Life Adrift: Evolution of Pelagic Lifestyle in Marine Gastropods

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ABSTRACT

The evolutionary history of pelagic gastropods remains poorly understood. To clarify relationships among major pelagic lineages, we constructed phylogenies of heteropods, pteropods, pelagic nudibranchs, and the snail, *Janthina,* using three genetic markers: cytochrome oxidase I (COI), 28S rRNA, and histone 3 (H3). We constructed phylogenies using Bayesian and maximum likelihood statistical methods. COI and 28S trees consistently support Thecosomata, Gymnosomata, and Heteropods as distinct monophyletic clades. H3 phylogeny showed weaker resolution, with some unexpected placements for *Glaucus* and *Janthina*. Together these results suggest that transitions to pelagic life in gastropods has been perpetuated by modifications of the shell. Our findings provide a comparative framework for pelagic gastropod evolution and highlight the need for improved taxon sampling and stronger support values in order to better identify when and how these lineages became pelagic.

INTRODUCTION

Gastropoda, a diverse class within the phylum Mollusca, occupy ecological zones from intertidal to deep-sea. Pelagic gastropods live their entire life cycle in the open ocean and are therefore highly specialised for life in the pelagic environment. The shell, a major component of Gastropod morphology, can be difficult to have while living in the free-floating, pelagic environment. As a result, many pelagic gastropod lineages have evolved to have a reduced shell or have lost it completely, which serves to lessen specific gravity and increase buoyancy (Lalli & Gilmer 1989). Of the approximately 40,000 marine gastropod species, only about 140 are holoplanktonic. They also play an important ecological role in the context of marine food webs. They graze on phytoplankton and prey upon smaller zooplankton and are themselves prey for larger animals such as seabirds, whales and commercially important species of fish such as mackerel, herring, and salmon (Lalli & Gilmer 1989) . The shelled pteropods and some heteropods also make contributions to sediments when their shells sink to the ocean floor. They build their aragonite shells using bicarbonate ions from the surface seawater. This removes dissolved inorganic carbon from the upper ocean. Thus when the shells dissolve or sink they are deposited on the seafloor, that carbon is stored as calcium carbonate in their shells (Berner 1977; Peijnenburg 2020). This reduces the amount of carbon dioxide that can be exchanged between the ocean and the atmosphere. Despite the ecological importance of pelagic gastropods, their phylogenetic relationships and evolutionary origins of their fully pelagic lifestyle remain poorly understood. In this study, we investigated the evolutionary relationships among four groups of pelagic gastropods: Heteropods, Pteropods, the snail species *Janthina janthina* and the Nudibranch, *Glaucus* sp. to understand their adaptations to the pelagic realm and the evolutionary pressures that shaped this transition.

Heteropods (Pterotracheoidea), often referred to as “sea elephants”, are found in all tropical and subtropical oceans of the world (Burridge 2017). They are primarily epipelagic, living at depths from just below the surface to about ~500 meters but since they are mobile carnivores, their population densities are relatively low (Lalli & Gilmer 1989; Richter and Seapy 1999). They have a large trunk-like proboscis where the mouth is located at the terminal end. Heteropods also have reduced often vestigial tentacles of unequal size located at the base of the proboscis (Seapy, Lalli, & Wells 2003). Additionally, at the posterior base of tentacles, they have

large eyes which serve to locate their prey which includes zooplankton, phytoplankton, and other small fishes. Beneath and anterior to the visceral mass lies the swimming fin (Lalli & Gilmer, 1989). This group has three distinct subfamilies: Atlantidae, with a fully coiled shell, Carinariidae, with a capped shell, and Pterotracheidae, with no shell. The Atlantids are considered to be the most primitive heteropods. Their microscopic body can be completely withdrawn into its shell and seal the aperture with the operculum when it is disturbed (Wrobel & Mills 1998; Seapy, Lalli, & Wells 2003).

Pteropods are defined by their pair of wing-like parapodia, which they use to "fly" through the water. They are found throughout the world's ocean. It is widely agreed upon that this group is divided into two orders: Thecosomata, "sea butterflies", and Gymnosomata, "sea angels". Thecosomes are generally small, omnivorous, and have thin, fragile, external shells or internal gelatinous conchae. They are unique among pelagic gastropods in developing a mucous web as a feeding mechanism for small phytoplankton (Lalli and Gilmer 1989). Thecosomata are further divided into suborders Euthecosomata and Pseudothecosomata and are distinctive by their shell. The euthecosomes have aragonite shells (shells composed of calcium carbonate) throughout their adult life while pseudothecosomes lose their shell during metamorphosis. Due to their aragonitic shell, pteropods have sparked an interest in environmental scientists to see how their shells can act as bioindicators of the effects of ocean acidification and climate change (Bednarsek 2012; Fabry 2008). However, the extent of shell dissolution is still up for debate because studies have demonstrated that certain species, such as *Limancina helicina* can maintain shell integrity by thickening their inner shell wall (Peck et al., 2016, Peck et al., 2018). The Gymnosomata are the most poorly known of the holoplanktonic gastropods due to their small size, patchy distribution, and soft, delicate bodies (Lalli & Gilmer 1989). They have been found in all major oceans and are mostly confined to epipelagic and mesopelagic zones. They lack a shell and mantle cavity. Most notably, their feeding habits are sharply different from the Thecosomata. Instead, they are highly specialized carnivores that have adapted for the capture and ingestion of thecosomes and other large zooplankton.

