*Phyllidiopsis krempfi*
336600		-	KX235981	*Phyllidiopsis krempfi*	2007	RAJ38	0°34'05.0"S; E130°38'31.5" E	Northeast Mansuar	*Phyllidiopsis krempfi*
336650		-	KX235977	*Phyllidiopsis krempfi*	2009	TER40	0°46'25.3"N; 127°32'22.0"E	Teluk Dodinga; West Karang Ngeli.	*Phyllidiopsis krempfi*
336598		-	KX235980	*Phyllidiopsis krempfi*	2007	RAJ28	0°46'46.7"S; 130°42'42.7"E	North Batanta, North Pulau Yarifi	*Phyllidiopsis krempfi*
336597		-	KX235978	*Phyllidiopsis krempfi*	2007	RAJ15	0°33'42.8"S; 130°39'40.4"E	Southwest Pulau Kri, Kuburan	*Phyllidiopsis krempfi*
18LePhni3	RB028	MN508646	MN507479	*Phyllidiopsis krempfi*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiopsis krempfi*
Phpu15Bu37	DS252	MT483983	MT478688	*Phyllidiopsis krempfi*	2015	Bunaken	1°35'46.8″N; 124°50'15.9″E	Tiwoho	*Phyllidiopsis krempfi*
Phpu16Sa93	NU228	MN244078	MN248655	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis burni*
Phpu16Sa47	NU181	MN244076	MN248654	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis burni*
Phpu16Sa83	NU219	MN244080	MN248657	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis burni*
Phpu16Sa55	NU189	MN244081	-	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis burni*
Phpu16Sa19	NU154	-	MN248652	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'59.40″N; 125°29'23.40″E	Tahuna Bay South	*Phyllidiopsis burni*
Phpu16Sa54	NU188	MN244077	MN248653	*Phyllidiopsis krempfi*	2016	Sangihe	3°32'08.87″N; 125°37'25.46″E	Manalu	*Phyllidiopsis burni*

