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ORIGINAL ARTICLE

Phylogenomic analysis and morphological data suggest left-right swimming behavior evolved prior to the origin of the pelagic Phylliroidae (Gastropoda: Nudibranchia)

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Abstract

Evolutionary transitions from benthic to pelagic habitats are major adaptive shifts. Investigations into such shifts are critical for understanding the complex interaction between co-opting existing traits for new functions and novel traits that originate during or post-transition. Gastropod mollusks are of particular interest in regard to benthic-pelagic evolutionary transitions, as shifts from benthic to pelagic habitats are uncommon. *Phylliroe* is one such pelagic lineage in Phylliroidae, a family of holoplanktonic nudibranchs with a highly aberrant morphology that appears to be adapted for life in the pelagic zone. However, the phylogenetic placement of this enigmatic group of pelagic nudibranchs has never been investigated. Here we present phylogenomic analyses which place *Phylliroe* within a group of nudibranchs called Dendronotida sensu stricto. We also discuss a subset of the morphological and behavioral features that *Phylliroe* shares with other closely related lineages (Dendronotidae, Tethyidae, and Scyllaeidae) and some that are unique to *Phylliroe*. Based on these data, and a literature review, we find a number of unique features found in *Phylliroe* that are adaptations to a pelagic environment, such as a fish-like body plan, highly reduced connective and muscular tissue in the notum, and elongated rhinophores. However, we were able to identify only a single commonality among *Phylliroe* and its closely related lineages, which is the presence of left-right swimming behavior. We further hypothesize that swimming behavior in this group likely represents an important trait that facilitated the transition from benthic to pelagic environments, and thus may provide evidence that major pelagic lifestyle transitions can rely on behavioral exaptations. These new insights into the origins of *Phylliroe* now provide a phylogenetic framework for testing for adaptations necessary for the benthic-pelagic transition in this group.

Keywords Nudibranch · Exaptation · Holoplanktonic · Swimming behavior · Cladobranchia

Introduction

Evolutionary transitions from benthic to pelagic habitats provide important insights into how organismal features evolve in relation to major adaptive shifts. Studies of such adaptive shifts allow researchers to identify exaptations crucial for the origin of such transitions, as well as how new adaptations advantageous for survival and reproduction in new habitats originate during or post-transition. Canonical examples of major adaptive shifts include the introduction of tetrapod vertebrates to terrestrial habitats during the Carboniferous, which required extensive changes in physiology, life history, and morphology (Long and Gordon 2004). Such water-land transitions are uncommon in most metazoan lineages (Vermeij and Dudley 2000). Adaptive shifts that allow a change from a benthic to pelagic life are probably more common, but also require extensive lifestyle modifications. Reduction of mass and body density (Chen 2000; Voskoboinikova et al. 2017) and elongate appendages known to be used as floating devices (Ce et al. 1979; Lalli and Gilmer 1989) are examples of such modifications.

Gastropod mollusks are of particular interest in regard to evolutionary transitions, as shifts to pelagic, terrestrial, and

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freshwater habitats have occurred in multiple groups (Klussmann-Kolb et al. 2008; Lalli and Gilmer 1989; Strong et al. 2007), though most lineages have diversified quite successfully among benthic marine habitats (Appeltans et al. 2012). However, the shift from benthic into a complete pelagic lifestyle is relatively rare within gastropods, and the only other mollusk group with such transitions are cephalopods (Lalli and Gilmer 1989). Gastropod lineages that have evolved to live in the open ocean include violet snails (Janthinidae), pteropods, heteropods (Pterotracheoidea, former Heteropoda), and several families and genera of nudibranchs (Scyllaeidae, Phylliroidae, *Glaucus*, *Fiona*) (Klussmann-Kolb and Dinapoli 2006; Lalli and Gilmer 1989). However, only the pteropods, heteropods, and Phylliroidae nudibranchs have transitioned to a completely pelagic existence. These transitions represent instances where lineages have overcome a number of unique pressures associated with living in the pelagic zone, including challenges maintaining buoyancy, finding adequate food sources, or carrying the weight of a calcium carbonate shell. All of these taxa have evolved adaptations—some more, some less—in response to such pressures, including modification of the foot for different tasks (especially in fully pelagic forms that are not attached to any drifting objects), as well as changes in reproductive modes, feeding mechanisms, color, and behavior (Lalli and Gilmer 1989). One advantage of studying benthic-pelagic transitions in gastropods is that these changes can be compared with those in the closely related cephalopods, whose pelagic species are relatively well understood by comparison (e.g., Doubleday et al. 2016; Ibáñez et al. 2019; Lansdell and Young 2007; Seibel et al. 1997).