Nudibranchs, also known as sea slugs, are found in marine environments worldwide, from the poles to the tropics, in both shallow and deep waters. All of the sea slugs lack a shell and a mantle cavity and their body is streamlined and flexible (Lalli & Gilmer 1989). This structure has resulted in some nudibranch species becoming truly holoplanktonic. Currently, the

only known pelagic nudibranch species are *Phylliroe bucephala*, *P. lichtensteinii, Cephalopgye trematoides, Glaucus atlanticus, G. marginata,*(Lalli & Gilmer 1989)*, Pleuropyge melaquensis* (Santiago-Valentín, et al. 2025), and *Bathydevius caudactylus* (Robison & Haddock 2024).

Understanding the multi-gene phylogenetic relationships of these pelagic gastropod lineages is crucial for revealing patterns of their evolutionary history and biogeography. We used the genes cytochrome-C oxidase subunit 1, Histone 3, and 28S rRNA to construct phylogenetic trees, this study aims to shed light on the relatedness of these various species. This analysis can provide insight into how different lineages are evolutionarily related, revealing their ancestral histories and patterns of divergence over time. Furthermore, clarifying these relationships can help us understand the selective pressures or ecological advantages that drove the transition to a pelagic lifestyle and how these lineages may continue to evolve in response to modern environmental pressures, such as ocean acidification and climate change.

MATERIALS AND METHODS

Sample Collection and Extraction

We extracted and sequenced 24 specimens which were collected during blue-water SCUBA diving expeditions in 2024 from the Gulf of California and Hawaii. We preserved the samples immediately in DNA/RNA Shield solution or flash frozen in liquid nitrogen to stabilize nucleic acids. We extracted genomic DNA extracted from tissue of each individual using the Omega Bio-Tek E.Z.N.A. Mollusc DNA Kit, following the manufacturer's protocol. To supplement our dataset, we retrieved ~110 publicly available sequences from GenBank (NCBI) for related taxa. These reference sequences included specimens collected from the Mediterranean Sea and Catalonia.

DNA Amplification and Sequencing

We amplified 1001 bp of the *28S* rRNA gene, 651 bp of mitochondrial cytochrome c oxidase subunit I (*COI*), and 328 bp of Histone-3 (*H3*) via PCR. We amplified the *COI* barcode fragment with primers jgLCO1490 (5′-TITCIACIAAYCAYAARGAYATTGG-3′) and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) (Geller et al., 2013) and COIF (5'-TCMACTAATCAYAARGAYATTGGNAC-3') and COIR (5'-CCDCTTAGWCCTARRAARTGTTGNGG-3'), (Nelson and Fisher, 2000). We amplified the

28S fragment with primers LSUD1F (5'-ACCCGCTGAATTTAAGCATA-3')) and D3AR (5'-ACGAACGATTTGCACGTCAG-3') (Scholin, 2004), and the *H3* fragment with primers H3F (5′-ATG GCT CGT ACC AAG CAG ACV GC-3′) and H3R (5′-ATA TCC TTR GGC ATR ATR GTG AC-3′) (Colgan, 2000).

PCR reactions (25 µL) contained 12.5 µL Red Taq Master Mix, 1.0 µL each of forward and reverse primers, 2.5 µL BSA, 7.0 µL nuclease-free water, and 2.0 µL template DNA. Thermocycling conditions were: initial denaturation at 95 °C for 10 min; 40 cycles of 94 °C for 1 min, 46 °C for 1 min, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. We visualized PCR products with agarose gel electrophoresis. We purified PCR products with the Multiscreen HTS 96 vacuum manifold system (Millipore Corp., Bilerica, MA).

We sequenced purified amplicons using Sanger dideoxy sequencing with the Big Dye Terminator v3.1 kit (ThermoFisher Scientific). Sequencing reactions (10 µL) contained 0.5 µL Big Dye, 1.75 µL 5× Sequencing Buffer, 0.5 µL primer (3.2 pmol/µL), 2.0 µL template DNA, and 5.25 µL nuclease-free water. Cycling conditions were: 96 °C for 1 min; 25–40 cycles of 96 °C for 10 s, 50 °C for 50 s, and 60 °C for 1 min 15 s; and a final hold at 4 °C for 4 min. Sequencing products were precipitated with ethanol/EDTA/sodium acetate and analyzed on an ABI genetic analyzer.

