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Phylogenomic reconstruction of Solenogastres (Mollusca, Aplacophora) informs hypotheses on body size evolution

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ABSTRACT

Body size is a fundamental characteristic of animals that impacts every aspect of their biology from anatomical complexity to ecology. In Mollusca, Solenogastres has been considered important to understanding the group's early evolution as most morphology-based phylogenetic reconstructions placed it as an early branching molluscan lineage. Under this scenario, molluscs were thought to have evolved from a small, turbellarian-like ancestor and small (i.e., macrofaunal) body size was inferred to be plesiomorphic for Solenogastres. More recently, phylogenomic studies have shown that aplacophorans (Solenogastres + Caudofoveata) form a clade with chitons (Polyplacophora), which is sister to all other molluscs, suggesting a relatively large-bodied (i.e., megafaunal) ancestor for Mollusca. Meanwhile, recent investigations into aplacophoran phylogeny have called the assumption that the last common ancestor of Solenogastres was small-bodied into question, but sampling of meiofaunal species was limited, biasing these studies towards large-bodied taxa and leaving fundamental questions about solenogaster body size evolution unanswered. Here, we supplemented available data with transcriptomes from eight diverse meiofaunal species of Solenogastres and conducted phylogenomic analyses on datasets of up to 949 genes. Maximum likelihood analyses support the meiofaunal family Meiomeniidae as the sister group to all other solenogasters, congruent with earlier ideas of a small-bodied ancestor of Solenogastres. In contrast, Bayesian Inference analyses support the large-bodied family Amphimeniidae as the sister group to all other solenogasters. Investigation of phylogenetic signal by comparing site-wise likelihood scores for the two competing hypotheses support the Meiomeniidae-first topology. In light of these results, we performed ancestral character state reconstruction to explore the implications of both hypotheses on understanding of Solenogaster evolution and review previous hypotheses about body size evolution and its potential consequences for solenogaster biology. Both hypotheses imply that body size evolution has been highly dynamic over the course of solenogaster evolution and that their relatively static body plan has successfully allowed for evolutionary transitions between meio-, macro- and megafaunal size ranges.

1. Introduction

Body size change over evolutionary time is incredibly important as body size has consequences for all aspects of an organism's biology such as distribution, ecological function and morphology (Peters, 1983; Schmidt-Nielsen, 1984). Miniaturization, or extreme phylogenetic size decrease, is an important trend in the evolution of body size (Hanken & Wake, 1993; Rundell & Leander, 2010). This evolutionary process is exemplified in minute benthic metazoans small enough to pass through a sieve with a 1 mm mesh size, collectively referred to as meiofauna (Higgins & Thiel, 1988; Ptatscheck et al., 2020; Swedmark, 1964). As meiofauna is an operational definition of body size, most lineages considered to be meiofaunal are generally in the range of 60 μm to 2 mm in size (Rundell & Leander, 2010). Often in the literature, the term meiofauna is used to describe the small metazoans that live in the interstitial spaces between sand grains. Marine sediments are some of the most widespread and taxonomically diverse habitats, with representatives from 23 phyla (Kennedy & Jacoby, 1999; Swedmark, 1964).

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This includes species from ancestrally small-bodied phyla such as Gastrotricha, Kinorhyncha, and Gnathostomulida and some representatives of primarily large-bodied phyla, such as Mollusca and Echinodermata, that have either evolved to attain a smaller adult size or are "temporary meiofauna" early in their life history. Although meiofaunal lineages are represented across the metazoan tree, some commonalities between these taxa as a potential consequence of microscopic size include direct development, relative simplification when compared to closely related macroscopic species often converging on a vermiform morphology, presence of adhesive organs, and reinforcement of the body wall through a well-developed cuticle or spicules (Laumer et al., 2015; Swedmark, 1964). The presence of meiofaunal lineages across Metazoa therefore presents an interesting context in which to study major themes of evolution such as convergence and reduction (Laumer et al., 2015; Rundell & Leander, 2010).