Phylliroe is one such holoplanktonic genus in Phylliroidae, a family of pelagic nudibranchs that has long been considered a member of the suborder Cladobranchia (originally as Cladohepatica) (Bergh 1884; Bouchet et al. 2017). In contrast to other pelagic nudibranchs from the families Glaucidae and Fionidae, of Aeolidida (*s.l.*, and not following the Aeolidioidea *s.str.* meaning, as is outlined in WoRMS; Horton et al. 2019), individuals of Phylliroidae have a highly aberrant morphology, including a laterally compressed body shape that allows individuals to swim in the water column. Species of *Phylliroe* are also bioluminescent, a rarity among gastropod lineages (Herring 1987), and they are capable of swimming via lateral undulations that allow the animals to move forward at speeds of up to 15 cm/s (Lalli and Gilmer 1989). Development has only been investigated in one species, *Phylliroe bucephala* Lamarck, 1816, whose veliger larvae and juveniles exhibit brood parasitism within the bell of the hydromedusa *Zanclea costata* Gegenbaur, 1857, and feed on the ring and radial canals and manubrium of the animal. After reaching the size of the medusa (usually then larger than 10 mm), the slug starts to consume the rest of the medusa, which remains attached to the foot of the slug for some time (Ankel 1952; Martin and Brinckmann 1963). Adult specimens have also been shown to prefer *Zanclea* medusae over offered Siphonophora (Martin and Brinckmann 1963), but feeding on both by adults has been recorded (Schmekel and Portmann 1982).

Beyond the assignment of Phylliroidae to Cladobranchia, there have been no attempts to resolve the phylogenetic placement of Phylliroidae, leading to uncertainty surrounding the origins of a pelagic lifestyle within this group. Here, we provide evidence for the phylogenetic placement of *Phylliroe* within Dendronotida, and discuss available information on morphological and behavioral features that unite members of this genus with non-Phylliroidae cladobranchs. We also discuss the features unique to *Phylliroe* that likely facilitated the transition to a pelagic existence or evolved in response to the various selective pressures found in the pelagic marine environment.

Methods

Organismal sampling

For molecular analysis, a mantle tissue sample was obtained from a specimen of *Phylliroe bucephala* collected on a blue water dive in the Gulf of California (< 15 m depth) on 24 June 2010 (collector: Meghan Powers; SRR12006002) and a head tissue sample from *Pteraeolidia ianthina* (Angas, 1864) (SRR12006001; Voucher, USNM 1461134; Orrell 2016) was obtained from Cairns Marine (Queensland, Australia) collected on 11 January 2016 (Australia, precise locality unknown). Both specimens were preserved in RNA later and identified by their respective collectors (and confirmed with molecular data). We generated RNA-Seq data for each of these samples, and downloaded data for 39 additional Cladobranchia species from the NCBI Sequence Read Archive (SRA), including a second transcriptome for *Phylliroe bucephala*. We also obtained data from two outgroup species from the SRA: *Pleurobranchaea californica* MacFarland, 1966 and *Prodoris clavigera* (Thiele, 1912). Specimen data and SRA numbers are listed in Tables S1, 2, 3, 4.

RNA extraction and sequencing

RNA extraction was performed using a modified TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) protocol established previously (Goodheart et al. 2015, 2017). Total RNA samples were submitted to the DNA Sequencing Facility at University of Maryland Institute for Bioscience and Biotechnology Research, MD, USA, where quality assessment, library preparation, and sequencing were performed. The facility completed RNA quality assessment with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and used the Illumina TruSeq RNA Library Preparation Kit v2 (Illumina, San Diego, CA, USA) for library preparation with 200 bp inserts. We sequenced 100 bp, paired-end reads with an Illumina HiSeq1000 sequencer (Illumina, San Diego, CA, USA).

Quality control and assembly of reads

Reads that failed to pass the Illumina "chastity" quality filter (under default parameters) were excluded from our analyses. Samples were de-multiplexed, and adapters were removed, at the sequencing facility prior to us obtaining the reads. Reads passing the quality filter were assembled de novo using Trinity version 2.4.0 (Grabherr et al. 2011) with default settings, which required assembled transcript fragments to be at least 200 bp in length. Assemblies were evaluated with a custom perl script (https://github.com/goodgodric28/phylliroe_phylogenomics; https://doi.org/10.5281/zenodo.3875677) and the Benchmarking Universal Single-Copy Ortholog assessment tool (BUSCO 3.0.2) (Simão et al. 2015)

Orthology assignment

Translated transcript fragments were organized into orthologous groups corresponding to a custom gastropod-specific core-ortholog set of 3854 protein models (Goodheart et al. 2015) using HaMStR version 13.2.6 (Ebersberger et al. 2009), which in turn used FASTA version 36.3.6 (Pearson and Lipman 1988), GeneWise version 2.2.0 (Birney et al. 2004), and HMMER version 3.1b2 (Eddy 2011). Following previous methods (Goodheart et al. 2015, 2017), the E-value cutoff defining an HMM hit was set to 1e-05 (the HaMStR default is 1.0), and retained only the top-scoring quartile of hits. This is intended to reduce the number of highly divergent, potentially paralogous sequences returned by this search.

Construction of data matrix and paralogy filtering

Protein sequences in each orthologous group were aligned using MAFFT version 7.407 (Katoh and Standley 2013) and converted the protein alignments to corresponding nucleotide alignments with a custom Perl script (https://github.com/goodgodric28/phylliroe_phylogenomics; https://doi.org/10.5281/zenodo.3875677). A maximum likelihood tree was inferred by using RAxML-NG version 0.9.0 (Kozlov et al. 2019) and the model GTR+G for each orthologous group where at least 75% of the taxa were present (703 orthologous groups). The majority-rule consensus tree was given as input to PhyloPyPruner version 0.9.3 (Thalén 2018) for paralogy filtering (parameters: –min-taxa 31 –trim-lb 5 –min-support 70 –min-gene-occupancy 0.3 –outgroup Pleurobranchaea_californica –root midpoint –prune LS). This process resulted in 292 orthologous groups eligible for inclusion in our data matrices, each with only a single sequence from any sample. Individual orthologous group alignments were concatenated with the script catfasta2phyml.pl (Nylander 2013), and codons not represented by sequence data in at least four taxa then removed.

