Molecular Phylogenetics and Evolution 201 (2024) 108183

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Between sea angels and butterflies: A global phylogeny of pelagic pteropod molluscs

Jose Vidal-Miralles [a], Peter Kohnert [b], Marina Monte [a], Xavier Salvador [c], Michael Schrödl [b,d], Juan Moles [a,e,*]

[a] Department of Evolutionary Biology, Ecology, and Environmental Science, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[b] SNSB-Bavarian State Collection of Zoology, Section Mollusca, Münchhausenstrasse 21, D-81247 Munich, Germany
[c] Department of Physical & Technological Oceanography (CSIC), Institute of Marine Sciences (ICM), Passeig Marítim de la Barceloneta 37, 08003 Barcelona, Spain
[d] Biozentrum Ludwig Maximilians University and GeoBio-Center LMU Munich, Germany
[e] Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Barcelona, Spain

ARTICLE INFO

Keywords:
Biodiversity
Holoplanktonic
Gastropoda
Heterobranchia
Taxonomy and systematics

ABSTRACT

Pteropods, holoplanktonic gastropods, play pivotal roles in marine ecosystems as integral components of food webs and carbon cycling. With global change threatening pelagic ecosystem equilibrium, conserving pteropod biodiversity is paramount. Here, we present the most extensive phylogenetic study of the order Pteropoda to date, utilizing a complete mitogenome phylogeny to support the suppression of Thecosomata, thus demonstrating the lack of relationship between Pseudothecosomata and Euthecosomata. Through multilocus Sanger-based taxon sampling with 411 specimens (92 newly sequenced), representing nearly 100 species (out of 163 valid) from various oceans, we elucidate robust support for higher taxonomic rankings. Despite strong support, relationships between the major groups Gymnosomata, Pseudothecosomata, and Euthecosomata remain contentious. Our study addresses unresolved taxonomic questions, identifying cryptic species complexes across vast biogeographic areas, and offering unprecedented insights into pteropod diversity. We shed light on several open questions in pteropod systematics, proposing the reclassification of *L. antarctica* stat. rest. and elucidating the position of *Thliptodon*, Heliconoididae, and Thieleidae. This systematic review enhances our understanding of pteropod diversity and underscores the urgency of conservation efforts in the face of changing oceanic conditions.

1. Introduction

Pteropods are widely distributed holoplanktonic marine gastropods and a key group due to their role as bioindicators of ocean acidification, as well as for their importance in food chains and carbon cycling in marine ecosystems (Bednaršek et al., 2012; Kohnert et al., 2020). Their aragonite shells are approximately 50 % more sensitive than calcite shells to rising ocean acidity, making them particularly vulnerable to climate change (Mucci, 1983). The current acidification process is expected to be much more abrupt than historical events, such as the Paleocene-Eocene thermal maximum (PETM) around 56 Mya, which could lead to a major shift in zooplanktonic communities (Zachos et al., 2005; Janssen et al., 2016). Pteropods possess the best fossil record among planktonic metazoans because they have successfully survived both the Cretaceous-Paleogene extinction event and the PETM crises. Therefore, it is hypothesized that such sharp environmental changes may have had a catalytic effect on the evolution of Pteropoda (Burridge et al., 2017a; Peijnenburg et al., 2020).

Morphologically, pteropods have wing-like parapodia as an adaptation for swimming in the water column. It is widely agreed that this adaptation results from a neotenic process, conserving typical characteristics of the veliger larva, such as the adaptation of the foot and shell to a pelagic life (Corse et al., 2013). The taxon Pteropoda Cuvier, 1804, is a monophyletic group composed of three suborders: the shell-less Gymnosomata Blainville, 1824, the shelled Euthecosomata Meisenheimer, 1905, and the Pseudothecosomata Meisenheimer, 1905, with heterogeneous shell conditions (Kohnert et al., 2020). Gymnosomes possess shells only during the larval stage (Lalli and Conover, 1976), while euthecosomes maintain the aragonitic shell in both larval and adult stages, and pseudothecosomes can lose their shells in the adult

* Corresponding author.
E-mail address: juan.moles@ub.edu (J. Moles).

https://doi.org/10.1016/j.ympev.2024.108183
Received 16 November 2023; Received in revised form 6 June 2024; Accepted 26 August 2024
Available online 3 September 2024

stage, adopting very different morphologies that correspond to the three families described within the suborder. These families are the shell-less Desmopteridae (Chun, 1889), Cymbuliidae (Gray, 1840) with a gelatinous pseudoconch, and the aragonitic-shelled Peraclidae (Tesch, 1913) (Lalli and Gilmer, 1989; Ramos-Silva et al., 2021). In terms of feeding habits, euthecosomes and pseudothecosomes create a mucus web to capture phytoplankton, while gymnosomes are species-specific feeders, specialized in preying upon thecosomes (Weldrick et al., 2019). Euthecosomata and Pseudothecosomata were recently confirmed as sister groups by using transcriptomic data, thus restoring the former taxonomic status, and encompassing them in the Thecosomata Blainville, 1824 (Peijnenburg et al., 2020).

The taxonomy of the group has been in constant flux, especially among euthecosomes at the superfamily levels of Limacinoidea (coiled shells) and Cavolinioidea (straight shells), where the positions of many families are unresolved (Burridge et al., 2017a). The latest molecular revision of the suborder (Rampal, 2017) classifies the three Limacinoidea genera into different families: Heliconoididae (*Heliconoides*), Thieleidae (*Thielea*), and Limacinidae (*Limacina*). Moreover, Limacinoidea has been recovered as paraphyletic in all multilocus phylogenies analysed to date (Corse et al., 2013; Burridge et al., 2017a; Rampal, 2017). Cavolinioidea was not found to be monophyletic, and neither *Styliola* (Creseidae) nor *Hyalocylis* (Hyalocylidae) has a certain position within the superfamily (Rampal, 2017). The systematics of Gymnosomata and Pseudothecosomata are poorly studied, with the evolutionary relationships of key taxa such as the gymnosome *Thliptodon* and the thecosomes *Desmopterus* and *Thielea* still to be resolved. Given their morphology and the need for a better understanding of the evolutionary history of the three suborders of pteropods, these taxa are truly crucial.

Previous phylogenetic studies using individual gene trees have shown to lack resolution at deep-node levels (Jennings et al., 2010; Corse et al., 2013). This is particularly true when using the mitochondrial marker cytochrome *c* oxidase subunit I (COI) due to its high heterogeneity (Lessios and Hendler, 2022). More recently, a multi-locus phylogeny using COI and the nuclear 28S and 18S rRNAs (Burridge et al., 2017a) still revealed uncertainty in family-level relationships. Phylogenomic studies have achieved a higher resolution but lacked key taxa useful for comprehending the evolution of Pteropoda (Peijnenburg et al., 2020). For instance, at the species level, only a few studies have dealt with certain genera such as *Creseis* (Gasca and Janssen, 2014) or *Cuvierina* (Burridge et al., 2015). Obtaining a broad overview of the genetic diversity of the group is essential to better understand its ecology and evolution (Burridge et al., 2017a). Such studies are particularly interesting from the perspective of biogeography and biodiversity. Although few studies have dealt with oceanographic barriers that structure pteropod populations, the determinant influence of currents on euthecosome populations has been reported, as well as their abundance and distribution patterns, mostly in the Atlantic (Burridge et al., 2017b; Choo et al., 2021).

Studies on diversity and abundance in shallow and warm waters are very limited in key areas such as the Red Sea or the Mediterranean Sea (Howes et al., 2015). The Mediterranean Sea is particularly sensitive to the effects of climate change due to the short residence time of its water masses and its semi-enclosed nature (Duarte et al., 1999; Mohan et al., 2006). During this century, the average temperature of the Western Mediterranean is expected to increase by 2 to 2.5 °C, along with a decrease in pH of 0.3 to 0.4 units due to the increase in CO_2 (D'Ortenzio et al., 2008; Lazzari et al., 2014; Lionello et al., 2014). As a result, pteropods could be used as a proxy to understand the effects of climate change in the Mediterranean. Thus, studying their diversity could help determine if actions to mitigate this process are sufficient to help the conservation of this group (Howes et al., 2015).

In this study, we present the most comprehensive multilocus phylogeny to date of pteropods, using three different molecular markers: COI, 28S rRNA, and histone 3 (H3), alongside a phylogeny constructed from 15 complete mitochondrial genomes. Drawing from an extensive taxon sampling across the Western Mediterranean and specimens gathered during oceanographic expeditions such as KOSMOS, MALASPINA, SokhoBio, and Kurambio II spanning the Atlantic, Arctic, Antarctic, and Pacific oceans, our study aims to achieve the following objectives: (1) Diversity overview, by sequencing new specimens and employing species delimitation tests, we aim to provide a comprehensive overview of pteropod diversity, addressing potential instances of cryptic speciation; (2) Systematics investigation, our study investigates the systematics of pteropods at family, genus, and species levels through a robust phylogenetic analysis incorporating multiple markers; and (3) Biogeographical and diversity patterns, through maximizing the inclusion of samples from different regions, we seek to identify and analyse potential biogeographical and diversity patterns among pteropod populations.

2. Material and methods

2.1. Taxon sampling

Eleven Mediterranean pteropod specimens (Fig. 1) were collected by snorkelling along different points of the Catalan coast (NE Spain) using small bottles of about 0.5 L. The water collected during the sampling was transferred to 5-litre bottles that were tied to the safety buoy. Sampling localities were selected seaward, perpendicular to the coast in places where the current was stronger, or the wind dynamics favoured sampling. The sampling was carried out during spring upwelling to favour diversity hotspots (Mohan et al., 2006; Howes et al., 2015). Only *Creseis acicula* was observed near the coast throughout the year. Specimens were photographed with a Nikon D7200 and D500 coupled with 60 and 90-mm macro lenses in the lab using 2 Youngnuo YN 560 III flashes. Samples were later anaesthetized using a 7.5 % $MgCl_2$ solution and fixed in 96 % EtOH for molecular purposes. Permits to collect samples were issued by the Catalan Government (permit no. DG/051201–371/2021).