The phylum Mollusca is the second most diverse metazoan phylum and shows incredible variation in body size and morphology. Of the eight classes of Mollusca, six (excluding Cephalopoda and Monoplacophora) have species considered meiofaunal for either a portion or the entirety of their life histories. Represented in the permanent meiofauna are species from Gastropoda, Solenogastres, Caudofoveata, and potentially Polyplacophora. Solenogastres (=Neomeniomorpha) Gegenbaur, 1878 is one of the two classes of aplacophoran molluscs that with chitons (Polyplacophora) form the sister group to all other molluscs, Aculifera. With comparably less variation in morphology to other molluscan groups, Solenogastres is characterized by a vermiform body shape, a narrow ventral foot, the lack of a shell, and presence of aragonitic spines and/or scales called sclerites. There are >300 formally described species of Solenogastres that are currently classified in 24 families and four orders (Pholidoskepia, Cavibelonia, Sterrofustia, and Neomeniamorpha; (Aplacbase, 2023; Cobo & Kocot, 2021; García-Álvarez & Salvini-Plawen, 2007; Todt, 2013). These exclusively marine animals occur primarily along the lower continental slope and shallow bathyal depths with fewer species described from shallow waters and the deep sea (Bergmeier et al., 2016, 2019; Cobo & Kocot, 2020; García-Álvarez et al., 2000).

While most solenogaster species are a few millimeters in length, i.e., visible to the naked eye and can be classified as macrofaunal (defined here as animals between 2 mm and 1 cm), published reports of body size range from that of the megafaunal (defined here as >1 cm) *Epimenia* Nierstrasz, 1908, which can grow to at least 30 cm (reviewed by Todt, 2013), to the meiofaunal (defined here as minute animals <2 mm) *Meiomenia* Morse, 1979, which are less than 1 mm (reviewed by García-Álvarez et al., 2000). Published records of meiofaunal solenogasters are largely from well-sampled areas around marine stations (e.g. Roscoff, France, Friday Harbor, USA, and Bermuda), but records of undescribed species reveal that they occur in clean, coarse sand worldwide (Bergmeier et al., 2016; García-Álvarez et al., 2000; Klink et al., 2015; Kocot & Todt, 2014; Morse & Norenburg, 1992; Neusser et al., 2021; Salvini-Plawen, 1985b, 1986; Salvini-Plawen, 1967; Vortsepneva et al., 2021; own unpublished records). Given their worm-shaped body and ability to contract, which allows them to squeeze through the mesh of a relatively fine sieve, Bergmeier & Jörger (2020) considered 23 species in nine families to be meiofaunal. Of these, ten are described from the interstitial habitat (Bergmeier & Jörger, 2020; García-Álvarez et al., 2000; Salvini-Plawen, 1985b) although it is difficult to confidently state whether these animals truly live in the interstitial habitat or if they glide along the sediment surface. However, morphological work has highlighted potential adaptations to the interstitial habitat. For example, members of the exclusively meiofaunal family Meiomeniidae Salvini-Plawen, 1985 have oval or rounded scales covering the body, triangular sclerites oriented towards the anterior, a pedal commissural sac, and an adhesive terminal gland making them particularly adapted to the interstitial habitat (Bergmeier et al., 2016; García-Álvarez et al., 2000). Oval, scale-like sclerites with a central depression are thought to allow for the movement of the scale in multiple directions as the animal pushes through the sand grains while anteriorly positioned triangular sclerites are thought to aid in fluid dispersal (García-Álvarez et al., 2000). The pedal commissural sac is thought to be a gravity-sensing organ, though ultrastructural differences clearly indicate that the structure is not homologous to the molluscan statocyst (Hazprunar, 1986; Bergmeier et al., 2016).