Phylogenetic analyses

For phylogenetic analysis, the final nucleotide data matrix from transcriptome data was partitioned by assigning independent model parameters and rates to the three types of codon positions. For each partition, a general time reversible (GTR) substitution model (Tavaré 1986) was assigned applying a rate heterogeneity model with a gamma distribution (+G) (Yang 1993). One phylogenetic analysis was conducted using RAxML-NG (Kozlov et al. 2019) using default settings (and the –all flag) and 30 parsimony-based starting trees. For our analysis, 1000 bootstrap replicates were generated and a best tree search was performed with 30 search replicates. We also ran a Bayesian phylogenetic analysis with MrBayes version 3.2.7a (Ronquist et al. 2012) through the CIPRES Science Gateway (Miller et al. 2010), which uses the Extreme Science and Engineering Discovery Environment (XSEDE) (Towns et al. 2014). The BEAGLE library (Ayres et al. 2012) was also implemented in this analysis to make use of highly parallel processors and speed up the core calculations for the phylogenetic analysis. We produced two separate runs for this analysis (with 4 chains and 5 million generations each), and sampled every 100 generations, with the first 25% of trees discarded as burn-in. MrBayes default settings were retained for the rest of the analysis parameters. Convergence was assessed in Tracer version 1.7.1 (Rambaut et al. 2018).

Morphological analysis

A specimen of *Phylliroe bucephala* provided by the Museum für Naturkunde in Berlin was examined histologically (ZMB/Moll 260,007) (length: 20 mm, height: 13 mm, preserved in formaldehyde of unknown concentration), as were specimens of *Melibe leonina* (Gould, 1852), *Melibe engeli* Risbec, 1937, *Crosslandia viridis* Eliot, 1902, and *Dendronotus frondosus* (Ascanius, 1774) (from the research collection of HW, preserved in 4–10% formaldehyde/seawater). The specimens were embedded in hydroxyethyl methacrylate for serial sectioning (2.5 μm) (Kulzer ®7100). Sections were stained with toluidine blue and investigated under a ZEISS AXIO, Imager.Z2M with ZEISS AxioCam HRc and the software AxioVision, Release 4.8.1 (11-2009).

Results

Transcriptome assembly and data matrix construction

The raw number of RNA-Seq reads for the new *Phylliroe bucephala* (A) and *Pteraeolidia* transcriptomes was roughly

45.3 and 48.8 million, respectively (Table S1). Once assembled, the number of contigs per sample ranged from 67,793 to 274,251 (mean = 137,418; Table S2) and completeness according to BUSCO scores ranged from 40.6 to 96.0% (*P. bucephala* A, 67,793 contigs, 40.6% complete; *P. ianthina*, 224,238 contigs, 87.7% complete). N50 ranged from 496 to 1609 bp (mean = 1028 bp; *P. bucephala* A, 496 bp; *P. ianthina*, 1327 bp). The number of transcript fragments from each assembly that matched the HaMStR database ranged from 893 to 1754 (mean = 1332; *P. bucephala* A, 1138; *P. ianthina*, 1772). However, the number of matches to unique orthologous groups ranged from 508 to 1086 (mean = 902; *P. bucephala* A, 577; *P. ianthina*, 1072) per assembly. The mean length of transcript fragment matches to the HaMStR database across all assemblies was 289 ± 75 amino acids (*P. bucephala* A, 106; *P. ianthina*, 346). HaMStR results are presented in Table S3. The final data matrix consisted of 292 orthologous groups and 454,608 nucleotide positions (0.006% ambiguous characters, 51.4% complete). Coverage for individual species ranged from 90,807 sites (20%, *Phylliroe bucephala* A) to 310,872 sites (68.4%, *Catriona columbiana*), with an average of 233,502 (Table S4) across all species.

Phylogenetic results

The results of our phylogenetic analysis (Fig. 1) are consistent with previous work utilizing the data from the SRA used here (Goodheart et al. 2015, 2017). The two *Phylliroe bucephala* samples included in our analysis form a clade (bootstrap support (BS) = 100, posterior probability (PP) = 1.0) within Dendronotida sensu stricto and are sister to a clade containing *Melibe* (Tethyidae) and *Scyllaea* (Scyllaeidae) (BS = 99, PP = 1.0). The *Melibe* + *Scyllaea* + *Phylliroe* clade is sister to *Dendronotus* (Dendronotidae) (BS = 100, PP = 1.0). In regard to the other new transcriptome in our analysis, *Pteraeolidia ianthina* is placed as the sister lineage to a clade containing other Facelinidae (BS = 100, PP = 1.0).

Convergence statistics supported convergence of the Bayesian analysis by multiple measures (MrBayes convergence statistics files in dryad repository; https://doi.org/10.6075/J04J0CHG). The average approximate topology effective sample sizes (ESS) were 5036 and 5178, well over the recommended ESS levels (> 200; Drummond et al. 2006). The parameter traces show well-mixed chains with the ESS for all parameters estimated at greater than 300. The topology of these trees is 100% consistent with the maximum likelihood topology from our RAxML-NG analysis.