Furthermore, 81 samples were collected during Russian-German oceanographic expeditions including KOSMOS, Kurambio II, MALASPINA, and Vema-TRANSIT spanning the years 2013 and 2017. The distribution of the sampled individuals covered different parts of the Atlantic, Pacific, Antarctic, and Arctic oceans. Individuals were collected using vertical hauls reaching depths of down to 5,900 m across 21 distinct localities. Upon collection, specimens were preserved in 96 % EtOH and 4 % formalin/seawater solution. All specimens are deposited at the Bavarian State Collection of Zoology, Munich (ZSM) (Kohnert et al., 2020). Additionally, targeted genes and whole mitogenomes from 15 specimens were extracted from in-lab genomic resources, amplified via a target enrichment protocol, and sequenced using Illumina NovaSeq technology (Moles and Giribet, 2021). GenBank accession numbers for Sanger-based genes and complete annotated mitogenomes are provided in Supplementary Table S1.

Due to the large number of specimens available in online repositories, we pre-analysed a COI tree of up to 1,000 specimens, and we performed a pre-selection based on species delimitation tests (SDT). The final dataset contained 411 sequences, 92 newly sequenced here and 319 of which belonged to specimens mined from GenBank and BOLD Systems (see Table S1).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted from tissue samples obtained mainly from the mantle or parapodia. Only in the case of the species *Styliola subula* was it necessary to use the whole individual. DNA extractions were performed with the Biotools kit and following the manufacturer's protocol (Standard Biotools Inc., CA, USA). Three genes were amplified: the mitochondrial gene cytochrome *c* oxidase subunit I (COI; primers: LCO1490, HCO2198; Folmer, 1994) and the nuclear genes 28S rRNA (28S) using the primers LSU5-F and 900-F (Littlewood et al., 2000), 900F (Olson et al., 2003), LSU1600-R (William et al., 2003), and ECD2S-

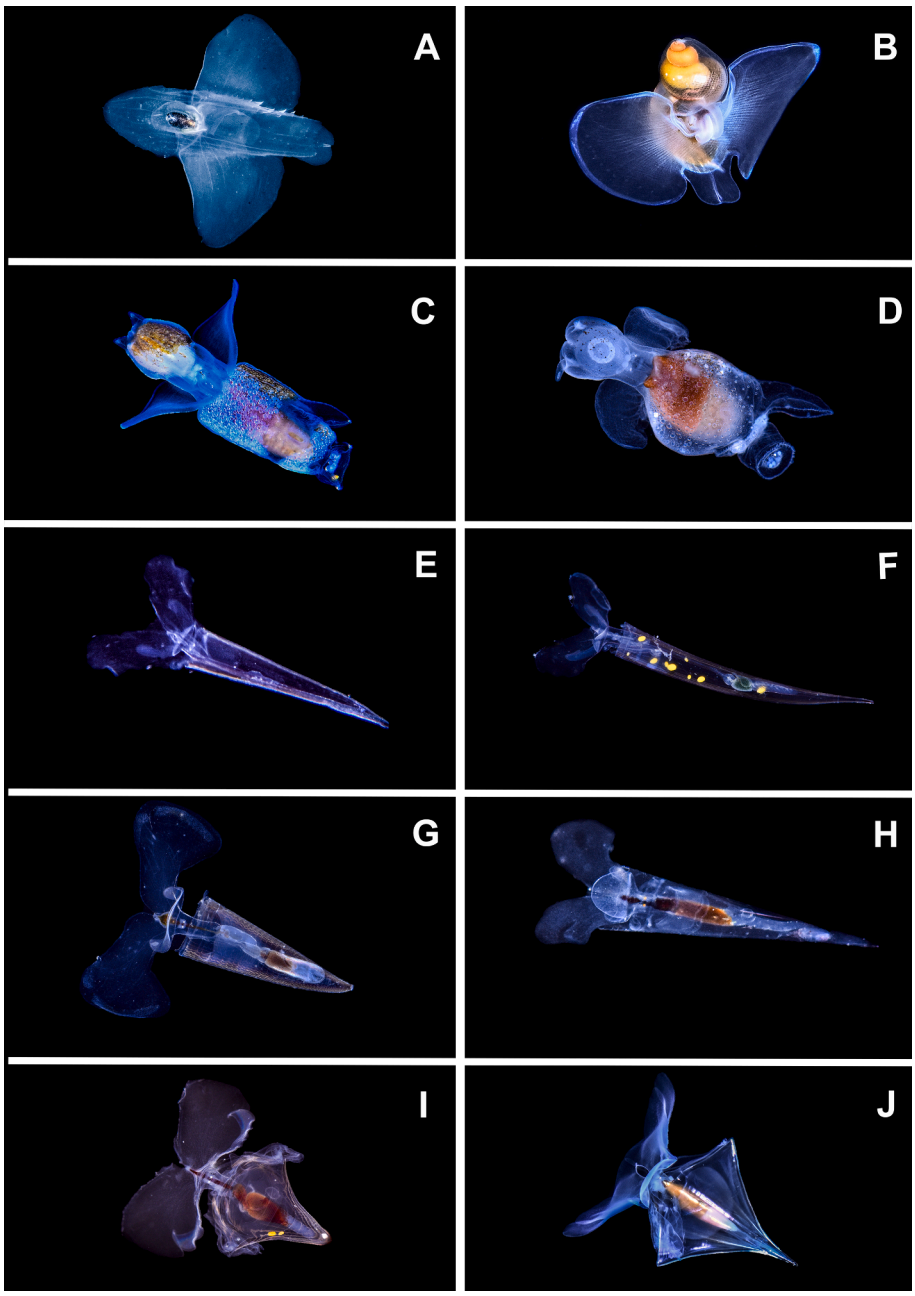

Fig. 1. Underwater photographs of Mediterranean sequenced specimens. **A**: *Cymbulia peronii* (voucher ZSM:Mol:20230822); **B**: *Peracle reticulata* (voucher ZSM:Mol:20230827); **C**: *Pneumodermopsis canephora* (voucher ZSM:Mol:20230828); **D**: *P. canephora* (voucher ZSM:Mol:20230829); **E**: *Creseis acicula* (voucher ZSM:Mol:20230819); **F**: *Creseis conica* (voucher ZSM:Mol:20230821); **G**: *Hyalocylis striata* (voucher ZSM:Mol:20230826); **H**: *Styliola subula* (voucher ZSM:Mol:20230820); **I**: *Cavolinia inflexa* (voucher ZSM:Mol:20230825); **J**: *Clio pyramidata* (voucher ZSM:Mol:20230824). Specimens were collected from various locations along the Catalan coast (NE Spain; see Table S1 for details).

R (primers modified from Littlewood et al., 2000) and the *histone H3* (H3) using the primer pair H3aF and H3aR (Colgan et al., 1998). PCRs were performed in 20 µl volume with 8 µl REDTaq® ReadyMix™ and 0.5 µl of each primer. For the amplified mitochondrial gene, conditions were an initial hot start step of 5 min at 95 °C; 35 cycles of 30 s at 95 °C (denaturation), 35 s between 50 and 52 °C (annealing), and 45 s at 72 °C (extension); and a final elongation of 5 min at 72 °C. For the nuclear gene H3, conditions were identical, except for the annealing temperature that ranged between 52 °C and 55 °C. In the case of 28S, conditions were as follows for both regions: 5 min at 94 °C; 35 cycles of 45 s at 94 °C (denaturation), 45 s at 52 °C (annealing), and 2 min at 72 °C (extension); and a final extension of 10 min at 72 °C. Possible errors and contaminations in the amplifications were checked using gel electrophoresis. Successful amplifications were purified and sequenced in Humanizing Genomics Macrogen Inc. (Seoul, South Korea).

For the 15 taxa used for the mitogenomic phylogeny, genomic DNA was extracted using the E.Z.N.A. mollusc extraction kit (Omega Bio-Tek, Doraville, USA) following the manufacturer's protocol. Extractions were sent to Daicel Arbor Bioscience (MI, USA) for target capture sequencing using a myBaits® probe set (see Moles & Giribet, 2021). Samples were sequenced on the Illumina NovaSeq 6000 platform on partial lanes to approximately 0.6 Gbps of data per sample.

2.3. Phylogenetic analysis and species delimitation tests

All sequences underwent verification using the BLASTn algorithm

(Altschul et al., 1997) and were confirmed not to be contaminated. Sequences were edited and assembled using Geneious R8.1.9. Subsequently, alignments were built in MAFFT v. 7 (Katoh and Standley, 2013) using the G- INS-i (COI and H3) and L-INS-i (28S) algorithms. The partial COI and H3 markers were translated into amino acids to check for possible misalignments. Intra- and interspecific distances were calculated by Geneious R8.1.9 software, based on sequence similarity. Single-gene trees were performed to check for potential discrepancies. These alignments were then concatenated to obtain a final dataset containing 413 individuals. Primer overlap was trimmed in gene-by-gene alignments for markers COI, 28S, and H3 to lengths of 658, 1140, and 330 bp, respectively. The final tree, composed of 411 specimens, included two sequences of Aplysiida, namely *Akera bullata* and *Aplysia dactylomela*, selected as the closest outgroups based on Wägele et al. (2014).

Phylogenetic analyses were run in the CIPRES Science Gateway v. 3.3 portal (http://www.phylo.org/). Both maximum likelihood (ML; Supplementary Figure S1) and Bayesian inference (BI; Supplementary Figure S2) approaches were employed, as described below. The ML analysis was performed using IQ-TREE v. 2.1.2 (Minh et al., 2020). The evolutionary model for the concatenated dataset was selected employing ModelFinder (Kalyaanamoorthy et al., 2017) for each gene partition, with consideration for codon positions for the protein-coding genes. Branch support was assessed using ultrafast Bootstrap with 1,500 replicates, with bootstrap support values (bs) depicted for each node (Hoang et al., 2018). For the BI analysis, MrBayes v. 3.2.7a (Ronquist et al., 2012) was utilized. A GTR+I+G evolutionary model per partition. The analysis comprised four parallel runs of 20 million generations, with sampling conducted every 1000 generations and a burn-in of 25 %. Topological robustness was evaluated using posterior probabilities (pp). Trees were visualized using FigTree v. 1.4.4 (Rambaut, 2014) and edited using Adobe Illustrator (Adobe Systems, CA, USA).