Statistical Methods

To examine within-order diversity, we assembled, edited, and aligned sequence fragments using Geneious Alignment in Geneious Prime (Geneious Prime 2025.2.1). We used ModelTest within Geneious to determine which model was most appropriate for each locus. We estimated phylogenies for all three loci separately with MrBayes (v3.2.7a, Ronquist et al., 2012) and IQ-Tree 2 web server. We estimated Bayesian phylogenies with six chains that ran for 5,000,000 generations sampled and printed every 1000 generations and had a burn-in of 10%. We estimated IQtree phylogenies with the ultrafast bootstrap for 1000 generations. We visualized and annotated phylogenies with FigTree (v1.4.4, tree.bio.ac.uk/software/figtree/). We reported Bayesian and maximum likelihood support values on the phylogenies.

Examples of Pelagic Gastropods

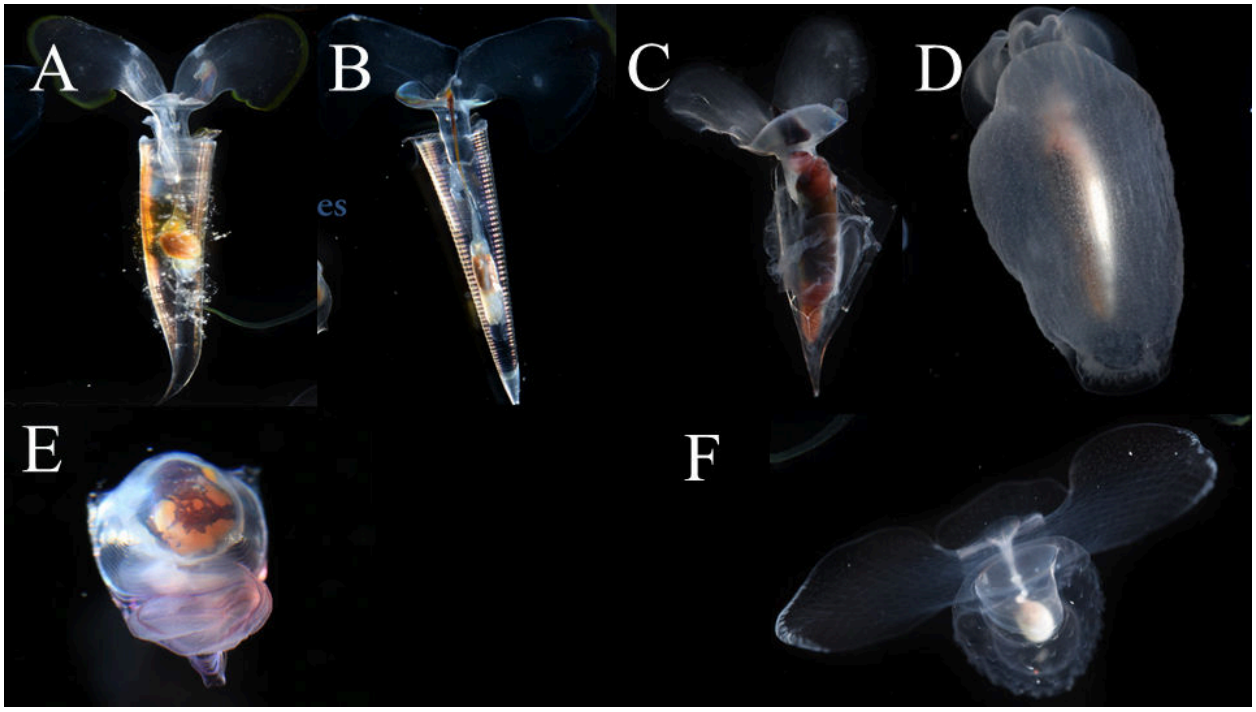

Image 1. Examples of pteropods photographed. (A) *Creseis* sp., (B) *Hyalocylis* sp., (C) *Clio* sp., (D) *Thliptodon* sp., (E) *Diacavolinia* sp., (F) *Corolla* sp. Photographs by Steven Haddock, Shannon Johnson, Jacob Church, and Jamie Brisbin (May 2024).