Morphological and histological analysis

Phylliroe shares a number of features with most other members of Dendronotida (including Scyllaeidae), such as the lack of an oral hood (Fig. 2a, d, g, j), a smooth velum without tentacles (Fig. 2a, d, j), and a thickened prostate that is a part of the vas deferens (Table 1). Members of this genus also share a few derived features with Scyllaeidae, including an undivided velum (Fig. 2d, j), teeth serrated on both sides (not analyzed here, but see Schmekel and Portmann 1982), and a gonad in 2–4 spherical lobules. In *Phylliroe*, these comprise a large central male part, with separate female follicles surrounding the male part (Fig. 3a). Small longitudinal muscle strips are arranged regularly along the lateral sides, and are restricted to just beneath the epidermis (Fig. 3e), thus reducing tissue to a great extent and rendering the animal completely transparent. We can see a similar trend in *Melibe* and *Crosslandia*, which also have reduced mantle tissue mainly at the lateral and dorsal body parts and muscles that are arranged in bundles. As is typical for cladobranchs, the investigated specimen also has a glandular stripe on the right side of the body starting behind the genital opening (Fig. 3g) (Wägele 1998). The animal sectioned here also showed remnants of a medusa consumed before collection (species unknown). Unexploded cnidocysts of the stenotele type were still visible in the digestive gland (Fig. 3d).

Phylliroe also has some unique and striking features in comparison with the other dendronotids evaluated here. The rhinophores exceed the length of any other heterobranch known so far, reaching until the posterior half of the body (Fig. 2). The notum margin separating the dorsal body from lateral body does not exist. The body is laterally compressed rendering the animal taller than it is broad (Fig. 2j). This has an impact on the organ systems, in so far that the main right and left digestive gland branches are shifted to dorsal and ventral positions (Fig. 3b). No distinct foot, with thxc 1e typical underlying mucus cells, can be seen; it has fused with the notum completely in most areas (Fig. 2b, e, h, k). The posterior of the animal is transformed into a tail-fin-like structure. The stomach is without a cuticularized layer, plates, or teeth (Fig. 3c) (a character that can be found in few other dendronotid species). The penis is located in a separate sac-like structure attached to the penial sheath (Fig. 2l) and is not a continuous muscular part of the vas deferens (Fig. 2c, f, i) (Schmekel and Portmann 1982). The vas deferens, before entering the penial sheath, is covered with tuberculate structures, not known from any other nudibranch taxon (Figs. 2l and 3f). Most strikingly, *Phylliroe* has nearly no connective tissue and muscle cells in the visceral cavity and the body wall (Fig. 3b, e). Another interesting feature is the highly reduced number of mucus cells in the epidermis (Fig. 3b, g). In some areas, we could observe globular structures that probably represent the bioluminescent organs, mainly distributed along the dorsal and ventral rim, as well as in the posterior lateral parts of the body. Unfortunately these structures were not well preserved (Fig. 3h). Table 1 summarizes a few of the main