In addition, species delimitation tests (SDT) were carried out based on COI alignments to determine the diversity of the order Pteropoda based on existing data. COI sequences were aligned by genus or family, as each group has a different threshold of species identity (Tobias et al., 2010). An assembled species by automatic partitioning (ASAP; Puillandre et al., 2021), was carried out by accessing the free access website https://bioinfo.mnhn.fr/abi/public/asap/. We apply Kimura (K80) TS/TV distance matrices with default parameters (TS/TV=2.0). Intra- (INTRA) and interspecific (INTER) distances were calculated using Geneious. The Poisson Tree Processes (PTP; Zhang et al., 2013) was conducted using the default parameters (100,000 generations, burn-in of 10 %). The multi-rate Poisson Tree Processes (mPTP; Kapli et al., 2017) were also performed but discarded due to the general tendency to over-split species boundaries. The latter two analyses were conducted using the web interface (https://species.h-its.org/ptp/). Relevant individuals corresponding to putative species or haplotypes with a single marker were also included after checking the preliminary results of the SDTs and single-gene trees.

2.4. Mitogenome assembly, annotation, and matrix construction

Phyluce v. 1.7.1 was used to process the raw reads (Faircloth, 2016). Raw reads were demultiplexed per individual, adapter contamination and low-quality bases were trimmed using Trimmomatic v.0.39 (Bolger et al., 2014) implemented in Illumiprocessor v.2.0.9 (Faircloth, 2013). Clean reads were assembled using Trinity v. 2.1.1 (Haas et al., 2013). We identified the mitogenomes via BLASTn search against a custom database, created using BLAST v.2.6.0 (Altschul et al., 1997), which included all available heterobranch mitogenomes from GenBank.

Despite high sequencing coverage, not all mitogenomes were recovered completely. Most of the newly sequenced mitogenomes were incomplete to some degree, possibly due to difficulties in sequencing through secondary structures associated with the 16S rRNA and the control region. However, most of the mitochondrial protein-coding genes were obtained for all species. Ribosomal sequences (rRNA) and transfer RNA (tRNAs) were annotated with the MITOS Web Server (Bernt et al., 2013). The 13 mt protein-coding genes were annotated using Geneious® 2023.0.4, where open reading frames (ORFs) were manually identified employing the invertebrate mitochondrial code. The limits of both the protein-coding and rRNA genes were adjusted manually based on the positions of adjacent genes.

The dataset included 13 mitochondrial protein-coding genes and two rRNA genes, all analysed at the nucleotide level. These were aligned separately using MAFFT v. 7.490 (Katoh and Standley, 2013) implemented in Geneious, using G-INS-I for protein-coding genes and L-INS-I for the rRNAs. The protein-coding genes' alignment was adjusted manually, removing short sequences, outlier taxa (likely contaminations), and a minimal number of sites, i.e., gaps created by insertions in the sequences of the outgroups and ambiguously aligned positions. Alignment accuracy was corroborated by translating nucleotide sequences into amino acids and inspecting for stop-codons.

Subsequently, alignments of all genes were concatenated in a single dataset. The mitogenomes of three species of *Aplysia* were downloaded from GenBank and used as outgroups. The resulting matrix contained nucleotide sequences curated manually, totalling 12.361 bp (4,7% of missing data). Partitions were designated for each marker, along with codon positions for the protein-coding genes.

3. Results

3.1. 3-gene sequence alignments

The gene markers COI, 28S, and H3 were concatenated into a single alignment for the 411 individuals of the order Pteropoda, which represented the largest number of individuals of both Pseudothecosomata (n = 37) and Gymnosomata (n = 72) included to date in the same phylogeny, together with samples of Euthecosomata (n = 302) (Fig. 2). No stop codons or frameshift mutations were detected in either the COI or H3 markers. In addition to the sequence characteristics previously described in Burridge et al. (2017a), in the COI alignments of the sequences of the undescribed species *Peracle* sp. from Kuril-Kamchatka Trench (Okhotsk Sea), all individuals exhibited the same missing codon (309–311 bp). Regarding the genus *Limacina*, all species shared the same three missing codons as the pseudothecosomes genera *Corolla*, *Cymbulia*, and *Gleba* (77–85 bp). *Limacina antarctica*, *L. helicina*, *L. rangii*, and *L. retroversa* each had one additional missing codon (352–354 bp), while *L. bulimoides*, *L. lesueurii,* and *L. trochiformis* possessed two consecutively missing codons (464–469 bp). Gene marker 28S sequences were trimmed to a length of 1140 characters, while maintaining the hypervariable regions of the gene, thus avoiding masking (see Moles et al., 2023). After a pre-selection, the final dataset consisted of specimens with more than two genes and/or those belonging to distant localities (for distribution range purposes).

3.2. Phylogenetic relationships among higher taxa

The best-fit model of evolution for the mitogenome phylogeny was determined as TIM3 + F+I+G4 for the third codon position of the genes COX1, COX2, COX3, CYTB, ND1, ND4L, and ND5, while GTR+F+I+G4 was used for the rest of the partitions, including codon positions. The mitogenome phylogenetic tree (Fig. 3) revealed *Peracle* sp. from the superfamily Cymbulioidea to be the sister taxon to both Gymnosomata and Euthecosomata. The gymnosome superfamily Clionoidea contained two monophyletic families, with Clionidae as the sister group to two non-classified gymnosomes (probably Notobranchaeidae). Euthecosomata contained one superfamily, Cavolinioidea, which comprised four families, with Hyalocylidae as the sister group to the rest, followed by Cavoliniidae as the sister group to both Cliidae and Creseidae.

Individual gene trees were conducted separately from the Sanger-based dataset for the three suborders to verify possible errors before

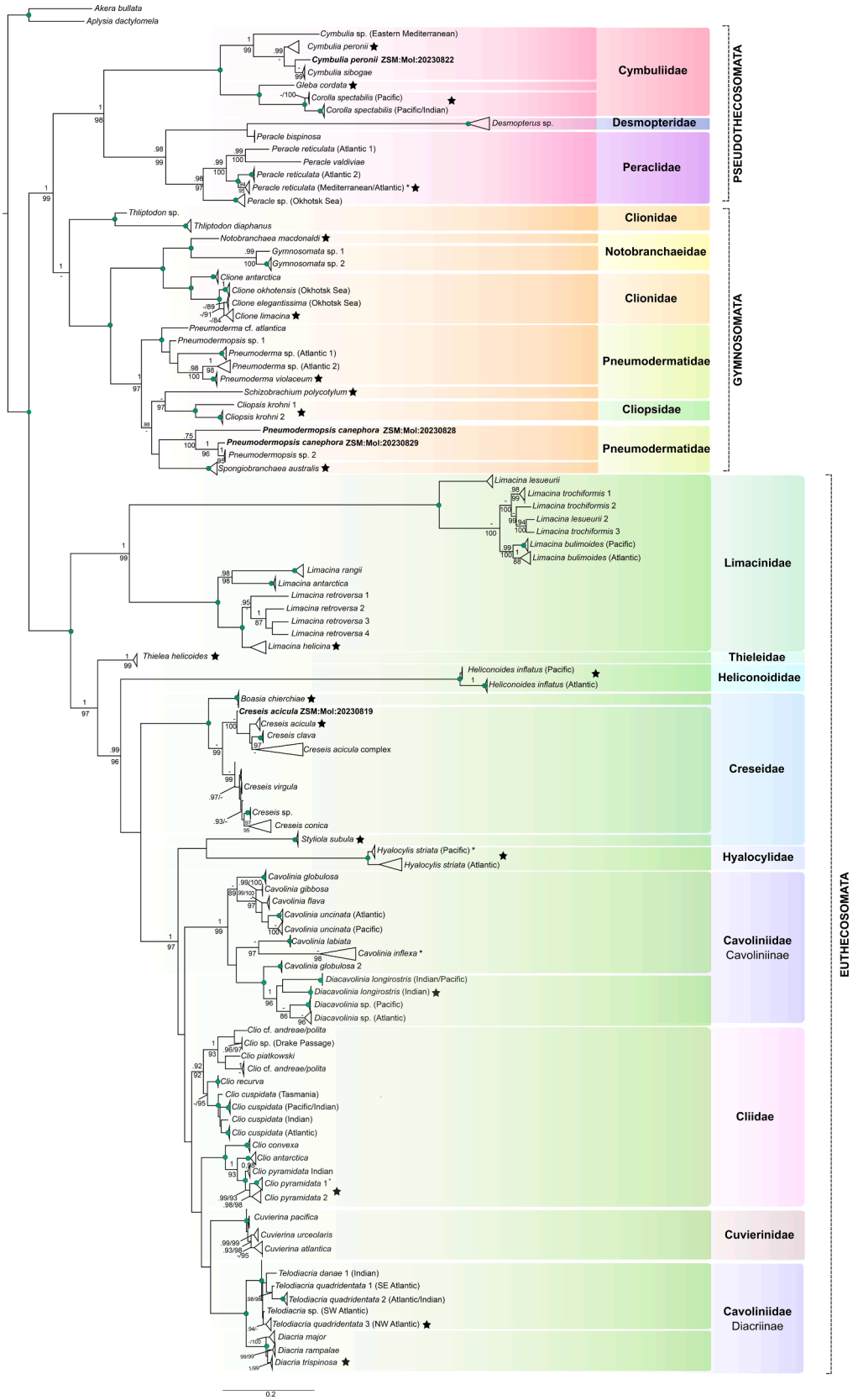

Fig. 2. Phylogenetic tree based on Bayesian inference for the order Pteropoda. The tree is constructed using concatenated COI, 28S, and H3 markers (2134 bp). The posterior probabilities (above or to the left) for BI and ML bootstrap support values (below or to the right) indicate branch support. Green dots at nodes indicate maximum support in both BI and ML analyses. Species recognized from all species delimitation tests were collapsed. Different genera are depicted in coloured boxes, with larger coloured boxes representing families. Suborders are delineated on the rightmeso. Specimens sequenced in this study are marked in bold or with an asterisk when pooled within a larger collapsed branch. Type species for each genus are denoted with a black star on the right side. The scale bar represents substitutions per site