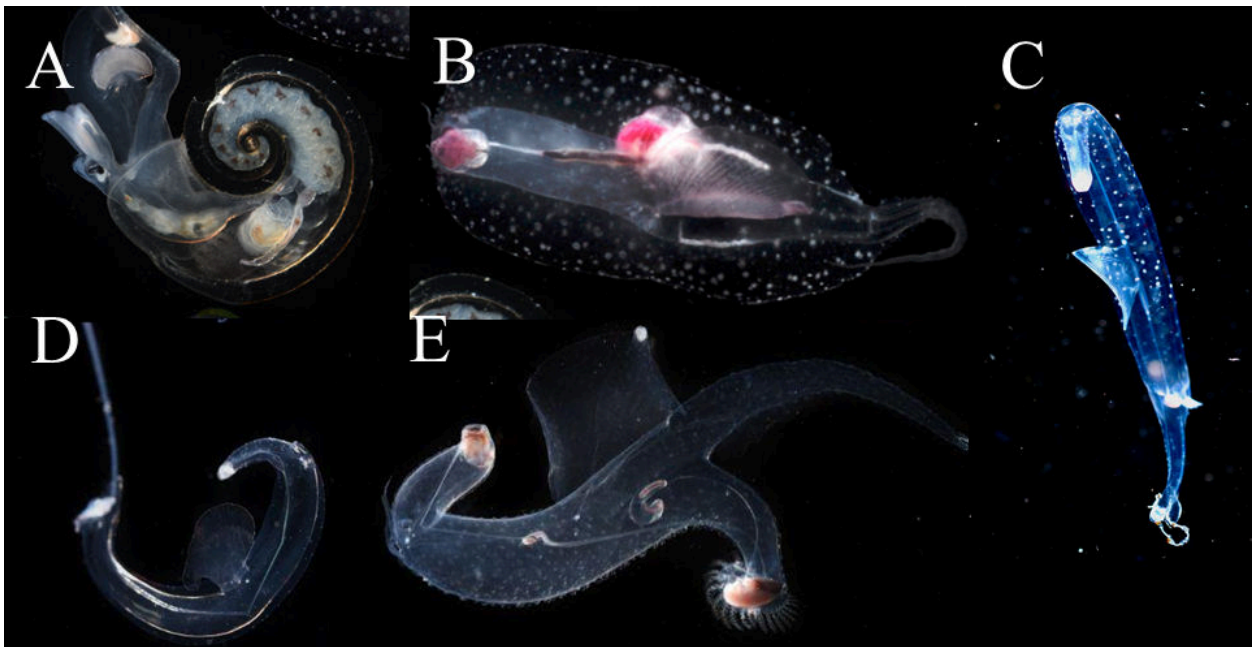

Image 2. Examples of heteropods photographed. (A) *Atlanta* sp., (B) *Pterosoma* sp., (C) *Pterotrachea* sp., (D) *Firoloida* sp., (E) *Cardiopoda* sp. Photographs by Steven Haddock, Shannon Johnson, Jacob Church, and Jamie Brisbin.

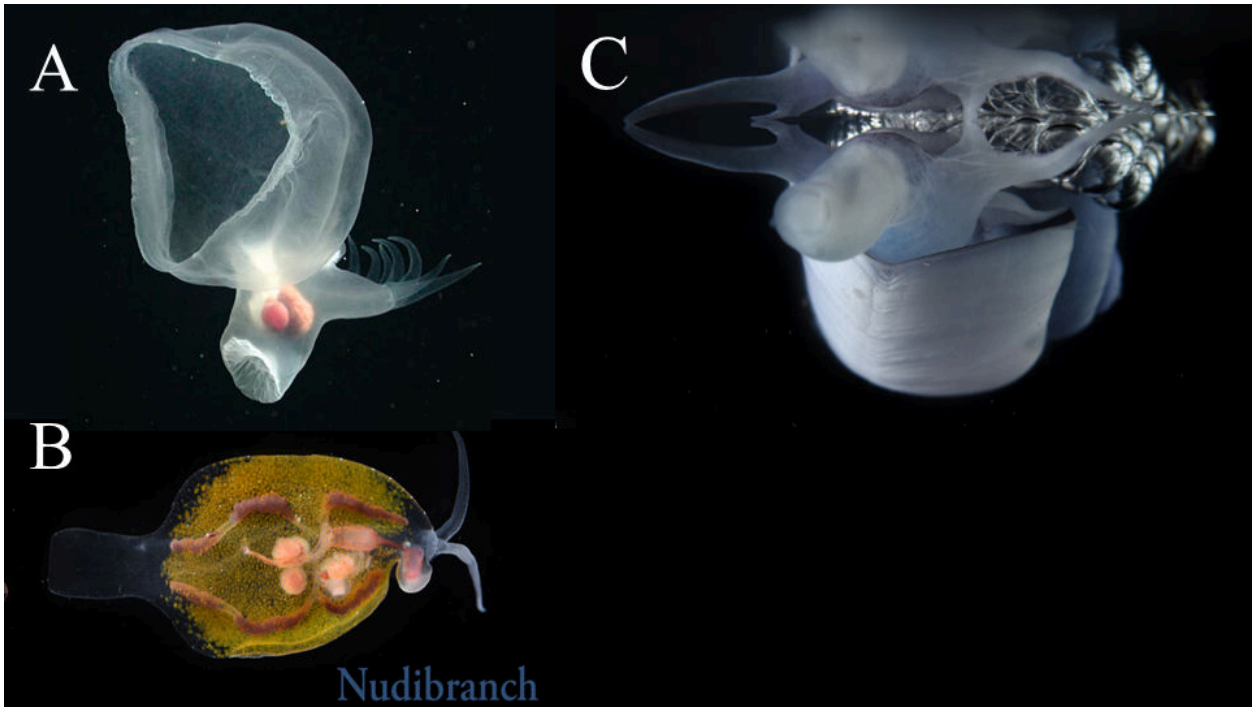

Image 3. Examples of pelagic gastropods photographed. (A) *Bathydevius caudactylus* (MBARI 2021) (B) *Phylliroe* sp., (C) *Janthina janthina* Photographs by Steven Haddock, Shannon Johnson, Jacob Church, and Jamie Brisbin.