Meiofaunal solenogasters present an interesting framework in which to study morphological adaptations to an interstitial lifestyle and reduction in body size, but a phylogenetic framework including representation of the diverse taxa that are meiofaunal is needed. Traditional hypotheses on the evolution of Solenogastres viewed the small-bodied solenogaster clade Pholidoskepia as the earliest branching lineage because of similarities to a hypothesized molluscan ancestor such as a thin cuticle, scale-like sclerites, and morphology of the ventral foregut glands (Salvini-Plawen, 1978). This hypothesis was first tested using a phylogenetic framework by Salvini-Plawen (2003) based on a large-scale cladistic analysis of 53 morphological characters with weighted and unweighted parsimony. The relative homogeny in the solenogaster body plan and suspected high degree of convergent evolution limited choice of characters for this analysis and therefore resulted in a lack of resolution (Salvini-Plawen, 2003). Further, character coding in this analysis was problematic as there are multiple issues of character compounding and non-additive binary coding, both of which can result in systematic errors (Brazeau, 2011). Kocot et al. (2019) performed the first large-scale molecular phylogenetic analysis of Aplacophora sampling 27 aplacophoran taxa, 21 from Solenogastres and six from Caudofoveata. For solenogasters, seven families with at least one representative of each order were included. Cavibelonia, a traditionally recognized order of Solenogastres characterized by a thick cuticle, hollow acicular sclerites, and generally macrofaunal to megafaunal body size was recovered polyphyletic, with the relatively large-bodied cavibelonian family Amphimeniidae Salvini-Plawen, 1972, rather than the small-bodied order Pholidoskepia, as the sister taxon of all other Solenogastres (Kocot et al., 2019). The distribution of large-bodied taxa throughout Solenogastres was interpreted as possible evidence for a large-bodied animal with hollow acicular sclerites as the last common ancestor. Although this study was the first to use phylogenomic techniques to investigate solenogaster evolution, it was limited in its sampling of solenogaster diversity. Only seven of 24 families were sampled with sampling biased towards larger species from which enough RNA could be obtained for the cDNA preparation technique used. Notably, just four meiofaunal species were sampled by Kocot et al. (2019): the pruvotinid *Hypomenia sanjuanensis* Kocot & Todt, 2014, the macellomeniid *Macellomenia schanderi* Kocot & Todt, 2014, the dondersiid *Micromenia fodiens* Schwabl, 1955, and a specimen identified as the meiomeniid *Meiomenia swedmarki* Morse, 1979. Bergmeier et al., (2019,2021) investigated the systematics of deep-sea solenogasters from the Northwest Pacific based on 16S and COI from 193 individuals. Interestingly, the recovered topology (albeit with limited support for many deeper nodes) reflected the findings of the transcriptome-based analysis of Kocot et al. (2019) with Amphimeniidae as the sister taxon of all other sampled solenogasters (Bergmeier et al., 2019). While this data set is based on broader taxon sampling including better representation of small-bodied taxa, it was limited to deep-sea taxa, lacking representatives of shallow-water and interstitial habitats.

Studies of solenogaster phylogeny to date have presented a framework that suggests multiple independent adaptations to an interstitial lifestyle and a possible large-bodied ancestor (Bergmeier et al., 2019; Kocot et al., 2019). However, it was recently shown that the specimen identified as *Meiomenia swedmarki* by Kocot et al. (2019) was actually an undescribed species belonging to the order Pholidoskepia, and therefore this work actually lacked representation of the family Meiomeniidae Salvini-Plawen, 1985 (Kocot et al., 2022). This family is important to understanding adaptations to an interstitial lifestyle in Solenogastres as it is the only family in which all known species live in the interstitial habitat and therefore have key characters thought to be involved in this

transition (i.e., adhesive organs, sclerite morphology, and presence of a pedal commissural sac). As other interstitial solenogasters lack some of these structures, Meiomeniidae represents an ideal family in which to study the evolution of interstitial and meiofaunal solenogasters. The exclusion of a true representative of Meiomeniidae in Kocot et al. (2019) therefore leaves unresolved questions concerning body size evolution and transitions into the interstitial habitat in Solenogastres. To address these questions, we built upon the taxon sampling of Kocot et al. (2019) to include a total of eleven (eight newly sampled) meiofaunal species.