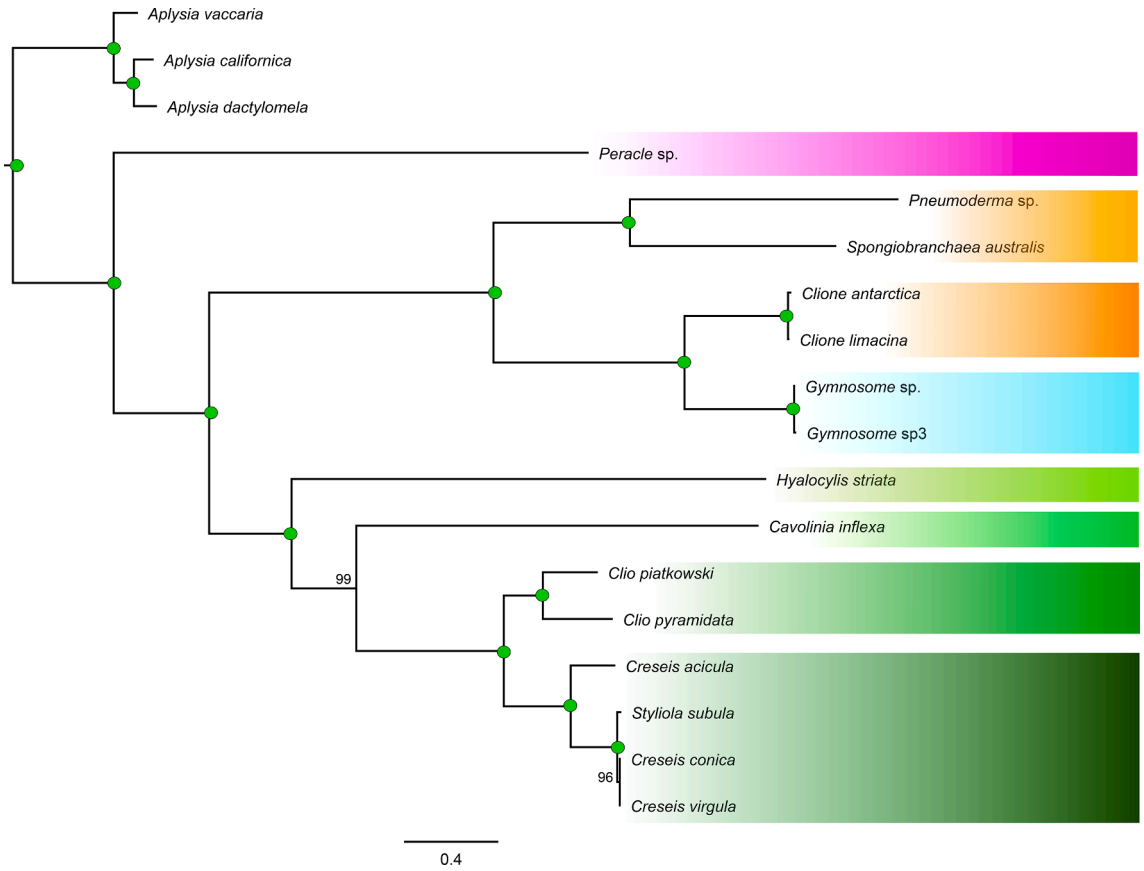

Fig. 3. ML phylogenetic tree based on complete mitogenomes for the order Pteropoda. Bootstrap support values are depicted on branches. Green dots at nodes indicate maximum support. The scale bar indicates substitutions per site

concatenation. The most suitable substitution model of the final concatenated dataset was determined to be GTR+F+I+G4 for each partition. Both BI and ML analyses provided high support the monophyly of Pteropoda (pp = 1, bs = 98), as well as suborders Euthecosomata (pp = 1, bs = 95) and Pseudothecosomata (pp = 1, bs = 93) (Fig. 2). Gymnosomata did not receive support as a clade due to the exclusion of the genus *Thliptodon*. This elusive gymnosome genus appeared in both analyses as the sister group to the rest of the pteropods. Concerning the phylogenetic relationships between the three suborders, they could not be resolved with the molecular data available.

Within pseudothecosomes, the three families described (Cymbuliidae, Desmopteridae, and Peraclidae) and all genera within them were strongly supported in both analyses. Regarding the Gymnosomata families, Notobranchaeidae and Cliopsidae emerged as the only ones that were monophyletic in the tree with maximum support in both analyses. However, the families Clionidae (*Clione* + *Thliptodon*) and Pneumodermatidae (*Pneumoderma* + *Pneumodermopsis* + *Schizobrachium* + *Spongiobranchaea*) were found to be paraphyletic. All genera received high support in both analyses (pp => 0.94, bs => 92) except for *Pneumodermopsis* and *Pneumoderma* in the ML and BI analyses, respectively. Regarding Euthecosomata, Limacinoidea was confirmed as monophyletic (pp = 1, bs = 95), and the three monotypic families were highly supported: Limacinidae (pp = 1, bs = 94), Thieleidae (pp = 1, bs = 100), and Heliconoidae (pp = 1, bs = 100). *Heliconoides* appeared as the sister group of Cavolinioidea (pp = 0.99, bs = 96).

Cavolinioidea failed to receive support as a clade in any analyses, so the relationship between Creseidae and the other families remained undetermined, as was the case for the other families in Cavolinioidea. Creseidae was divided into three genera (*Boasia*, *Creseis,* and *Styliola*) and appeared paraphyletic herein, with the genus *Styliola* emerging as the sister group to *Hyalocylis* in the BI analysis. All other genera within Cavolinioidea proved monophyletic except *Clio* and *Cavolinia*. In the case of the former, there was a well-supported main clade consisting of (1) *C. cuspidata, C. recurva,* and the related deep-sea species *C. piatkowski* and *C.* cf. *andreae/polita* and (2) with no clear position composed of *C. antarctica*, *C. convexa*, and *C. pyramidata*. Concerning the genus *Cavolinia*, all species were positioned with good support except for some individuals identified as *C. globulosa*, which were grouped without support together with the genus *Diacavolinia*.

3.3. Diversity patterns

Our taxon sampling included sequences from all described genera of Pseudothecosomata and Euthecosomata, as well as from all extant families of the gymnosome superfamily Clionoidea. To date, no individuals from the superfamily Hydromyloidea have been sequenced. Type species from all genera have been incorporated except for the genera *Desmopterus*, *Pneumodermopsis,* and *Thliptodon* (see Table 1).

Species delimitation tests confirmed the presence of 100 species: 14 pseudothecosomes, 21 gymnosomes, and 65 euthecosomes. Within the Pseudothecosomata, we identified two species of the genus *Corolla* for the first time and three different species classified as *Peracle reticulata*. In this species complex, one species was related to the meso-bathypelagic species *Peracle valdiviae* (Roberts et al. 2014), while the other two formed a distinct sister clade in the Atlantic Ocean, with one also present in the Mediterranean Sea. Both SDTs recognized the *P. reticulata* species complex, with INTRA ranging from 1.24–4.17 % for the Mediterranean species and 1.37 % for the Atlantic *P. reticulata* 2. According to the INTER, the latter two species were closer to each other (12.94–14.35 %) than to *P. reticulata* 1 from the Atlantic (20.27–22.22 %), which is more related to *P. valdiviae*.

Regarding the gymnosomes, the family Notobranchaeidae contained

Table 1
Type species included in the phylogenetic analyses, with type and sampling localities.

Type species	Type locality	Sampling locality
Boasia chierchiae (Boas, 1886)	Panama, Pacific Ocean (110°E, 10°N).	Gulf of Aden and Caribbean Sea
Cavolinia tridentata (Forsskål, 1775)	Unknown	Southwest of Tasmania Island, Southern Ocean
Clio pyramidata Linneaus, 1767	Unknown ('in oceano')/ Neotype located in Jamaica, Caribbean Sea	Global distribution
Clione limacina (Phipps, 1774)	Svalbard, Arctic Ocean	Arctic Ocean
Cliopsis krohnii (Troschel, 1854)	Strait of Messina, Mediterranean	Northwest Atlantic and Northeast Pacific Ocean
Corolla spectabilis (Dall, 1871)	North Pacific Ocean (42°50′N 147°25′E)	Northeast Pacific and Indian Ocean
Creseis acicula (Rang, 1828)	Indian Ocean	Western Mediterranean and North Atlantic Ocean
Cymbulia peronii (Blainville, 1818)	Nice, Western Mediterranean	Nice, Western Mediterranean
Diacavolinia longirostris (Blainville, 1828)	North Atlantic Ocean (22° 9′N)	North Atlantic Ocean
Diacria trispinosa (Blainville, 1821)	Caribbean Sea (15°58′N 56°44′W)	Caribbean Sea and Atlantic Ocean
Gleba cordata (Forsskål, 1776)	Western Mediterranean	Northwest Atlantic Ocean
Heliconoides inflatus (d'Orbigny, 1835)	North Atlantic Ocean	North Atlantic Ocean
Hyalocylis striata (Rang, 1828)	Atlantic and Indian Ocean	Mediterranean, Atlantic, Indian and Pacific Ocean
Limacina helicina (Phipps, 1774)	Svalbard, Arctic Ocean	Arctic and Northwest Pacific Ocean
Notobranchaea macdonaldi (Pelseneer, 1886)	Northwest Pacific Ocean	Northwest Pacific Ocean
Peracle reticulata (d'Orbigny, 1835)	Southeast Pacific Ocean (20°S 89°W)	Western Mediterranean and Caribbean Sea
Pneumoderma violaceum (d'Orbigny, 1835)	North Atlantic Ocean (4°N 27°W)	Atlantic Ocean
Schizobrachium polycotylum (Meisenheimer, 1903)	Northeast Atlantic Ocean	Liberia, Atlantic Ocean
Spongiobranchaea australis (d'Orbigny, 1836)	Falkland Islands	Antarctic Ocean
Styliola subula (Quoy & Gaimard, 1827)	Canary Islands	North Atlantic Ocean
Telodiacria quadridentata (Blainville, 1821)	Barbados, Caribbean Sea	Caribbean Sea and Atlantic Ocean
Thielea helicoides (Jeffreys, 1877)	West Ireland, Atlantic Ocean	North Atlantic Ocean

three undescribed sister species (maximum INTER of 12.58 %) that appeared evolutionarily distant from the type species *Notobranchaea macdonaldi* (21.20–22.42 %), with a long branch. The genus *Clione* included all four described species: *C. antarctica*, *C. okhotensis*, *C. elegantissima*, and *C. limacina*. The former species appeared as a sister species to the other three (INTER of 15.13–17.54 %), while *C. okhotensis*, *C. elegantissima*, and *C. limacina* had an INTER of 4.53–10.97 %. Most species of the genera *Pneumoderma* and *Pneumodermopsis* were listed as unidentified here. *Pneumoderma* included *P. violaceum* (INTRA of 0.76–1.06 %) and other unidentified species except for *P.* cf. *atlantica*. In the case of *Pneumodermopsis*, we reported only an unidentified species collected off the California coast (possibly related to *P. cicimarensis* also from the Gulf of California; Angulo-Campillo & Aceves-Medina, 2018). Two specimens of *Pneumodermopsis canephora* were also included in the tree. However, since COI sequences were unavailable, we could not confirm their identity using SDT. Alongside this species were two other unidentified species from the Pacific Ocean. Both ML and BI analyses distinguished two species in the monotypic genus *Cliopsis* plus an unidentified one, with an INTER of 18.06–18.24 %. Regarding *Thliptodon*, an unidentified species from California and the mesopelagic species *T. diaphanus* have been included (Van der Spoel, 1987a).