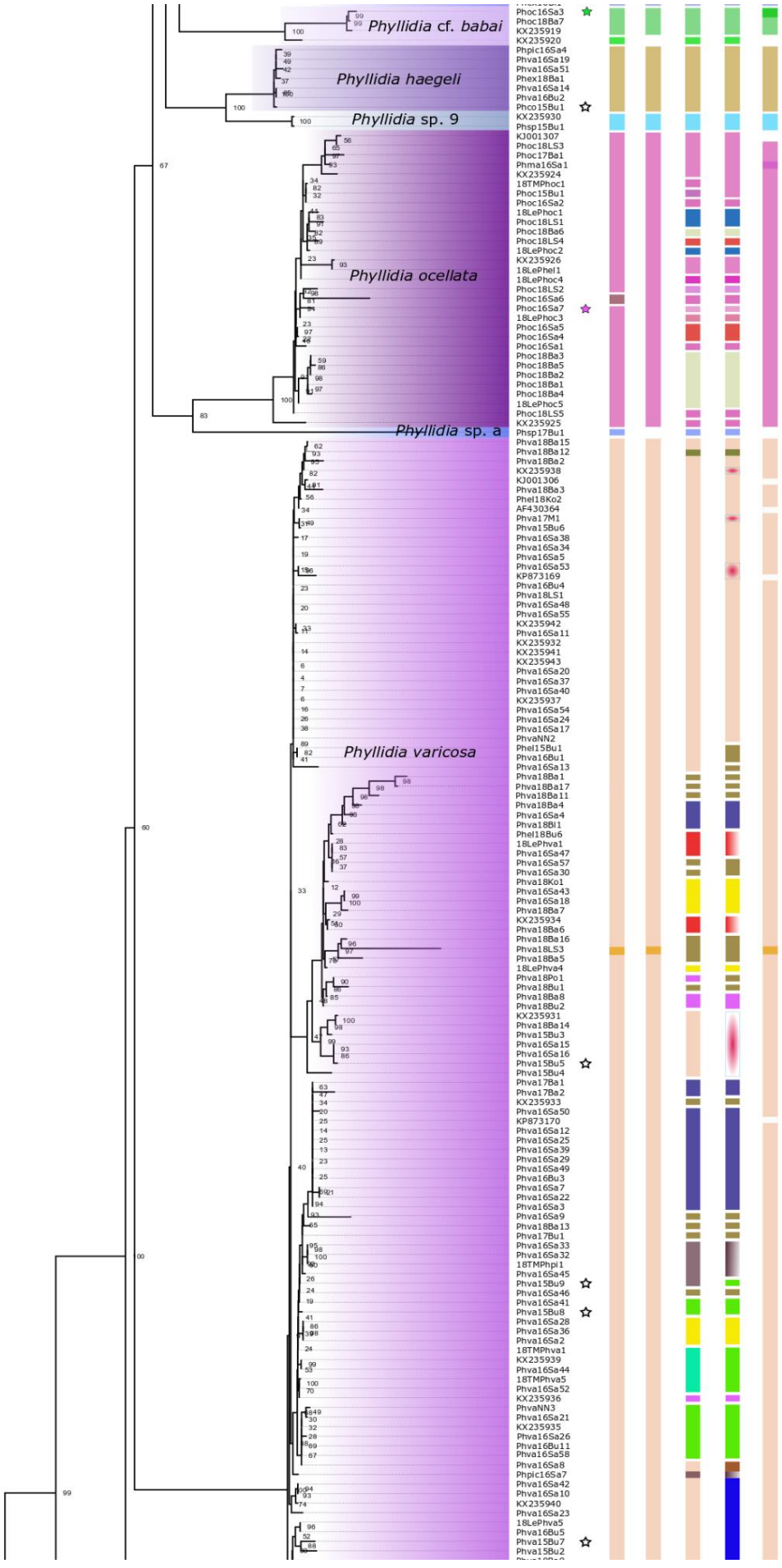
Phpu16Sa82	NU218	-	MN248658	*Phyllidiopsis krempfi*	2016	Sangihe	3°35'18.92″N; 125°34'26.67″E	Palahanaeng	*Phyllidiopsis burni*
18LePhysp3	RB025	MN507483	MN508648	*Phyllidiopsis annae*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiopsis annae*
18LePhysp2	RB053		MN507482	*Phyllidiopsis annae*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiopsis annae*
18LePhysp1	RB024	-	MN507481	*Phyllidiopsis annae*	2018	Lembeh	1°28'56.4"N 125°14'19"E	Nudi Retreat	*Phyllidiopsis annae*
CASIZ186138		MF958283		*Phyllidiopsis annae*			-		*Phyllidiopsis annae*
AF430368		AF430368	-	*Phyllidiopsis sphingis*	2000	New Caledonia	-	Baie du Santal, Lifou Island,	*Phyllidiopsis annae*
336591		-	KX235987	*Phyllidiopsis xishaensis*	2007	Raja Ampat RAJ05	0°30'57.1"S; 130°40'22.1"E	Southeast Gam, Pulau Kerupiar, Mike's Point	*Phyllidiopsis xishaensis*
336592		-	KX235988	*Phyllidiopsis xishaensis*	2007	RAJ07	0°33'22.2"S; 130°41'28.7"E	East Pulau Kri, Cape Kri	*Phyllidiopsis xishaensis*
336593		-	KX235989	*Phyllidiopsis xishaensis*	2007	RAJ44	0°25'44.3"S; 130°33'56.8"E	Eastern entrance of passage	*Phyllidiopsis xishaensis*
AF430369		AF430369	-	*Phyllidiopsis xishaensis*	2000	New Caledonia	-	Baie du Santal, Lifou Island	*Phyllidiopsis xishaensis*
Phsh16Sa2	NU232	MN244082	MN248659	*Phyllidiopsis shireenae*	2016	Sangihe	3°34'55.81″N; 125°34'49.04″E	Sapaeng	*Phyllidiopsis shireenae*
336451		-	KX235985	*Phyllidiopsis shireenae*	2009	TER06	0°47'15.0″N; 127°23'25.0"E	Kampung Cina / Tapak 2	*Phyllidiopsis shireenae*
336652		-	KX235986	*Phyllidiopsis shireenae*	2009	TER41	0°46'32.8″N; 127°33'43.4"E	Teluk Dodinga; East Karang Luelue	*Phyllidiopsis shireenae*
18LePhsh1	RB048	MN508647	MN507480	*Phyllidiopsis shireenae*	2018	Lembeh	1°27'40.9"N 125°13'37"E	Nudi Falls	*Phyllidiopsis shireenae*
AF430367		AF430367	-	*Phyllidiopsis cardinalis*	2000	Lifou, New Caledonia	-	Baie du Santal	*Phyllidiopsis cardinalis*
		-	KJ001308	*Phyllidiopsis cardinalis*	(2014)	Queensland, Australia	26°40'S; 153°7'E	Mooloolaba	*Phyllidiopsis cardinalis*
CASIZ 181231		MF958307	MF958434	*Dendrodoris atromaculata*			-	Janao Bay, Luzon, Philippines	*Dendrodoris atromaculata*
CASIZ 182859		KP871697	KP871649	*Phyllidia larryi*			-		*Phyllidia larryi*