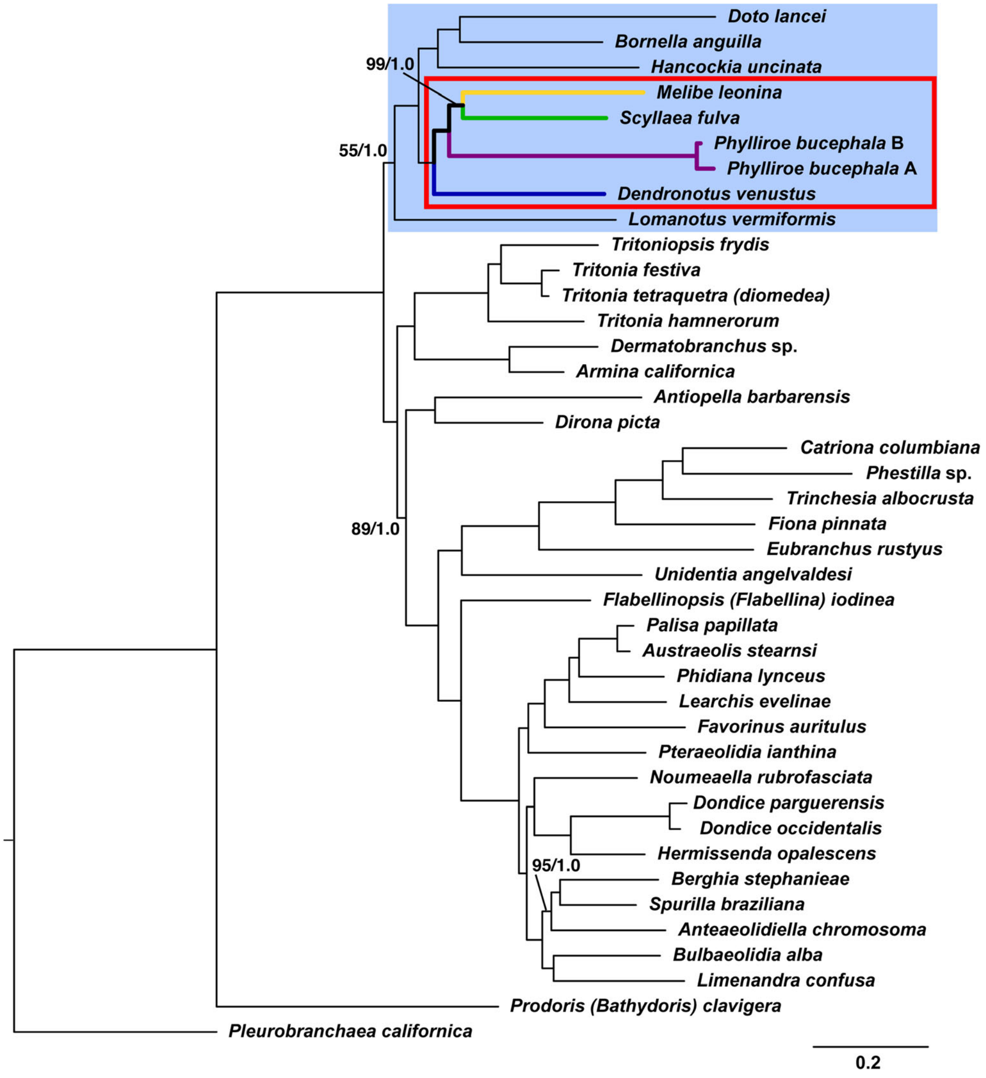

Fig. 1 Maximum likelihood phylogeny of Cladobranchia from RAxML-NG using a concatenated nucleotide matrix of 292 genes partitioned by codon position. All nodes with no support values indicated have 100% bootstrap support and a posterior probability of 1.0 in our analyses. The blue box indicates Dendronotida sensu stricto and the red outline shows the closest relatives to *Phylliroe* in our analysis (*Melibe leonina*, Tethyidae; *Scyllaea fulva*, Scyllaeidae; *Dendronotus venustus*, Dendronotidae), highlighted further in Fig. 2

characters that either are unique to Phylliroidae or that members of this family share with Scyllaeidae, Tethyidae, and/or Dendronotidae.

Discussion

The phylogenomic analyses presented here (Fig. 1) are consistent with prior transcriptome-based phylogenies for Cladobranchia (Goodheart et al. 2015, 2017) which indicate that Dendronotida *s.s.* (excluding Tritoniidae) forms the earliest branching clade in the Cladobranchia tree (though with lower bootstrap support than prior analyses). Regardless, it is clear that Phylliroidae falls well within this group, including the closely related Tethyidae as the sister lineage to Scyllaeidae, as well as Dendronotidae. Although we were unable to include *Cephalopyge* in either our morphological or molecular analysis (the other genus within Phylliroidae), we are confident of the assignment of this genus to Phylliroidae due to the overall similarity in morphology between *Cephalopyge* and *Phylliroe* with few differences (e.g., the position of the penis, see Steinberg 1956).

In addition to the morphological data presented here, we also evaluated available information for other ecological, behavioral, and morphological characters to assess whether any represent synapomorphies for the clade containing Phylliroidae, Dendronotidae, Scyllaeidae, and Tethyidae. The only apomorphic feature that appears to unite this clade is left-right swimming behavior, characterized by body flattening in the sagittal plane and repeated, alternating bending left–right near the midpoint of the body axis (Newcomb et al. 2012). The behavior is characterized as left-right undulation in Phylliroidae and considered a refinement of the left-right flexion found in the other three families. Other species within Dendronotida show a similar swimming behavior, including *Bornella* and *Lomanotus*, but the presence of swimming in those lineages may be independent (Newcomb et al. 2012). Furthermore, the dorso-ventral swimming behavior found in

Table 1 Morphological similarities and differences between *Phylliroe* and its closest lineages, including Scyllaeidae, Tethyidae, and Dendronotidae. Coding scheme based on prior characterization of ancestral characters in Dendronotida (Wägele and Willan 2000): 0, ancestral; 1, derived; 2, additional derived state

	Plesiomorphic	Differences (apomorphies)	*Phylliroe*	Scyllaeidae	Dendronotidae	Tethyidae
1	No oral hood	Head in a hood (A)	0	0	0	1
2	Smooth velum without small tentacles	Velum with many separated and small tentacles	0	0	0	1
3	Velum not divided	divided	1	1	1	0
4	Rhinophores lamellate	Rhinophores smooth	1	0	0	0
5	Rhinophoral sheath smooth	Rhinophoral sheath with oblique lamella	0	1	0	1
6	Gill-like structures (branchial tufts) absent on the notum	Gill-like structures present on notum	0	1	1	1
7	Notum with cerata-like structures as typical for many Cladobranchia	Simple notum margin with no appendages	1	0	0	0
8	Round cross-section	Laterally compressed	1	0	0	0
9	Radula present	Radula absent	0	0	0	1
10	First lateral smooth or with denticles only on one side	First lateral with denticles on both sides	1	1	0	Not applicable
11	Cuticular structures in the stomach present	Cuticular structures in the stomach absent	1	0, with plates	0, only cuticular layer	0, usually with plates
12	Digestive gland rather compact with no branches extending to the notum margin	Digestive gland with processes reaching into dorsal appendages	0	0	1	1
13	Prostate is a thickened part of the vas deferens	Prostate is separate forming a ball-like structure at the proximal part of vas deferens	0	0	1	1
14	Gonad compact	Gonad separated in many ball like structures (1) Gonad in 2 to 4 spherical lobules (2) (additionally in *Hancockia* and *Lomanotus*)	2	2	0	1
15	Penis continuous with vas deferens and sitting in the penial sheath	Penis located in a sac-like structure attached to vas deferens at the transition into penial sheath	1	0	0	0