All six species of the coiled euthecosomes of the genus *Limacina* were present in this study, namely *L. bulimoides*, *L. helicina*, *L. lesueurii*, *L. rangii*, *L. retroversa*, and *L. trochiformis*. Additionally, the species *L. antarctica* stat. rest. (INTRA of 0–0.72 %) was recovered, which had been confirmed as a distinct taxonomical unit in both phylogenetic and SDT analyses. For the most closely related and historically confused species, INTER ranged between 28.96 to 35.52 % for *L. rangii* (INTRA of 0.32–7.04 %) and 25.81–28.23 % for *L. helicina* (INTRA of 0–1.25 %). Both *L. retroversa* and *L. trochiformis* appeared as species complexes in both ASAP and PTP due to the very high INTRA (14.83–18.83 % for *L. retroversa* and 6.55 –17.56 % for the two possible species of *L. trochiformis*). Analyses of *L. bulimoides* distinguished between two species, one from the Atlantic and the other from the Pacific Ocean (INTER of 12.77–15.25 %). Within Limacinoidea *sensu lato*, *Thielea* remained a monotypic clade represented by the species *T. helicoides* (INTRA of 0.15–2.32 %). The monotypic *Heliconoides inflatus* presented two distinct species supported by all analyses, one found in the Atlantic and the other in the Pacific Ocean (INTER of 9.59–10.34 %).

Concerning the uncoiled euthecosomes, *Boasia* was confirmed as a monotypic genus and the sister group to *Creseis*. In all analyses, *Creseis* contained the three currently described species *C. acicula*, *C. conica*, and *C. virgula*, as well as five other possible species. These included the currently unaccepted *Creseis clava* from the *C. acicula* species complex, an undescribed species from California, one located offshore the Azores Archipelago, and another one from the Vema-TRANSIT in the mid-Atlantic. INTER ranged from 9.28–10.50 % between *C. acicula* and *C. clava*, 4.27–5.34 % between the unidentified species and *C. virgula*, and 5.33–11.74 % within the *C. acicula* complex. *Styliola* was confirmed as monospecific. Two hidden species of *Hyalocylis striata* were differentiated: one from the Pacific Ocean and the other present in distant localities such as the Mediterranean, the Red Sea, and the Atlantic Ocean. The same applied to species of the genera *Cavolinia* (*C. uncinata*) and *Diacavolinia* (*Diacavolinia* sp.) where there were two species identified as such, differentiated by their specific presence in the Atlantic and Pacific Oceans. Two hidden species appeared as *D. longirostris* too. INTER between *C. uncinata, Diacavolinia* sp., and *D. longirostris* species ranged from 8.84–10.77 %, 14.89–16.58 %, and 23.29–23.55 %, respectively. It was also remarkable the large INTER between *C. inflexa* and the rest of the species of the genus (22.74–30.97 %).

Regarding the family Cliidae, we recovered the same number of currently described species (12), but with possible misidentifications that did not allow us to recognize all described species. Four distinct species were ascribed to *C. cuspidata* and three to *C. pyramidata* (INTER within these complexes of 5.42–7.60 % and 3.50–5.26 %, respectively). Additionally, two deep-sea species (an unidentified species from the Drake Passage in the Antarctic Peninsula and *C.* cf. *andrea/polita*) were included with an INTER ranging from 13.95–14.48 %. We also recovered *C. antarctica*, which appeared as the sister group to *C. pyramidata* with an INTER of 6.50–10.31 %. The clade of *C. convexa* emerged as the basal group of the unsupported branch of *Clio* and sister to the rest of Cliidae. The interspecific distance with *C. antarctica* ranged from 9.83–10.31 %. The genus *Cuvierina* presented only three species out of the six currently described: *C. atlantica*, *C. pacifica*, and *C. urceolaris*, with an INTER of 3.65–6.23 %. Regarding the subfamily Diacriinae, we found three clearly defined species for the genus *Diacria* (*D. major*, *D. rampalae*, and *D. trispinosa*), while for *Telodiacria*, all analyses identified five different species out of the four currently accepted. This genus presented four different species identified as *T. quadridentata* and one as *T. danae*. The first group of species contained two particularly close species, which were supported only in the ASAP analysis: one from the Southwest Atlantic and another from the Caribbean Sea (INTER of 2.54–3.21 %). The second group had an INTER with the rest of the species of the genus ranging from 6.70–10.35 %.

Mediterranean individuals sequenced did not show cryptic speciation, grouping with individuals described as the same species. For the sequenced COIs, the maximum INTRA within each group was as follows: 4.17 % between *Peracle reticulata* ZSM:Mol:20230827 and *P. reticulata* 196 from the Caribbean Sea within *Peracle reticulata* Mediterranean/Atlantic. The INTRA between *Cavolinia inflexa* ZSM:Mol:20230825 and *C. inflexa* 168 from the Caribbean Sea was 2.04 %. The INTRA between *Clio pyramidata* ZSM:Mol:20230824 and *C. pyramidata* AMT_18_27_1 within *C. pyramidata* 1 was 1.89 %. The remaining individuals with 28S and H3 fit perfectly in the tree to their described species and, in the case of insufficient sampling taxa, to their genera.

4. Discussion

With approximately 100 different species recovered out of 163 extant species, our multilocus phylogeny encompasses much of the diversity within the order Pteropoda, offering new insights into long-lasting systematic controversies. The three suborders Euthecosomata, Gymnosomata, and Pseudothecosomata, as recognized by Bouchet et al. (2017), are supported here both in the mitogenomic and the Sanger-based analyses. Recently, transcriptomic analysis has provided a resolution to their relationships, reaffirming Blainville's (1824) classification by grouping pseudothecosomes and euthecosomes (Thecosomata), while rendering gymnosomes as the sister group (Peijnenburg et al., 2020). However, these three approaches remain controversial, with the Pseudothecosomata appearing as the sister group to both Gymnosomata and Euthecosomata in the mitogenomic phylogeny (Fig. 3), while in the Sanger-based phylogeny, the sister group to all the rest were the euthecosomes (Fig. 2). Until a comprehensive genomic study encompassing key taxa from all three major groups is conducted to clarify these relationships, we will focus on species, genus, and family relationships hereafter.

4.1. Pseudothecosomata systematics

For the first time, molecular data recovered the monophyly of the shell-less Desmopteridae within pseudothecosomes, contradicting previous morphological assessments (Lalli and Gilmer, 1989). This suggests that the loss of the shell occurred independently in Gymnosomata and Pseudothecosomata. Interestingly, the embryonic development of *Desmopterus* exhibits similarities in cell cleavage patterns with that of the coiled-shelled euthecosome *Limacina* (Wakabayashi, 2017), highlighting the particularly interesting evolutionary relationships of this genus from anatomical and developmental perspectives. Pseudothecosomata appears to be divided into the three classical families based on morphology, specifically by the composition and presence or absence of the shell in the adult state (Corse et al., 2013). However, this group is poorly studied and documented at the species level, particularly Cymbuliidae and Desmopteridae, which hampers inference of interspecific morphological adaptations.

4.2. Pseudothecosomata diversity

Although environmental features and thermal or oceanographic gradients are known to be involved in genetic differentiation among pteropods, their diversity and speciation remain poorly studied (Burridge et al., 2016; Choo et al., 2021). Within Cymbuliidae, two different species identified as the type species of the genus *Corolla*, *C. spectabilis*, are reported herein for the first time: one from the Indo-Pacific and the other exclusive to the Pacific Ocean. The unidentified specimens found in the Indian Ocean could potentially belong to *C. intermedia* or *C. ovata* (Newman, 1998), needing a morphological assessment for confirmation.

The shelled genus *Peracle* currently lacks molecularly confirmed species-level classification, with some species described solely from shells, such as the Mediterranean *P. diversa* (Monterosato, 1875). We identified three species attributed to *P. reticulata* from the Atlantic Ocean and our specimens from the Mediterranean Sea. The sister species of *P. valdiviae*, named *P. reticulata* Atlantic 1, was found offshore Bermuda, which may indicate that it could represent *P. philiporum* (Gilmer, 1990). The sister species of *P. reticulata* Atlantic 2 could potentially be *P. diversa* or a currently undescribed species. *Peracle diversa* was described and reported based on dredged dead shells in the Mediterranean (Monterosato, 1875), and its distribution in the Atlantic could be explained by the Atlantic currents (Gulf Stream North Atlantic Drift, and Portugal Current), like *P. reticulata*. Further morphological and population genomic studies are necessary to ascertain their biogeographic and taxonomic status.