Table. 3 Type materials for external investigation

Old name	Type	Reg.Num	Deposit in	Locality	Species
Phyllidia nobilis	syntype	NHMD-91773	Copenhagen, ZMUC	Burias, Philippines	*Phyllidiella pustulosa*
Phyllidia nobilis	paralectotype	NHMD-633658	Copenhagen, ZMUC	Philippines	*Phyllidiella pustulosa*
Phyllidia nobilis	non-type	NHMD-633656	Copenhagen, ZMUC	(from things left behind by Prof. Berg)	*Phyllidiella pustulosa*
Phyllidia nobilis	non-type	NHMD-633657	Copenhagen, ZMUC	Fidji, øerne	*Phyllidiella pustulosa*
Phyllidia pustulosa	lectotype	MNHN-IM-2000-35147	Paris, MNHN	Timor	*Phyllidiella pustulosa*
Phyllidia melanocera	holotype	NHMUK 1985206/1	BMNH London	Red Sea	*Phyllidiella pustulosa*
Phyllidiella lizae	holotype	C159493	Australian Museum Sydney	Heron Island Reef, Capricorn Group, GBR	*Phyllidiella lizae*
Phyllidiella lizae	paratype	C162784	Australian Museum Sydney	Heron Island Reef Capricorn Group GBR	*Phyllidiella lizae*
Phyllidiella lizae	paratype	C159492	Australian Museum Sydney	Norman Reef Cairns 16°37'S, 146°0'E	*Phyllidiella lizae*
Phyllidiella annulata	nontype	C159519	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiella annulata*
Phyllidiella annulata	nontype	C159520	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiella annulata*
Phyllidiella annulata	nontype	C159520b	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiella annulata*
Phyllidiella annulata	nontype	C159522	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiella annulata*
Phyllidiella annulata	nontype	C159526	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiella annulata*
Phyllidiopsis pipeki	holotype	C162772	Australian Museum Sydney	Madang Lagoon	*Phyllidiopsis pipeki*
Phyllidiopsis burni	holotype	C159452	Australian Museum Sydney	Bile Bay, Guam. 13°16'N, 144°40'E.	*Phyllidiopsis burni*
Phyllidiopsis krempfi	nontype	RMNH.Mol.336469	Naturalis Biodiversity Center, Leiden	West Maitara, Ternate	*Phyllidiopsis krempfi*

S1. Full result of maximum likelihood phylogenetic tree of Phyllidiidae.