the nudibranch family Tritoniidae (Newcomb et al. 2012), which has traditionally been placed within Dendronotida, is distinct from left-right swimming behavior, which further supports the exclusion of Tritoniidae from Dendronotida *s.s.* The only other group of slugs with a pelagic lifestyle in Dendronotida *s.s.* is the genus *Scyllaea* Linnaeus, 1758 (Scyllaeidae), but these animals are not found drifting in the water column. Instead they are usually attached to floating algae (e.g., *Sargassum*), which they use as substrate. *Scyllaea* also swims with lateral threshing, but their external appearance resembles much more a typical dendronotoidean nudibranch (Thompson and Brown 1981). Interestingly, all scyllaeid genera (*Scyllaea*, *Notobryon*, and *Crosslandia*) possess a medio-dorsal posterior crest, which is used as a "paddle" to swim by vigorously waving it from side to side (Rudman 2000) (observation for *Crosslandia* by HW). This is similar to the tail-fin-like structure and undulation in *Phylliroe*. Given that left-right swimming behavior represents the only feature that unites this clade of nudibranchs, we hypothesize that it was an important exaptation for the ultimate transition of Phylliroidae into the pelagic zone.

Phylliroe also seems to have evolved further adaptations to a pelagic lifestyle, and is able to drift and swim in the water without being attached to secondary hard substrate or floating food organisms. The most important feature appears to be their fish-like body plan, which certainly increases effectiveness in swimming. But other adaptations are likely necessary for a pelagic existence, including a different arrangement of the digestive gland system due to a flattened shape, the highly reduced connective and muscular tissue in the notum, the lack of cuticle in the stomach, which helps to keep buoyancy, and the elongated rhinophores, which may be used as floating devices, like appendages in many other small planktonic organisms, such as the planktonic young of some pipefishes (Ce et al. 1979). Additional features shared with other organisms that are found in pelagic environments include the reduction of the foot (in gastropods) and bioluminescence (Lalli and Gilmer 1989). Bioluminescence is a particularly common feature of pelagic life forms, as nearly 80% of all metazoan taxa living in the pelagic zone produce bioluminescence (Haddock et al. 2010; Martini and Haddock 2017). However, few gastropods are known to produce bioluminescence, and most are