4.3. Gymnosomata systematics

Consistent with Burridge et al., (2017a), our ML analysis excludes *Thliptodon* from the suborder Gymnosomata, although it received full support in the BI analysis. Regardless, our topology suggests the potential exclusion of *Thliptodon* from the family Clionidae. A thorough taxonomic revision of the genus may revisit earlier classifications such as the discrimination of the subfamily Thliptodontinae Kwietniewski, 1902 from Clioninae Rafinesque, 1815, or even elevating *Thliptodon* to family status. Notably, a distinguishing characteristic of the family Clionidae is the presence of cephaloconi, adhesive buccal tentacles absent in *Thliptodon* (Newman, 1998). This poorly studied genus also exhibits mixed morphological characters shared with *Cliopsis* and *Clione*, as evidenced by the presence of gullet bladders (Van der Spoel, 1972). Our analysis based on both Sanger (Fig. 2) and mitogenomic data (Fig. 3) supports the classical distinctiveness of gymnosomes, highlighting: (1) *Thliptodon* presents two species; (2) Clionidae (only *Clione*) + Notobranchaeidae characterized by the absence of gills and the presence of cephaloconi; and (3) Pneumodermatidae + Cliopsidae with the presence of a posterior gill (Van der Spoel, 1972; Lalli and Gilmer, 1989). Although the positioning of Cliopsidae within Pneumodermatidae suggests ambiguity regarding the monophyly of the latter family. Incorporating molecular data on *Pruvotella* could provide insights into the taxonomic significance of lateral and posterior gills (Newman, 1998).

4.4. Gymnosomata diversity

Among gymnosomes, *Clione* is divided into Antarctic and Arctic clades. The latter exhibits low interspecific variation among *C. elegantissima* and *C. limacina*, with the only differentiation being body size and seasonality, and *C. okhotensis* is notably smaller and possesses a characteristic barrel shape (Yamazaki and Kuwahara, 2017; Yamazaki et al., 2018). The genus *Paraclione* (Tesch, 1903) was included in our dataset (Osborn *et al.* 2021) and clustered within the species *C. okhotensis*. Therefore, the existence of the genus *Paraclione* may be controversial due to its morphological similarity with *C. okhotensis* (Eydoux and Souleyet, 1852), contrasting with clear morphological differences between *C. okhotensis* and the other species of the genus *Clione*. The new molecular evidence expands the distribution of *C. okhotensis* to the coast of Portland, possibly influenced by Pacific currents (e.g., North Japan, North Pacific, California, and North Equatorial currents). Interestingly, within the monotypic genus *Cliopsis,* we identified two hidden species. Molecular clock analysis revealed an early divergence between Atlantic and Pacific populations of *C. krohni* before the formation of the Isthmus of Panama 2.8 Mya (Burridge et al., 2017a). However, allopatric speciation does not appear to be the primary driver here, as we found at least one of the two species in both the Atlantic and the Pacific oceans. Given that gymnosomes are specialized feeders targeting specific thecosomes, regardless of their ability to capture other zooplankton species (Lalli, 1970; Dadon & Chauvin, 1998; Newman, 1998), it would be intriguing to investigate this key driver of speciation and distribution.

4.5. Euthecosomata systematics

Euthecosomata comprises the two superfamilies Limacinoidea and Cavolinioidea, distinguished by their coiled or uncoiled shell, respectively. As Rampal (2017), we found the monophyly of Cavolinoidea, albeit without support, contrasting with the high support for monophyly reported by Burridge et al. (2017a). Limacinoidea was found to be paraphyletic, as *Thielea* and *Heliconoides* emerged as sister groups to the remaining members of Cavolinioidea. Under this scenario, the presence of a coiled shell appears to be a plesiomorphic trait that was secondarily lost during the formation of a straight teleoconch in Cavolinoidea *sensu stricto*. Our phylogenetic analysis is the first to include all three families of Limacinoidea with at least two genes. Consequently, we propose that *Thielea* and *Heliconoides* belong to distinct groups separate from both Cavolinioidea and the genus *Limacina*. Various authors support the idea that the unwinding of the shell is homoplastic (Rampal, 1973), raising doubts regarding the monophyly of groups such as Cavolinioidea. According to Corse et al. (2013), a single process of shell uncoiling occurred through evolution, supporting the monophyly of uncoiled euthecosomes, which aligns with our findings. These new systematic results within Euthecosomata warrant further investigation, particularly focusing on the poorly studied species of Thieleidae and Heliconoididae, as other characteristics such as shell morphology or embryonic development may provide insight into the classification of euthecosomes (Rampal, 2017).

The current classification of Cavolinioidea is primarily based on shell shapes: (1) Cavoliniidae comprises the subfamilies Cavoliniinae (*Cavolinia* and *Diacavolinia*) and Diacriinae (*Diacria* and *Telodiacria*), characterized by a rounded shell; (2) Cliidae (*Clio*) presents a triangular shell; (3) Creseidae (*Boasia*, *Creseis*, and *Styliola*) and (4) Hyalocylidae (*Hyalocylis*) have a conical shell; (5) Cuvierinidae (*Cuvierina*) exhibits a cylindrical shell (Van der Spoel, 1968; Rampal, 2017). While we have support at the genus level for most of these taxa, their interrelationships remain unclear. Creseidae was found to be paraphyletic, with *Styliola* as the sister group to *Hyalocylis*. The slight curvature or curved indentations in the shells of both genera could be considered synapomorphic (Rampal, 2017). The current relationship between *Boasia* and *Creseis* is clearly defined by the morphology of the proto- and teleoconch, in agreement with Rampal's (2017) revision. Both genera exhibit a conical and elongated shell, considered plesiomorphic within Cavolinioidea. Regarding Cavoliniinae, we recovered *Cavolinia* as paraphyletic, with individuals described as *C. globulosa* appearing as sister to all *Diacavolinia* species (Corse et al., 2013; Burridge et al., 2017a), although they might be misidentified and belong to the genus *Diacavolinia*. Similarly, *Clio* was found to be paraphyletic in the Sanger-based tree, with two differentiated and well-supported clades: (1) one consisting of deep-sea species (*C.* cf. *andrea/polita* and *C. piatkowski*) and the shallow-water *C. recurva* and the *C. cuspidata* species complex; and (2) including *C. convexa*, *C. antarctica*, and the *C. pyramidata* species complex. The distinction of these two clades within the genus is supported by shell morphology, as *C. recurva* and *C. cuspidata* have a rounded protoconch with a sharp transition to the apical spine, while species of clade 2 have a more elongated protoconch with a smoother continuation to the apical spine (Janssen, 2012). This is the first time that the clade of deep-sea species of *Clio* is included and found related to *C. recurva* and *C. cuspidata*. This clade shares a swollen posterior foot lobe and a dorsally curved shell (Kohnert et al. 2020; Van der Spoel et al. 1992). Similarly, the cylindrical-shelled genus *Cuvierina* also exhibits distinctive characteristics, such as the teleoconch shape, a bean-shaped aperture, and the absence of a protoconch in the adult stage (as in *Diacria* and *Diacavolinia*; Lalli & Gilmer, 1989; Burridge et al., 2015; Rampal, 2017). Diacriinae is confirmed to comprise *Diacria* and *Telodiacria*, with a perennial protoconch and a V-shaped teleoconch (with or without conspicuous spines; Rampal 2017). Despite being the most studied of the three groups, further research on relatively recent groups such as *Hyalocylis*, *Styliola*, or *Clio* would be essential for a better understanding of the Euthecosomata, to determine with certainty their position within the suborder, as well as the species within each of the genera.

4.6. Euthecosomata diversity

Limacinoidea is predominantly represented by the genus *Limacina*, with well-resolved phylogenetic relationships observed in this extensive analysis. We identified two distinct clades: (1) a low-latitude clade comprising *L. lesueurii*, the *L. trochiformis* species complex, and the two species described as *L. bulimoides*; and (2) a high-latitude clade consisting of the species *L. antarctica*, *L. rangii* (Southern Hemisphere), *L. helicina*, and the species complex of *L. retroversa* (Northern Hemisphere). The existence of clade 1 was previously established by Rampal (2017) based on geographic distribution, while clade 2 exhibits morphological similarities within the Northern and Southern Hemisphere groups. Clade 2, which includes the type species *L. helicina*, encompasses the two Antarctic species *L. antarctica* and *L. rangii*, here differentiated for the first time (Burridge et al., 2017a; Rampal, 2017). *Limacina antarctica* was previously synonymized as *L. rangii*, but evidence suggests they are distinct species, as argued by Hunt et al. (2010), with an early divergence estimated from the establishment of Oligocene cold-water regions, approximately 30 Mya. This distinction applies to their prey gymnosomes *Clione limacina* and *C. antarctica*, respectively, albeit with less interspecific variability. A definitive naming of *L. helicina antarctica* as *L. antarctica* stat. rest., apart from its later synonym *L. rangii*, is here proposed to facilitate the incorporation of both Antarctic species into future classifications. Despite confirmation by the analyses performed, the species complexes *L. retroversa* and *L. trochiformis* suggest an overestimation of their diversity. High intraspecific variability due to population isolation cannot be ruled out, but based on reported localities, population studies of both species appear crucial to understanding their true genetic diversity, as seen in *L. bulimoides* (Choo et al., 2020). Finally, *Thielea*, *Heliconoides,* and *Limacina* exhibit greater evolutionary divergence than other members of Cavolinioidea, likely stemming from an earlier origin (Burridge et al., 2017a; Peijnenburg et al., 2020). Among their biological differences, the three genera are distinguished by their reproductive mode, with *Limacina* being oviparous, *Thielea* ovoviviparous, and *Heliconoides* viviparous (Lalli and Gilmer, 1989; Corse et al., 2013). The latter two strategies have been proposed as adaptations to deep-sea habitats (Roberts et al., 2014).

In Limacinoidea *sensu stricto*, two distinct species groups showing evidence of hidden speciation can be identified within *Creseis*: (1) *C. acicula* + *C. clava* and (2) *C. conica* + *C. virgula* (Corse et al., 2013; Rampal, 2017). The genetic variability of *C. clava* compared to *C. acicula* was substantial enough to classify it as a separate species, a conclusion supported by our SDTs, conducted here for the first time (Gasca and Janssen, 2014). All *Creseis* species exhibit broad distributions (Janssen et al., 2019); for instance, *C. acicula* appears to have a global distribution, potentially obscuring significant cryptic speciation events yet to be resolved (Rampal, 2017), as evidenced by our findings. Allopatric speciation is evident in the case of *C. uncinata*, where the Pacific clade is confirmed as a distinct species from its Atlantic counterpart (Janssen et al., 2019). The taxonomy of *Diacavolinia* remains unresolved, with an apparent overestimation of species since the genus was described by Van der Spoel in 1987b (Maas et al., 2013; Burridge et al., 2019; Janssen et al., 2019). The species attributed to *Diacavolinia* may align with the four groups outlined by Burridge et al. (2019), although definitive species assignments within the genus remain elusive, with three species from the Indo-Pacific Ocean and one from the Atlantic, likely *D. longirostris*.