RESULTS

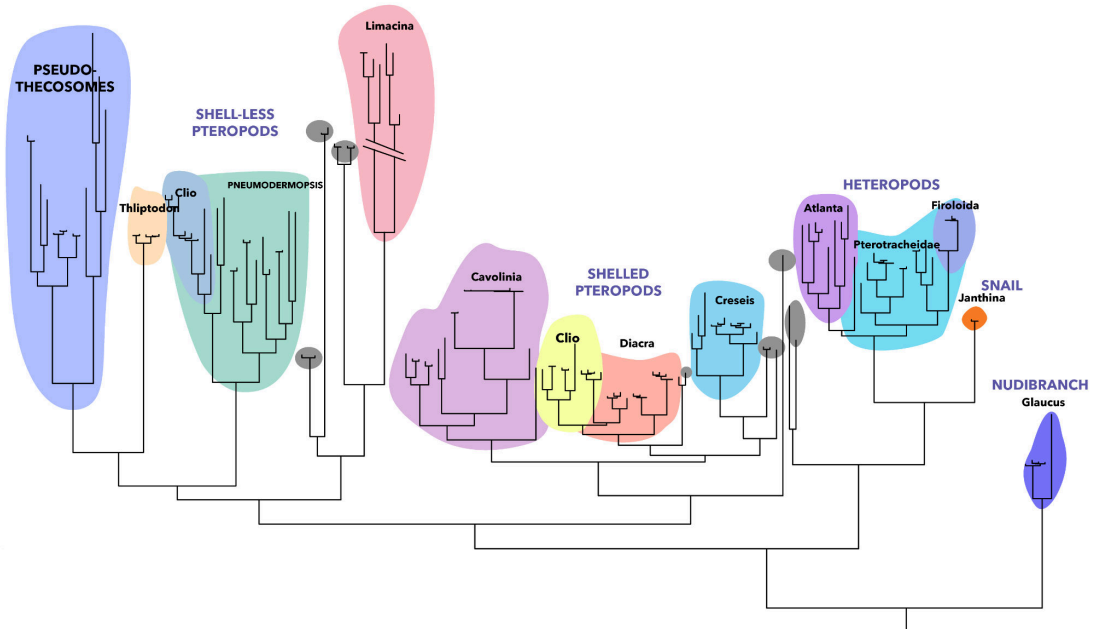

Figure 1. Bayesian and maximum likelihood estimated phylogenies for 651 bp *COI* fragments. Coloured shapes indicate similar genera or similar families.

The phylogenetic reconstruction of pelagic gastropods based on COI (Figure 1) recovered major taxonomic groups as distinct clades. Shelled pteropods (Euthecosomata) clustered together, with clear subclades corresponding to genera such as *Cavolinia, Limancina, Clio, Creseis* and *Diacra*. Shell-less pteropods (Gymnosomata) were also recovered as a clustered lineage, represented by *Clione* and *Thliptodon*. Heteropods, including genera *Atlanta, Firoloida*, and *Pterotrachea*, grouped separately from pteropods. The nudibranch *Glaucus atlanticus* was placed outside of these pelagic gastropod lineages as expected.