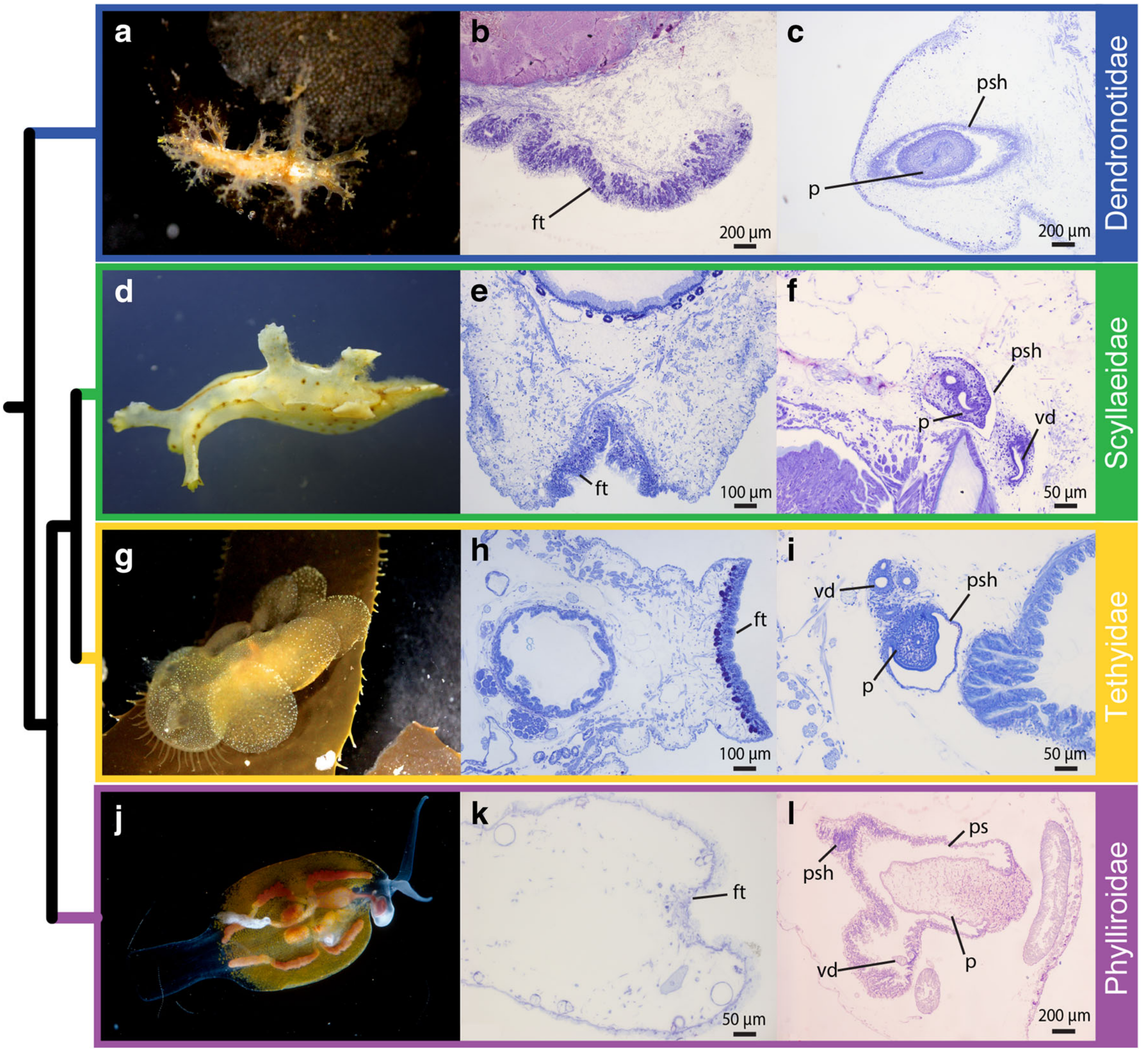

Fig. 2 Subtree of *Phylliroe* and its closest relatives from Dendronotida *s.s.*, Dendronotidae, Scyllaeidae, and Tethyidae. Images from life animals and histological slides. **a** *Dendronotus venustus*. **b**, **c** *Dendronotus frondosus*. **d–f** *Crosslandia viridis*. **g–i** *Melibe leonina*. **j–l** *Phylliroe bucephala* (photo credit live animal: Steve Haddock). **b**, **e**, **h**, **k** Histological sections of the foot. **c**, **f**, **i**, **l** Histological sections of the penis and associated structures for each species. ft., foot; p, penis; ps, penial sac; psh, penial sheath; vd, vas deferens

not pelagic (Herring 1987). In nudibranchs, bioluminescence is only produced in two other distantly related genera, *Kaloplocamus* and *Plocamopherus* (Polyceridae: Triophinae), as a defense when disturbed (Vallès and Gosliner 2006). Wägele et al. (2006) described several globular structures in the appendages of *Plocamopherus ceylonicus* (Kelaart, 1858), which could represent the luminous structures. The structures in our *Phylliroe* specimen are similar in shape but appear to differ in the overall contents of the cells. However, these structures are not well preserved in this specimen of *Phylliroe* and should be re-evaluated in the future. In the ocean, light emission by organisms is considered one of the most effective ways to communicate, including finding mating partners, attracting prey, or escaping predators (Martini et al. 2019). In *Phylliroe*, the arrangement of the bioluminescent structures along the dorsal and ventral body rim, along with its transparency, leads to the disruption of body lines and thus likely disguises the animal from potential predators, as has been shown in other pelagic organisms (Johnsen et al. 2004).

All closely related taxa investigated, despite their morphological adaptations with regard to swimming and mobility, still show the typical slug-like structure with a distinct foot still recognizable. However, these taxa are not floating freely in the water column, and are usually attached to their drifting food organism or a benthic hard substrate. With regard to other traits, the more closely related families Scyllaeidae and Tethyidae show a similar tendency to reduce connective tissue and muscle fibers in the notum, which cannot be seen in the more distantly related Dendronotidae. *Phylliroe* is simply the most extreme in this regard, appearing as a nearly transparent organism.

Even when compared with more distantly related gastropod taxa living in the pelagic zone, *Phylliroe* can still be