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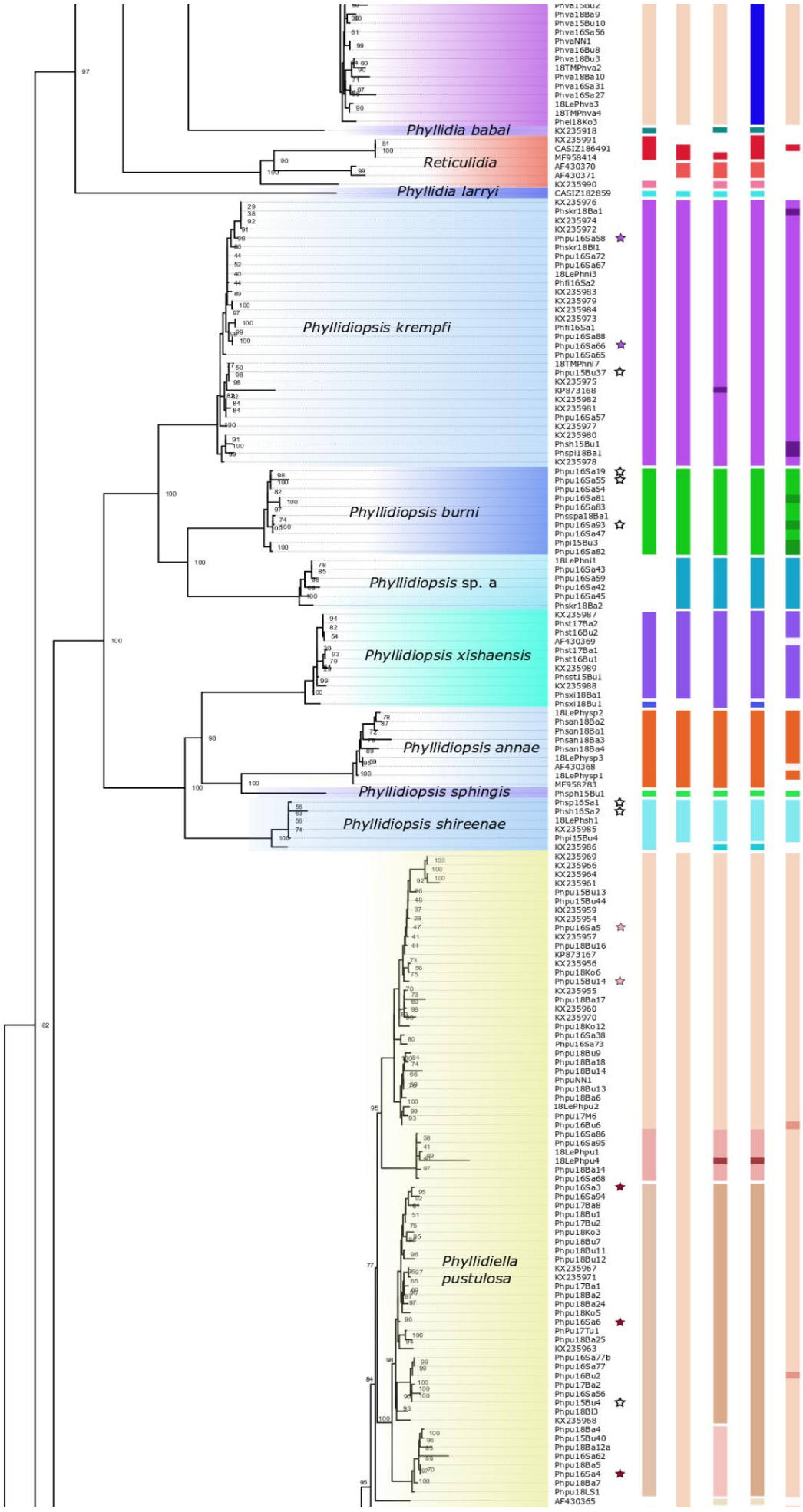
Phyllidia haegeli
Phyllidia sp. 9
Phyllidia ocellata
Phyllidia sp. a
Phyllidia varicosa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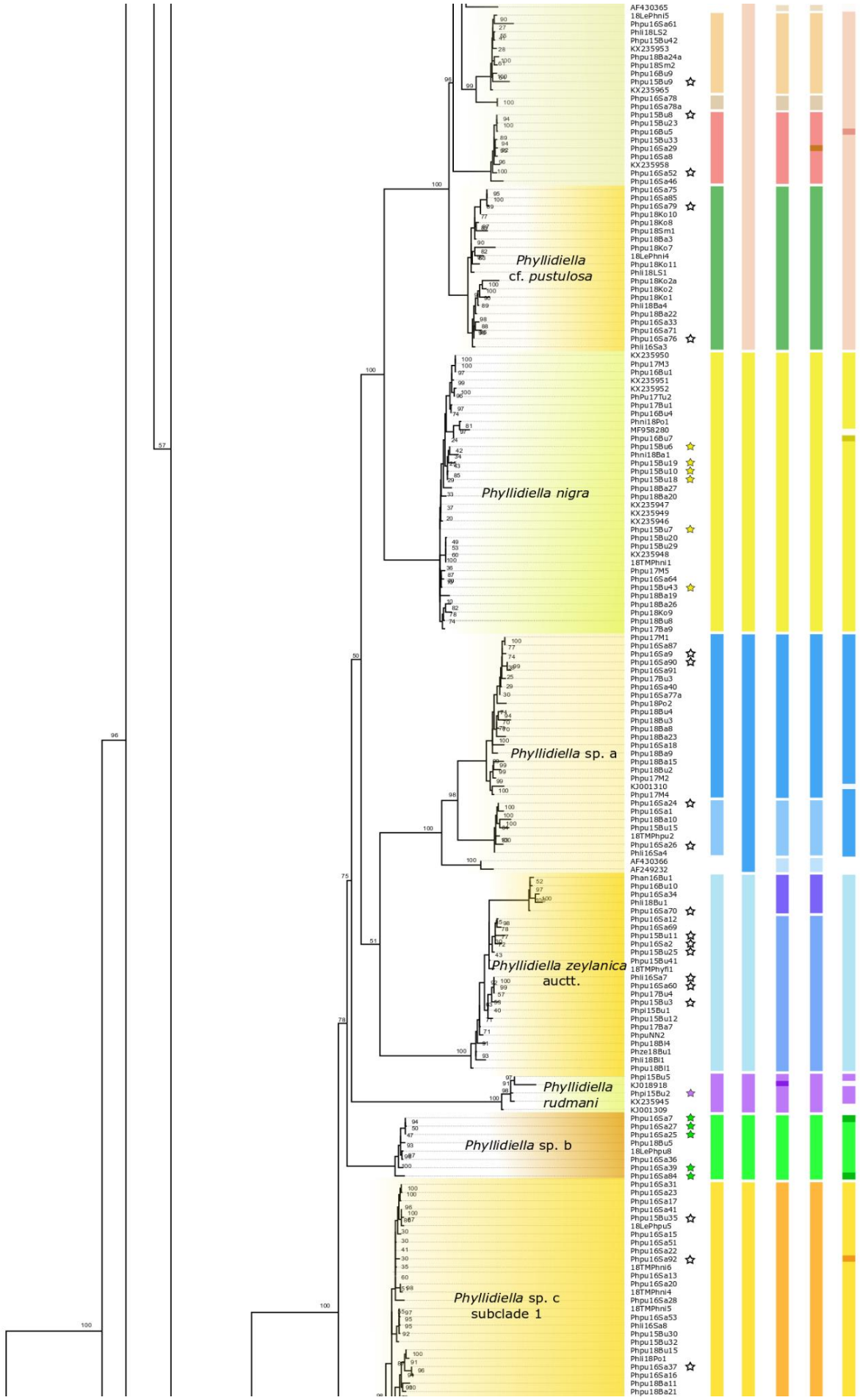