2. Methods

2.1. Specimen collection and imaging

To broadly sample the diversity of meiofaunal lineages of Solenogastres, we collected animals from the Azores, Curaçao, Florida, Hawaii, and Panama. Sand was collected for animal extraction by van Veen grab, SCUBA, or free diving. Sand samples were put into buckets and just barely covered with sea water. Subsamples of the sand were taken from the surface of the bucket, placed in a 2L Erlenmeyer flask, and treated with 7.5% $MgCl_2$ mixed 1:1 with filtered sea water to relax the meiofaunal animals. After 5–15 min, the flask was inverted several times or gently swirled to suspend meiofaunal animals, which were then elutriated onto a 60–100 μm sieve. Material sieved from the sediment was examined under a dissecting microscope with overhead illumination, which reflects off the sclerites of scale-bearing aplacophorans making them easier to see. Live animals were imaged in the field when possible and fixed in RNAlater for transcriptome sequencing. Additional specimens were preserved in 95% ethanol, 2.5% glutaraldehyde, and/or 4% formaldehyde. Specimens preserved in ethanol were imaged using an Olympus SZX16 stereomicroscope and an Olympus BX53 compound microscope with an Olympus SC50 camera. These specimens were then dried, mounted, and imaged using scanning electron microscopy on a Phenom Pro scanning electron microscope using a low-vacuum specimen holder.

2.2. Taxon sampling for phylogenomic analysis

We built upon the aplacophoran taxon sampling of Kocot et al. (2019) with publicly available data for *Gymnomenia pellucida* (Gymnomeniidae) and newly sequenced transcriptomes from eight species. Taken together, we sampled 30 species of Solenogastres representing at least 13 families and all four of the traditionally recognized orders. Of these, we sampled nine meiofaunal species representing at least seven families: Dondersiidae, Lepidomeniidae, Macellomeniidae, Meiomeniidae, Pruvotinidae, a new lineage closely related to Lepidomeniidae, and two additional species that could not be identified more specifically than as members of the order Pholidoskepia. Three caudofoveates, eight chitons, and three conchiferans were sampled as outgroups (Supplementary Tables 1-2).

2.3. Transcriptome sequencing and assembly

Whole animals were used for cDNA synthesis directly from lysed cells using the Takara SMART-Seq HT kit using 16 PCR cycles. The only exception was *Tegulaherpia tasmanica*(?) from which RNA was extracted using the Ambion RNAqueous Micro Kit and sent to Macrogen (South Korea) for cDNA synthesis with the Clontech SMART-Seq v4 kit. Molecular weight distribution and concentration of the cDNA prepared in-house were determined using an Agilent Fragment Analyzer with the HS NGS Fragment Kit for fragments 1–6000 bp. Dual-indexed Illumina sequencing libraries were then made from cDNA using the Nextera XT DNA Library Preparation Kit with 0.15 ng of cDNA. Molecular weight distribution and concentration of the final libraries was assessed using an Agilent Fragment Analyzer with the HS NGS Fragment Kit for fragments 1–6000 bp and the libraries were then sent to Psomagen (Cambridge, MA) for sequencing on a NovaSeq S4 flowcell to a depth of roughly 100 million reads each. The library for *Tegulaherpia tasmanica* (?) was sequenced on an Illumina HiSeq 2500 with 2 X 100 bp paired-end reads at Macrogen. Raw transcriptomic data for *Gymnomenia pellucida* and the outgroups were downloaded from NCBI SRA.

Quality filtering, quality trimming, adapter trimming and assembly were performed using Trinity v2.8.4 (Grabherr et al., 2011) with the flags –trimmomatic and –normalize_reads on the University of Alabama UAHPC cluster. TransDecoder v5.5.0 (Haas & Papanicolaou, 2017) was used to translate transcripts using the UniProt SwissProt database (accessed September 21, 2016; The Uniprot Consortium) and PFAM version 27 (Finn et al., 2016).

2.4. Orthology inference and matrix husbandry

For orthology inference, the 21 translated aplacophoran transcriptomes from Kocot et al. (2019) were used along with the peptide sequences inferred by TransDecoder from the newly sequenced species and those downloaded from NCBI using OrthoFinder v. 2.5.4 (Emms & Kelly, 2018) with an inflation parameter of 2.1. The output of this tool (Orthogroup_Sequences folder) yields groups of homologous sequences, which we term HomoGroups, that may contain paralogous sequences. HomoGroup sequences were further refined by first removing all sequences shorter than 100 amino acids. Redundant sequences were then removed using uniqHaplo.pl (https://raven.wrrb.uaf.edu/~ntakebay/teaching/programming/perl-scripts/uniqHaplo.pl) and aligned in MAFFT (Katoh & Standley, 2013) using the flags –auto and –maxiterate 1000. Potentially mistranslated or otherwise low-quality sequences were filtered using HmmCleaner with the default settings (Di Franco et al., 2019). Alignments were then trimmed using BMGE v. 1.12 to remove ambiguously aligned regions and sequences that did not overlap with others by at least 20 amino acids were removed. HomoGroups were then further filtered by the exclusion of any group containing less than 75% of the taxa.