Within *Clio*, the undescribed deep-sea species group found in the Drake Passage is sister to *Clio* cf. *andraea/polita* from the Kuril-Kamchatka Trench (Kohnert et al. 2020). The described species of the bottle-shaped genus *Cuvierina* are grouped into three clades differentiated geographically: Atlantic (*C. atlantica* and *C. cancapae*), Indo-Pacific

(*C. columnella*, *C. pacifica* N, and *C. urceolaris*), and South Pacific (*C. pacifica* S) (Burridge et al., 2015). Since Burridge et al. (2016), *C. pacifica* N was established as *C. tsudai*, but here we do not observe enough genetic variability between *C. columnella*, *C. tsudai*, and *C. urceolaris*. Therefore, we suggest all three species to be junior synonyms of *C. pacifica*. Concerning the recently divided subfamily Diacriinae, the species complex described as *T. quadridentata* from the Atlantic Ocean contains numerous misidentified species, perhaps actually belonging to a species complex of *T. danae*. The former species occurs only in the Indo-Pacific Ocean, while the latter has a global distribution (40°N-40°S) (Pierrot-Bults and Van der Spoel, 2003). With such a wide distribution, it seems likely that different barriers allow the speciation of species like *T. danae*. There appear to be two lineages, one with both Atlantic and Indian localities and one exclusive to the Atlantic. In the latter, there are two well-localized species, one from the Caribbean Sea influenced by the Gulf Stream and one from the Southwest Atlantic probably influenced by the subtropical gyre present in the area between 15°-18°S described by Choo *et al.* (2020).

Our individuals sequenced from the Mediterranean, despite not showing cryptic speciation, have confirmed their belonging to species from the Atlantic and, in some cases, the Red Sea. This corroborates the entry of individuals from the Atlantic and the flow with the Red Sea into the Mediterranean through the Portugal Current and the Suez Canal, respectively (Moraitou-Apostolopoulou, 1985; de Puelles et al., 2003).

5. Conclusions

In this study, we performed the widest multilocus phylogeny of Pteropoda to date, aiming to maximise genetic and geographic variability. For the first time in a study of this magnitude within Pteropoda, we performed species delimitation tests to reflect the existing diversity based on molecular markers. The phylogenetic analyses yielded approximately 100 species out of the 71 determined species in the sequences included, confirming the presence of hidden diversity and the rediscovery of species such as *L. antarctica* stat. rest., which was previously synonymized as *L. rangii* and should be reinstated as a distinct species. These cryptic species result from both limited knowledge in certain geographical areas, especially in the Southern Hemisphere, and likely misidentifications. In some groups, such discrepancies could obscure species such as *Peracle diversa* (within the *Peracle reticulata* complex) or, conversely, species that have yet to be identified. Furthermore, by including 204 out of the 411 individuals with at least two genes and at least one individual in each genus, we achieved high resolution at both the between- and within-genus levels.

As a result, we have optimized support among groups without the need to disproportionately increase the taxon sampling, maintaining our priority to represent the existing diversity of the order Pteropoda (Sanderson et al., 2003). Our extensive systematic review across the three suborders of Pteropoda has enabled us to confirm the positions of some uncertain taxa and to suggest important changes within some groups, such as *Thliptodon*, the families Thieleidae and Heliconoididae, and the possible suppression of the genus *Paraclione*. Additionally, we have shown broad biogeographical patterns that can serve as a foundation for future in-depth studies, which will be crucial for a comprehensive understanding of gene flow and its determinants within this order. However, it is imperative to expand taxon sampling, particularly for key taxa and underrepresented regions, especially in the Southern Hemisphere. By doing so, we can identify potential barriers crucial for the genetic differentiation of populations and ultimately for speciation. This is essential for achieving a comprehensive understanding for implementing effective conservation strategies for these planktonic marine gastropods in the face of challenges such as ocean acidification and global change.

CRediT authorship contribution statement

Jose Vidal-Miralles: Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Peter Kohnert:** Resources, Data curation, Writing – review & editing. **Marina Monte:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Xavier Salvador:** Resources, Writing – review & editing. **Michael Schrödl:** Funding acquisition, Resources, Writing – review & editing. **Juan Moles:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors sincerely thank the KOSMOS, MALASPINA, and Vema-TRANSIT cruise crew and participants for their invaluable support. Special appreciation is extended to Jose Fernández-Simón and the members of Slug Lab (@slug_lab) for their assistance in the molecular laboratory. The constructive feedback from two anonymous reviewers is greatly appreciated. Funding for this project was generously provided by the association Artenvielfalt erforschen und retten UG and the Malacological Society of London, to JVM's research. Additional support was received from the World Congress of Malacology 2022, facilitating an oral communication and participation in the Symposium in honour of Philippe Bouchet. JM acknowledges support from the Spanish Government through the HETGEN1000 project (PID2021-127037NA-I00/MCIN/AEI/10.13039/501100011033/ and by FEDER una manera de hacer Europa).

Author Agreement.

This statement certifies that all authors have reviewed and approved the final version of the manuscript being submitted. By signing this agreement, authors warrant that the article is their original work, has not been previously published, and is not under consideration for publication elsewhere.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2024.108183.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Angulo-Campillo, O., Aceves-Medina, G., 2018. Two new species of gymnosomatous pteropods from the Gulf of California (Gymnosomata: Pneumodermatidae). Hidrobiologica 28, 231–237.

Bednaršek, N., Tarling, G.A., Bakker, D.C.E., Fielding, S., Jones, E.M., Venables, H.J., Murphy, E.J., 2012. Extensive dissolution of live pteropods in the Southern Ocean. Nature Geoscience 5 (12), 881–885.

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., Stadler, P.F., 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Molecular phylogenetics and evolution 69 (2), 313–319.

Blainville, H.M.D., 1824. Mollusques, Mollusca (Malacoz.), in: Cuvier, F. (Ed.), Dictionnaire des Sciences Naturelles, vol. 32. Levrault, Strasbourg et Paris, & Le Normant, Paris, pp. 1–392.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.

Bouchet, P., Rocroi, J.P., Hausdorf, B., Kaim, A., Kano, Y., Nützel, A., Parkhaev, P., Schrödl, M., Strong, E.E., 2017. Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia 61, 1–526.

Burridge, A.K., Goetze, E., Raes, N., Huisman, J., Peijnenburg, K.T., 2015. Global biogeography and evolution of *Cuvierina* pteropods. BMC Evol. Biol. 15, 1–16.