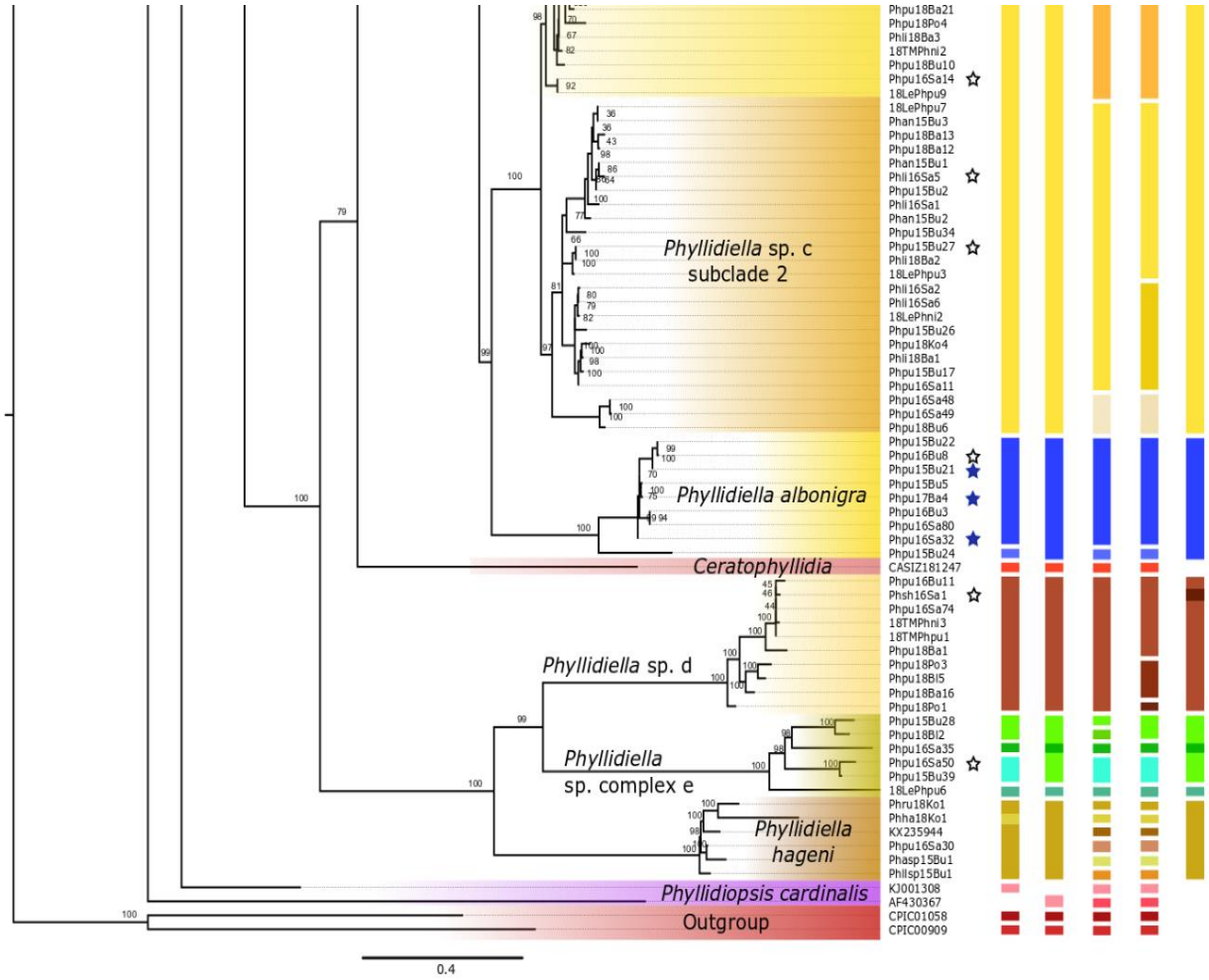
Reticulidia
Phyllidia larryi
Phyllidiopsis krempfi
Phyllidiopsis burni
Phyllidiopsis sp. a
Phyllidiopsis xishaensis
Phyllidiopsis annae
Phyllidiopsis sphingis
Phyllidiopsis shireenae
Phyllidiella pustulosa