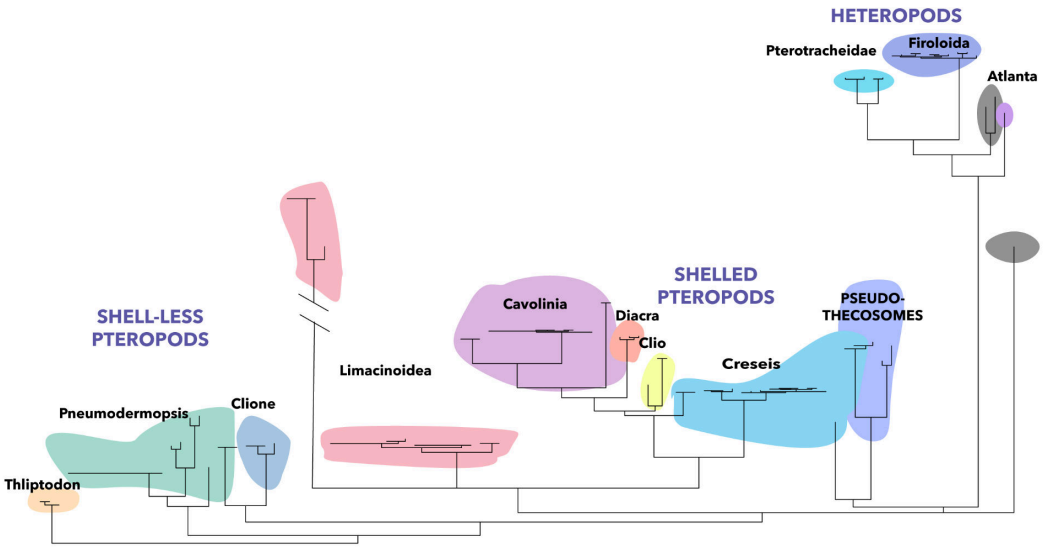

Figure 2. Bayesian and maximum likelihood estimated phylogenies for 1001 bp *28S* rRNA fragments. Coloured shapes indicate similar genera or similar families.

Phylogenetic analyses of pelagic gastropods using 28S (Figure 2; Supplementary Figure 1) recovered the major pelagic gastropods lineages in distinct clades. Shelled pteropods (Thecoosmata) formed a cohesive monohyletic group, with well supported subdivisions corresponding to *Cavolinia, Limancina, Clio, Creseis*, and *Diacra* (UFB=97 (SFig.1)). Within the shell-less pteropods were several monophyletic clades. *Spongiobrachea australis*, *Pneumodermopsis*, and *Cliopsis* formed a strongly supported clade (UFB=100 (SFig. 1)) and *Thliptodon* was recovered as an independent lineage. Heteropods (*Atlanta, Firoloida, Pterotrachea)* also formed a strongly supported monophyletic clade (UFB=100 (SFig. 1)).

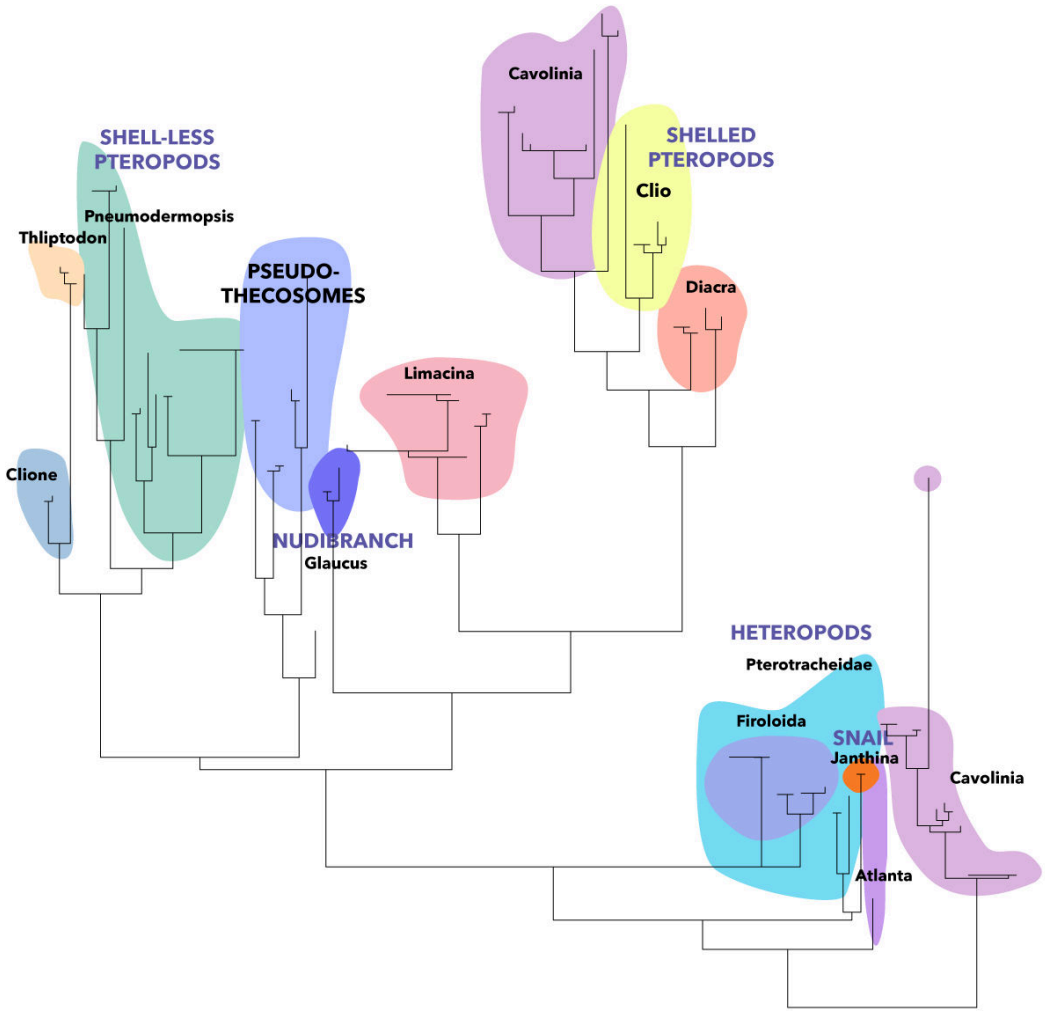

Figure 1. Bayesian and maximum likelihood estimated phylogenies for 328 bp *H3* fragments. Coloured shapes indicate similar genera or similar families.