Burridge, A.K., Janssen, A.W., Peijnenburg, K.T., 2016. Revision of the genus *Cuvierina* Boas, 1886 based on integrative taxonomic data, including the description of a new species from the Pacific Ocean (Gastropoda, Thecosomata). ZooKeys 619, 1.
Burridge, A.K., Hörnlein, C., Janssen, A.W., Hughes, M., Bush, S.L., Marlétaz, F., Gasca, R., Pierrot-Bults, A.C., Michel, E., Todd, J.A., Young, J.R., Osborn, K.J., Menken, S.B.J., Peijnenburg, K.T., 2017a. Time-calibrated molecular phylogeny of pteropods. PLoS ONE 12, e0177325.
Burridge, A.K., Goetze, E., Wall-Palmer, D., Le Double, S.L., Huisman, J., Peijnenburg, K. T., 2017b. Diversity and abundance of pteropods and heteropods along a latitudinal gradient across the Atlantic Ocean. Prog. Oceanogr. 158, 213–223.
Burridge, A.K., Van Der Hulst, R., Goetze, E., Peijnenburg, K.T., 2019. Assessing species boundaries in the open sea: an integrative taxonomic approach to the pteropod genus *Diacavolinia*. Zool. J. Linn. Soc. 187, 1016–1040.
Choo, L.Q., Bal, T.M., Choquet, M., Smolina, I., Ramos-Silva, P., Marlétaz, F., Peijnenburg, K.T., 2020. Novel genomic resources for shelled pteropods: a draft genome and target capture probes for Limacina bulimoides, tested for cross-species relevance. BMC genomics 21, 1–14.
Choo, L.Q., Bal, T.M., Goetze, E., Peijnenburg, K.T., 2021. Oceanic dispersal barriers in a holoplanktonic gastropod. Journal of Evolutionary Biology 34 (1), 224–240.
Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46, 419–437.
Corse, E., Rampal, J., Cuoc, C., Pech, N., Perez, Y., Gilles, A., 2013. Phylogenetic analysis of Thecosomata Blainville, 1824 (Holoplanktonic Opisthobranchia) using morphological and molecular data. PLoS One 8, e59439.
D'Ortenzio, F., Antoine, D., Marullo, S., 2008. Satellite-driven modeling of the upper ocean mixed layer and air–sea CO_2 flux in the Mediterranean Sea. Deep Sea Res. Part I Oceanogr. Res. Pap. 55, 405–434.
Dadon, J.R., Chauvin, S.F., 1998. Distribution and abundance of Gymnosomata (Gastropoda: Opisthobranchia) in the southwest Atlantic. J. Molluscan Stud. 64, 345–354.
de Puelles, M.F., Grás, D., Hernández-León, S., 2003. Annual cycle of zooplankton biomass, abundance and species composition in the neritic area of the Balearic Sea, Western Mediterranean. Mar. Ecol. 24, 123–139.
Duarte, C.M., Agustı, S., Kennedy, H., Vaqué, D., 1999. The Mediterranean climate as a template for Mediterranean marine ecosystems: the example of the northeast Spanish littoral. Prog. Oceanogr. 44, 245–270.
Eydoux, J.F.T., Souleyet, L.F.A., 1852. Voyage autour du monde exécuté pendant les années 1836 et 1837 sur la corvette La Bonite commandée par M. Zoologie, Tome Deuxième. Zoologie. Bertrand, Paris, Vaillant, p. 664.
Faircloth, B.C., 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. Bioinformatics 32 (5), 786–788.
Folmer, O., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Gilmer, R.W., 1990. *Procymbulia philiporum* new species, with a discussion of the genus *Procymbulia* Meisenheimer, 1905 (Gastropoda: Thecosomata). Nautilus 104, 111–119.
Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature protocols 8 (8), 1494–1512.
Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. Mol. Biol. Evol. 35, 518–522.
Howes, E.L., Stemmann, L., Assailly, C., Irisson, J.O., Dima, M., Bijma, J., Gattuso, J.P., 2015. Pteropod time series from the North Western Mediterranean (1967–2003): impacts of pH and climate variability. Mar. Ecol. Prog. Ser. 531, 193–206.
Hunt, B., Strugnell, J., Bednarsek, N., Linse, K., Nelson, R.J., Pakhomov, E., Seibel, B., Steinke, D., Würzberg, L., 2010. Poles apart: the "bipolar" pteropod species *Limacina helicina* is genetically distinct between the Arctic and Antarctic oceans. PLoS ONE 5, e9835.
Janssen, A.W., 2012. Late Quaternary to recent holoplanktonic Mollusca (Gastropoda) from bottom samples of the eastern Mediterranean Sea: systematics, morphology. Boll. Malacol. 48, 1–105.
Janssen, A.W., Sessa, J.A., Thomas, E., 2016. Pteropoda (Mollusca, Gastropoda, Thecosomata) from the Paleocene-Eocene Thermal Maximum (United States Atlantic Coastal Plain). Paleontol. Electron. 19, 1–26.
Janssen, A.W., Bush, S.L., Bednaršek, N., 2019. The shelled pteropods of the northeast Pacific Ocean (Mollusca: Heterobranchia, Pteropoda). Zoosymposia 13, 305–346.
Jennings, R.M., Bucklin, A., Ossenbrügger, H., Hopcroft, R.R., 2010. Species diversity of planktonic gastropods (Pteropoda and Heteropoda) from six ocean regions based on DNA barcode analysis. Deep Sea Res. Part II: Top. Stud. Oceanogr. 57, 2199–2210.
Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589.
Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., Flouri, T., 2017. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33, 1630–1638.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kohnert, P.C., Cerwenka, A.F., Brandt, A., Schrödl, M., 2020. Pteropods from the Kuril-Kamchatka Trench and the Sea of Okhotsk (Euopisthobranchia; Gastropoda). Prog. Oceanogr. 181, 102259.
Lalli, C.M., 1970. Structure and function of the buccal apparatus of *Clione limacina* (Phipps) with a review of feeding in gymnosomatous pteropods. J. Exp. Mar. Bio. Ecol. 4, 101–118.
Lalli, C.M., Conover, R.J., 1976. Microstructure of the veliger shells of gymnosomatous pteropods (Gastropoda: Opisthobranchia). Veliger 18, 237–240.
Lalli, C.M., Gilmer, R.W., 1989. Pelagic snails: the biology of holoplanktonic gastropod mollusks. Stanford University Press, California.
Lazzari, P., Mattia, G., Solidoro, C., Salon, S., Crise, A., Zavatarelli, M., Oddo, P., Vichi, M., 2014. The impacts of climate change and environmental management policies on the trophic regimes in the Mediterranean Sea: Scenario analyses. J. Mar. Syst. 135, 137–149.
Lessios, H.A., Hendler, G., 2022. Mitochondrial phylogeny of the brittle star genus *Ophioderma*. Sci. Rep. 12, 1–13.
Lionello, P., Abrantes, F., Gacic, M., Planton, S., Trigo, R., Ulbrich, U., 2014. The climate of the Mediterranean region: research progress and climate change impacts. Reg. Environ. Change. 14, 1679–1684.
Littlewood, D.T.J., Curini-Galletti, M., Herniou, E.A., 2000. The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. Mol. Phylogenetics Evol. 16, 449–466.
Maas, A.E., Blanco-Bercial, L., Lawson, G.L., 2013. Reexamination of the species assignment of *Diacavolinia* pteropods using DNA barcoding. PLoS One 8, e53889.
Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol. 37, 1530–1534.
Mohan, R., Verma, K., Mergulhao, L.P., Sinha, D.K., Shanvas, S., Guptha, M.V.S., 2006. Seasonal variation of pteropods from the Western Arabian Sea sediment trap. Geo-Mar. Lett. 26, 265–273.
Moles, J., Giribet, G., 2021. A polyvalent and universal tool for genomic studies in gastropod molluscs (Heterobranchia). Mol. Phylogenetics Evol. 155, 106996.
Moles, J., Brenzinger, B., Berning, M.I., Martynov, A.V., Korshunova, T., Schrödl, M., 2023. Systematic rearrangements in an all-genus phylogeny of side-gilled slugs (Heterobranchia: Pleurobranchida). Zool. J. Linn. Soc. https://doi.org/10.1093/zoolinnean/zlad162.
Monterosato, T.A., 1875. Nuova rivista della conchiglie mediterranee. Atti Accad. Sci. Lett. Arti Palermo II 5, 11–50.
Moraitou-Apostolopoulou, M., 1985. The zooplankton communities of the Eastern Mediterranean (Levantine basin, Aegean Sea); influence of man-made factors, In: Moraitou-Apostolopoulou, M., Kiortsis, V. (Eds.), Mediterranean Marine Ecosystems. NATO Conference Series, vol 8. Springer, Boston, MA., pp. 303–331.
Mucci, A., 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. Am. J. Sci. 283, 780–799.
Newman, L., 1998. Opisthobranchia, Chapter 16, Order Thecosomata, in: Beesley, P.L., Ross, G.J.B., Wells, A., Mollusca: the southern synthesis. Fauna of Australia. Vol. 5, part B. CSIRO Publishing, Melbourne, pp. 980–985.
Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A., Littlewood, D.T.J., 2003. Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int. J. Parasitol. 33, 733–755.
Peijnenburg, K.T., Janssen, A.W., Wall-Palmer, D., Goetze, E., Maas, A.E., Todd, J.A., Marlétaz, F., 2020. The origin and diversification of pteropods precede past perturbations in the Earth's carbon cycle. Proc. Natl. Acad. Sci. u.s.a. 117, 25609–25617.
Pierrot-Bults, A.C., Van der Spoel, S., 2003. Macrozooplankton diversity: how much do we really know? Zool. Verh. 345, 297–312.
Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. Mol. Ecol. Res. 21, 609–620.
Rambaut, A., 2014. Figtree, a graphical viewer of phylogenetic trees.
Ramos-Silva, P., Wall-Palmer, D., Marlétaz, F., Marin, F., Peijnenburg, K.T., 2021. Evolution and biomineralization of pteropod shells. Journal of Structural Biology 213 (4), 107779.
Rampal, J., 1973. Phylogénie des Ptéropodes Thécosomes d'après la structure de la coquille et la morphologie du manteau. C. r. Acad. Sci. Série II 277, 1345–1348.
Rampal, J., 2017. Euthecosomata (Mollusca. Gastropoda, Thecosomata), Taxonomic review. bioRxiv, p. 098475.
Roberts, D., Hopcroft, R.R., Hosie, G.W., 2014. Southern Ocean Pteropods, in: De Broyer, C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d'Acoz, C. d', Van de Putte, A. P., Danis, B., David, B., Grant, S., Gutt, J., Held, C., Hosie, G., Huettmann, F., Post, A., Ropert-Coudert, Y. (Eds.), Biogeographic Atlas of the Southern Ocean. SCAR, Cambridge, 276–283.
Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.
Sanderson, M.J., Driskell, A.C., Ree, R.H., Eulenstein, O., Langley, S., 2003. Obtaining maximal concatenated phylogenetic data sets from large sequence databases. Mol. Biol. Evol. 20, 1036–1042.
Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D., Collar, N.J., 2010. Quantitative criteria for species delimitation. Ibis 152, 724–746.
Van Der Spoel, S., 1968. The shell and its shape in Cavoliniidae (Pteropoda, Gastropoda). Beaufortia 15, 185–189.
Van Der Spoel, S., 1972. A taxonomical outline of the Gymnosomata (Mollusca). Basteria 36, 75–88.
Van der Spoel, S., 1987a. *Diacavolinia* nov. gen. separated from *Cavolinia* (Pteropoda, Gastropoda). Bull. Zool. Mus. 11, 77–79.
Van Der Spoel, S., 1987b. Five Pteropod species new for the Gulf of Alaska. Biol. Oceanogr. 5, 29–42.
Van der Spoel, S., Schalk, P., Bleeker, J., 1992. *Clio piatkowskii*, a mesopelagic pteropod new to science (Gastropoda, Opisthobranchia). Beaufortia 43, 1–6.

Wägele, H., Klussmann-Kolb, A., Verbeek, E., Schrödl, M., 2014. Flashback and foreshadowing—a review of the taxon Opisthobranchia. Org. Div. Evol. 14, 133–149.
Wakabayashi, K., 2017. Embryonic development of the sea butterfly *Desmopterus papilio* (Gastropoda: Thecosomata). Invertebr. Reprod. Dev. 61, 142–146.
Weldrick, C.K., Trebilco, R., Davies, D.M., Swadling, K.M., 2019. Trophodynamics of Southern Ocean pteropods on the southern Kerguelen Plateau. Ecol. Evol. 9, 8119–8132.
Williams, S.T., Reid, D.G., Littlewood, D.T.J., 2003. A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. Mol. Phylogenetics Evol. 28, 60–86.
Yamazaki, T., Kuwahara, T., 2017. A new species of *Clione* distinguished from sympatric *C. limacina* (Gastropoda: Gymnosomata) in the southern Okhotsk Sea, Japan, with remarks on the taxonomy of the genus. *J.* Molluscan Stud. 83, 19–26.
Yamazaki, T., Kuwahara, T., Takahashi, K.T., 2018. Genetic differences in spatially and temporally isolated populations: winter and spring populations of pelagic mollusk *Clione* (Mollusca: Gymnosomata), Southern Okhotsk Sea, Japan. Thalassas 34, 447–458.
Zachos, J.C., Röhl, U., Schellenberg, S.A., Sluijs, A., Hodell, D.A., Kelly, D.C., Thomas, E., Nicolo, M., Raffi, I., Lourens, L.J., McCarren, H., Kroon, D., 2005. Rapid acidification of the ocean during the Paleocene-Eocene Thermal Maximum. Science 308, 1611–1615.
Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876.