Phyllidiella cf. pustulosa
Phyllidiella nigra
Phyllidiella sp. a
Phyllidiella zeylanica auctt.
Phyllidiella rudmani
Phyllidiella sp. b
Phyllidiella sp. c subclade 1


Phyllidiella sp. c subclade 2
Phyllidiella albonigra
Ceratophyllidia
Phyllidiella sp. d
Phyllidiella sp. complex e
Phyllidiella hageni
Phyllidiopsis cardinalis
Outgroup
0.4

S2. Tabel of Matrix Distance of *Phyllidia* CO1 gene.

	P. elegans	*P. coelestis*	*P.* sp.3	*P. picta*	*P. exquisita*	*P.*cf.*babai*	*P. haegeli*	*P.* sp. 9	*P. ocellata*	*P.* sp. a	*P. varicosa*	*P. babai*
P. elegans	0-3.75	-	-	-	-	-	-	-	-	-	-	-
P. coelestis	11.09-13.66	0-2.01	-	-	-	-	-	-	-	-	-	-
P. sp.3	12.21-13.39	11.44-13.58	0	-	-	-	-	-	-	-	-	-
P. picta	14.06-16.95	11.96-14.6	13.29-14.49	0-8.63	-	-	-	-	-	-	-	-
P. exquisita	12.21-13.39	11.13-12.88	0.47-10.52	11.54-13.09	0-0.17	-	-	-	-	-	-	-
*P.*cf.*babai*	12.64-16.13	12.68-15.47	11.98-13.82	13.22-14.96	11.09-12.07	0-5.49	-	-	-	-	-	-
P. haegeli	9.08-10.91	11.045-12.85	11.52-12.28	10.94-15.49	8.82-9.48	12.95-14.98	0-0.71	-	-	-	-	-
P. sp. 9	10.1-11.85	11.1-12.67	11.4-11.77	11.36-14.92	12.25-12.52	13.41-16.06	7.1-7-44	0-0.33	-	-	-	-
P. ocellata	12.17-20.23	12.37-19.55	10.60-16.27	12.19-19.03	11.23-16.37	13.35-17.98	12.63-18.26	11.77-18.42	0-5.76	-	-	-
P. sp. a	17.82-18.94	15.66-17.11	16.25	16.98-19.04	16.10-16.45	17.5-19.21	17.88-18.68	16.57	14.82-18.65	0	-	-
P. varicosa	11.3-25.17	10.75-22.75	11.91-21.99	13.99-25.6	12.83-24.13	13.28-26.62	13.03-23.2	13.71-24.13	13.50-27.5	17.61-28.57	0-9.1	-
P. babai	13.16-14.58	13.15-14.495	0.14	14.31-16.14	12.98-13.18	12.34-14.13	15.21-15.93	15.55	14.45-18.3	17.19	13-24.32	0

S3. Tabel of Matrix Distance of *Phyllidiopsis* CO1 gene.