PhyloPyPruner v. 1.2.4 (Thalen, 2019) was then used to exclude paralogous sequences and exogenous contamination. The following options were used: –min-len 100 –min-taxa 19 –min-support 0.9 –mask pdist –trim-lb 3 –trim-divergent 0.75 –min-pdist 0.01 –prune LS. Briefly, while orthology is homology due to a speciation event, paralogy is homology due to gene duplication (Fitch, 1970). Phylogenetic analysis of paralogous sequences can produce a tree that reflects the history of gene duplication events rather than speciation events (Fitch, 1970; Struck, 2013). Therefore, PhyloPyPruner examines single-HomoGroup trees for evidence of paralogy, such as presence of two or more sequences from the same taxon that do not form a clade (as may occur if multiple paralogs were present or if a transcriptome contained exogenous contamination) and prunes out the offending sequences to produce a final set of OrthoGroups. This tool also calculates a paralogy frequency metric, which reflects the number of paralogs (or e.g., contaminant sequences) present per HomoGroup, normalized by the number of HomoGroups in which each species is present, which can shed light on problematic transcriptomes that may have undergone genome duplications or contain significant contamination (Thalen, 2019). Single-gene trees were produced for each HomoGroup using IQ-TREE 2 (Minh et al., 2020).

2.5. Phylogenomic subsampling

Phylogenomic subsampling is a sensitivity testing strategy to combat false phylogenetic signal through the selection of fewer genes that are considered reliable (Mongiardino Koch, 2021). To test the robustness of the topology of the maximum likelihood tree we used the program genesortR (https://github.com/mongiardino/genesortR) for subsampling of genes based on average pairwise distance, compositional heterogeneity, level of saturation, root-to-tip variance, Robinson-Foulds distance to a reference topology, average bootstrap support, and

proportion of variable sites. The tree recovered in the IQ-TREE 2 analysis of the complete matrix was used as the reference topology for the Robinson-Founds comparisons. We generated three reduced data sets based on the best 100 and 200 genes according to the results of a PCA based on all seven criteria assessed by genesortR and the 100 genes with the slowest evolutionary rate (Supplementary Figures 2–4).

2.6. Phylogenetic analyses

OrthoGroups retained by PhyloPyPruner were concatenated (hereafter referred to as the "complete matrix") and used to construct a maximum likelihood (ML) tree in IQ-TREE 2 (Minh et al., 2020) with the best-fitting model for each partition and 1000 rapid bootstraps using the flags -T AUTO -B 1000 -m MFP –wbtl. Similarly, output alignments from genesortR were concatenated and used as input for additional phylogenetic analyses. ML analysis was performed in IQ-TREE 2 as described above for all three subset matrices. Further ML analysis was performed on the best 100 gene matrix using the site-heterogeneous PMSF model fitting the C60 profile mixture model, (-m LG+C60+F+G) with the best 100 gene ML tree as the guide tree (-ft).

Bayesian inference (BI) analysis was performed using PhyloBayes MPI (Lartillot et al., 2013) with the site-heterogenous CAT-GTR model with the following options: -np 4 pb_mpi -dc -cat -gtr -dgam 4. Because of the computational intensity of BI only the matrix consisting of the best 100 genes identified by genesortR was analyzed using this method. Four parallel chains were run for up to 9821 cycles with the first 1000 trees discarded as burn-in. A 50% majority rule consensus tree was computed from the remaining trees from each of the chains. Tree convergence was indicated by PhyloBayes bcomp maxdiff of 0.264108 and meandiff of 0.00350121. Conchifera was used to root the tree for all analyses.