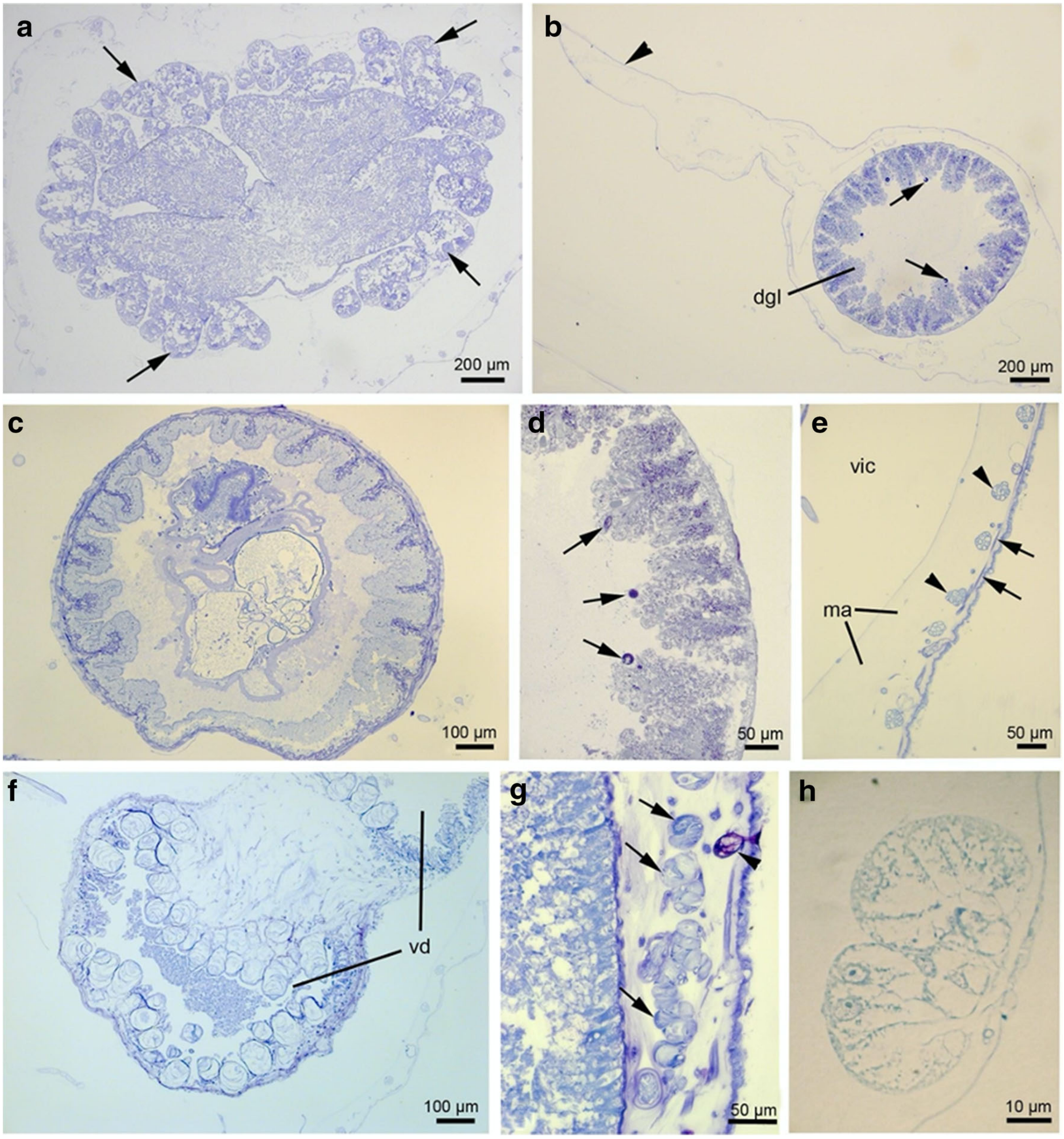

Fig. 3 Anatomical details of *Phylliroe bucephala*. **a** Cross section of one of the 4 gonad lobules. Note the male part in the middle of the lobule, and the female part at the periphery (arrows). **b** Cross section of dorsal hind part with dorsal digestive gland branch. Note the lack of mucus glands in the epidermis. Arrows indicate cnidocysts, arrowhead points towards dorsal fin-like mantle. **c** Cross section of stomach, with remnants of food. **d** Digestive gland epithelium with 3 cnidocysts (arrows). **e** Cross section of longitudinal muscle bundles (arrowheads). Arrows indicate circular muscle fibers. **f** Cross section of distal part of vas deferens before the sac housing the penis enters. **g** Glandular stripe, composed of few internal lying gland cells (arrows). Note the single epithelial mucus cell in the epidermis (arrowhead). **h** One globular structure from posterior part of the body, probably responsible for bioluminescence. Abbreviations: dgl, digestive gland; ma, mantle; vd, vas deferens; vic, visceral cavity.

considered unique. *Glaucus* (Nudibranchia: Aeolidida) is a neustonic nudibranch usually attached to its major food source, the floating *Velella* (Hydrozoa). The animal is dorso-laterally flattened, and the cerata are broadened, like large wings, in contrast to the fish-like shape in *Phylliroe* (Fig. 2). The neustonic janthinids produce bubble rafts from mucus secretions on the foot (Lalli and Gilmer 1989) that allow them to float just beneath the surface of the water. Importantly, neither of these lineages is truly holoplanktonic. Pteropods, however, are considered true holoplanktonic forms, and these species also show adaptations to free swimming in the water column (e.g., a greatly reduced shell and foot). These gastropods also have two parapodial wings for swimming, which they use both for maintaining buoyancy and as an escape response (Borrell et al. 2005; Gilmer and Harbison 1986; Satterlie et al. 1990). However, there are no species within Pteropoda that show similar re-arrangements of the anatomy to those seen in *Phylliroe*.

Based on our phylogeny, it is clear that left-right swimming behavior evolved well before the transition to a pelagic lifestyle. Though swimming behavior is often used as an escape response in closely related taxa such as *Dendronotus* and *Melibe leonina* (Lawrence and Watson 2002; Sakurai et al. 2011), as well as in the more distant *Tritonia* (Wyeth and Dennis Willows 2006), *Melibe leonina* is known to also swim spontaneously (Lawrence and Watson 2002). Our results indicate that behavioral exaptations such as swimming behavior may be critical for major habitat transitions, similar to the alternating "gait" found in the tetrapod transition (Lister 2014). Furthermore, with these new insights into the benthic origins of Phylliroidae, we now have a framework in which to test possible mechanisms underlying further morphological, molecular, and behavioral adaptations necessary for major benthic-pelagic transitions.

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Authors' contributions JAG and HW conceived the study and participated in study design; HW collected morphological data; JAG collected molecular data; JAG and HW participated in morphological and molecular data analysis, helped draft the manuscript, and gave final approval for publication.

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Data availability Aligned data matrices, tree files, and images for morphological data are available in the Dryad Digital Repository (https://doi.org/10.6075/J04J0CHG). Newly sequenced transcriptome data are available in the NCBI Sequence Read Archive (SRR12006001 & SRR12006002; see Tables S1, 2, 3, 4).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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