	P. krempfi	*P. burni*	*P. shireenae*	*P. xishaensis*	*P. sphingis*	*P. annae*	*P. cardinalis*
P. krempfi	0-2.73						
P. burni	8.52-10.9	0-1.27					
P. shireenae	12.34-15.39	13.76-18.26	0-3.74				
P. xishaensis	13.34-15.69	17.15-18.75	11.57-14.36	0-1.34			
P. sphingis	13.87-15.59	14.45-15.68	12.73-14.67	13.22-14	0		
P. annae	16.39-19.2	16.62-19.87	13.12-15.73	15.65-17.67	12.79-14.36	0-1.84	
P. cardinalis	12.88-14.32	12.56-13.68	15.03-16.7	16.84-18.28	16.5	18.37-19.46	0

S4. Tabel of Matrix Distance of *Phyllidiella* CO1 gene.

	P. pustulosa	*P.* cf. *pustulosa*	*P. nigra*	*Phyllidiella* sp. a	*P.* cf. *zeylanica*	*P. rudmani*	*Phyllidiella* sp. b	*Phyllidiella* sp. c subclade 1	*Phyllidiella* sp. c subclade 2	*P. albonigra*	*Phyllidiella* sp. d	*Phyllidiella* sp. e	*P. hageni*
P.pustulosa	0-9.87	-	-	-	-	-	-	-	-	-	-	-	-
P. cf. *pustulosa*	5.5- 8.64	0-2.52	-	-	-	-	-	-	-	-	-	-	-
P. nigra	9.77-13.83	10.71-13.27	0-1.68	-	-	-	-	-	-	-	-	-	-
Phyllidiella sp. a	13.92-20.6	14.73-18.92	13.36-16.72	0 - 7.3	-	-	-	-	-	-	-	-	-
P. cf. *zeylanica*	13.67- 18.36	13.66-15.47	13.15-14.31	14.13-16.94	0-5.48	-	-	-	-	-	-	-	-
P. rudmani	15.99-19.19	15.55-17.63	13.52-15.78	15.38-18.39	15.42-17.68	0-1.45	-	-	-	-	-	-	-
Phyllidiella sp. b	10.7-14.64	10.31-11.84	11.12-12.09	11.35-14.87	13.09-15.85	14.33-18.03	0-1.5	-	-	-	-	-	-
Phyllidiella sp. c subclade 1	11.09-15.81	11.09-13.4	9.63-11.78	11.73-14.28	12.31-13.71	14.28-15.87	8.83-10.3	0-1.27	-	-	-	-	-
Phyllidiella sp. c subclade 2	13.1-18.17	13.25-16.42	10.6-12.85	12.33-14.84	11.53-14.71	14.3-16.52	9.78-12.22	2.93-5.84	0-6.18	-	-	-	-
P. albonigra	12.99-17.67	14.01-15.83	11.51-12.68	13.28-15.67	14.6-16.25	17.19-18.45	11.61-12.4	10.09-11.99	9.96-13.32	0-1.84	-	-	-
Phyllidiella sp. d	14.24-18.18	17.71-23.09	16.49-21.66	17.74 -24.72	17.92-21.73	22.1-24.82	18.49-22.6	18.31-20.21	18.33-20.91	21.07-23.29	0-4.6	-	-
Phyllidiella sp. e	14.24-18.19	21.27-22.53	19.4-20.88	19.75-24.12	20.02-23.77	23.64-25.25	19.41-21.32	20.5-22.21	20.53-22.94	20.53-21.89	16.47-19.73	0-7.83	-
P. hageni	14.24-18.20	18.7-21.76	16.58-19.87	19.97-24.93	18.54-22.3	21.09-24.16	18.77-20.9	18.37-22.53	17.72-20.29	20.72-22.44	17.26-21.63	17.31-21.93	0 - 8.62