H3 phylogeny recovered the pelagic gastropod lineages in several distinct clades, but with inconsistent placement of some groups. The shelled pteropods form their own stringy supported monophyletic clade (UFB=98 (SFig.2)). The shell-less pteropods, Gymnosomata, (*Clione, Thliptodon, Pneumodermopsis*) form their own monophyletic clade (UFB=99(SFig.2)) and appear to be sister to the Pseudothecosomata. The nudibranch *Glaucus* sequences were shown to be sister to the shelled pteropods and the *Janthina* snail was also placed within the heteropods.

DISCUSSION

Phylogenetic analyses using COI, 28S, and H3 provide perspective on the evolutionary history of pelagic gastropods and the role of shell morphology in their speciation. Across all three gene markers, the major pelagic lineages, Thecosomata, Gymnosomata, and Heteropoda, were consistently seen across the three gene trees. The COI and 28s trees both support the monophyly of shelled pteropods and of Gymnosomata. Gymnosomata branches separately from the shelled forms which is consistent with the interpretation that Gymnosomata represent a lineage that experienced a loss of the shell from a shelled ancestor. The placement of Pseudothecosomata as sister to Gymnosomata in the trees suggests a stage of shell reduction or modification. Heteropods, on the other hand, were consistently recovered as a single lineage across both markers.

The H3 phylogeny provided weaker resolution of taxonomic groups with some unexpected placements. In particular, *Glaucus* was recovered alongside shelled pteropods and *Janthina* was nested within heteropods. These placements conflict with morphological expectations. This highlights the limitations H3 had for this study for resolving higher-level relationships for this group. Altogether though, these results show that the transition to pelagic life in gastropods has been perpetuated by modifications of the shell.

CONCLUSIONS

This study provides a comparative phylogenetic framework for pelagic gastropods based on 3 genetic markers. They highlight well supported clades and areas of uncertainty. Our results suggest that determining when marine gastropods became pelagic will require broader sampling of benthic lineages. This would help to pinpoint the timing of the transition and identify specific benthic ancestors. Based on the COI tree, clades defined by shell presence or absence are recovered as monophyletic, so we can infer that shell loss or gain occurred at the level of a shared ancestor. However, accurate resolution depends on improved data quality (checking for saturation and including higher-quality alignments) and increased taxon sampling in order to increase bootstrap and maximum likelihood support values.

Future work will be crucial to resolving deeper nodes and to evaluate whether evolutionary strategies in shell form and pelagic adaptation are linked to selective pressures in different oceanic environments. Also, building time-calibrated phylogenies that include fossil

data could help us to understand when these lineages diverged. Adding more genomic data will also make it possible to resolve uncertain parts of the tree.

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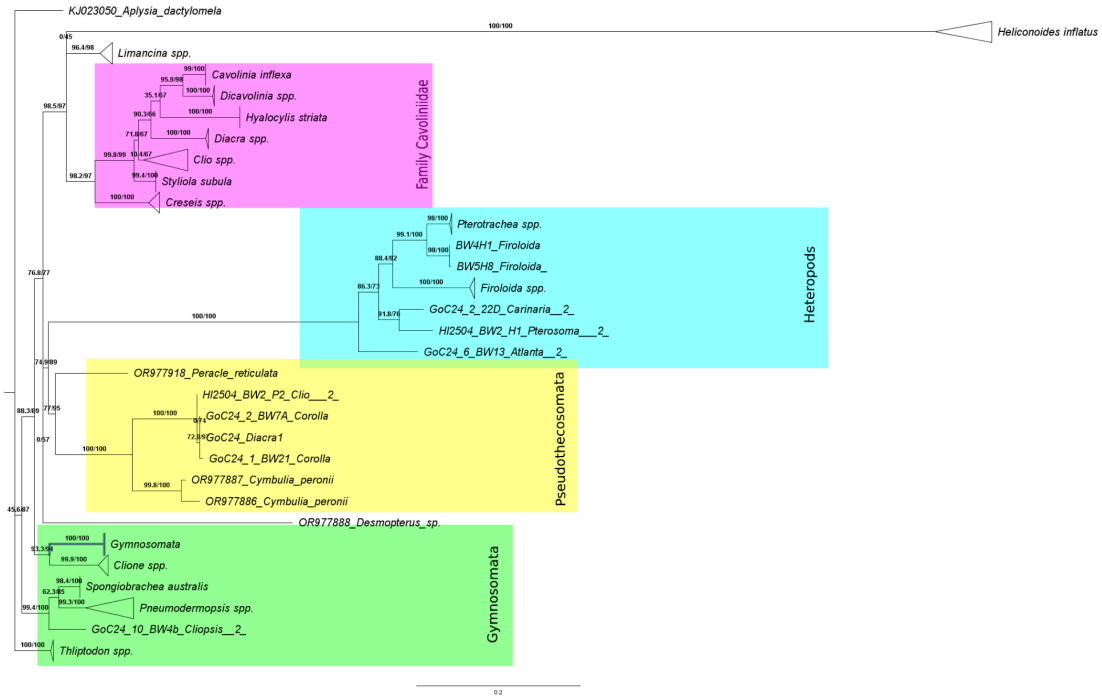
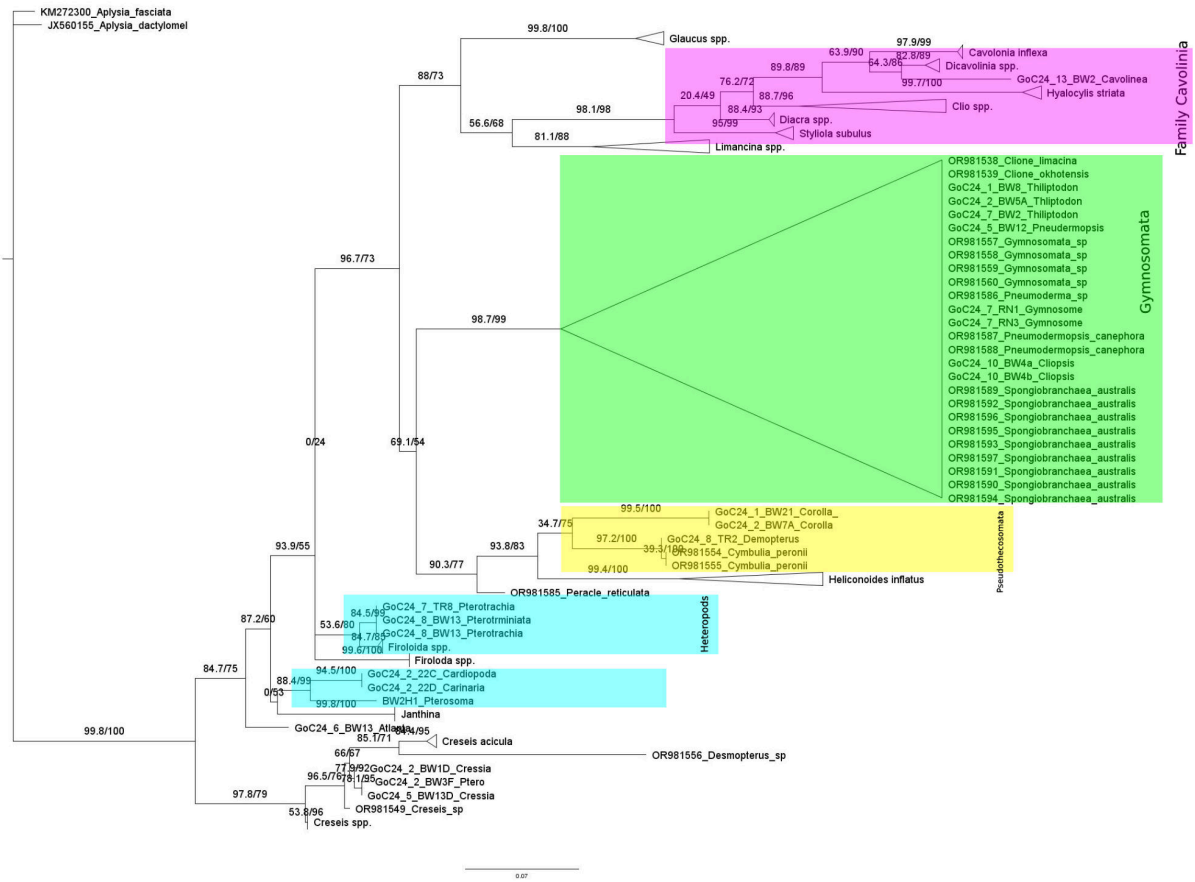
Supplementary Figures



Supplementary Figure 1. Bayesian and maximum likelihood estimated phylogenies for 1001 bp of 28S rRNA fragments. Coloured shapes indicate similar class , genera, or similar families.



Supplementary Figure 1. Bayesian and maximum likelihood estimated phylogenies for 328 bp of H3 rRNA fragments. Coloured shapes indicate similar class , genera, or similar families.