2.7. Hypothesis testing

We conducted hypothesis testing to evaluate conflicting hypotheses about the first-branching group of Solenogastres in IQ-TREE 2 using the options: -z -zw -au -zb 10,000 -n 0. This was performed on both the complete and the reduced best 100 gene matrices for the conflicting Amphimeniidae-first (T1) and Meiomeniidae-first (T2) topologies. Tests were performed using the RELL approximation (Kishino et al., 1990) including bootstrap proportion (BP), Kishino-Hasegawa test (Kishino and Hasegawa, 1989), Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999), expected likelihood weights (Strimmer and Rambaut, 2002) and approximately unbiased (AU) test (Shimodaira, 2002) with 10,000 RELL replicates.

2.8. GLS calculations

To investigate the distribution of phylogenetic signal within the complete matrix and best 100 gene matrix we calculated site-wise log-likelihood scores across each supermatrix considering each hypothesis (T1, Amphimeniidae-first, and T2, Meiomeniidae-first) following the general approach of Li et al., 2021. Site-wise log-likelihood was calculated using IQ-TREE 2 (option -wsl) given the LG+C60+F+G model, and a concatenated file of the two different phylogenetic hypotheses (option -z). The Meiomeniidae-first (T2) tree, produced based on either the full or best 100 gene matrices via IQ-TREE 2 and a tree constrained to have Amphimeniidae-first (T1) (option -g) were used as input. Next, given the site-wise log-likelihood scores for each topology, the Perl script Phylogenetic_signal_parser.pl (Shen et al., 2020) was used to generate the gene-wise log-likelihood scores for every gene, and indicate the supported topology. Next, the absolute difference in log-likelihood for each gene (ΔGLS) was calculated using a custom R script, removing outlier genes as defined by Shen et al. (2017). The absolute differences in gene-wise log-likelihood scores (abs[ΔGLS]) and supported tree topology with outliers removed were plotted using the R programming environment (R Core Team, 2022).

2.9. Ancestral character state reconstruction

Stochastic character mapping (SIMMAP) was performed using the R package phytools (Revell, 2012) to investigate the evolution of body size, sclerite and radular type morphology in Solenogastres. For each analysis the complete matrix ML tree and 100 best-gene BI trees were pruned to include only Caudofoveata + Solenogastres for character mapping. Body size was categorized as megafaunal if greater than 1 cm in size, macrofaunal if less than 1 cm in size and meiofaunal following the definition of Bergmeier & Jörger (2020) if 2 mm or less in size. Sclerite coding was simplified to either scale, hollow or solid and radular morphology was coded as either distichous, biserial, monostichous, polystichous, or absent based on García-Álvarez & Salvini-Plawen (2007).

The best-fitting model for stochastic mapping analysis was found for each set of characters and tree using the ace function in the R package Ape (Paradis & Schliep, 2019). Three models – equal rates (ER), symmetrical (SYM), and all-rates-different (ARD) – were evaluated using the fitMk function in phytools. Akaike Information Criterion (AIC) values and weights were used to select the best-fitting model for each analysis. Stochastic character mapping was performed using the phytools function make.simmmap based on a fixed transition rate matrix of each state for 10,000 simulations.

3. Results

3.1. Data matrices

Our bioinformatic pipeline resulted in a complete matrix of 949 genes with a total of 33,352 amino acid positions and 22.6% missing data (Table 1, Supplementary Table 3). Paralogy frequency scores calculated by PhyloPyPruner were less than 0.2 for all species prior to pruning paralogs with PhyloPyPruner (Supplementary Figure 1), which indicates no pervasive problems with paralogy or exogenous contamination. Statistics for the complete matrix and subsampled matrices are reported in Table 1.

3.2. Higher-level relationships of Solenogastres

We sampled representatives of all four traditionally recognized orders of Solenogastres: Cavibelonia (10 species), Neomeniamorpha (2 species), Pholidoskepia (17 species), and Sterrofustia (1 species). Because ML analyses of the complete dataset (Fig. 1) yielded similar results as that of the reduced datasets (Supplementary Figures 5–10) we focus our discussion on the ML analysis of the complete dataset and the BI analysis of the 100 best genes identified by genesortR (Fig. 1) and highlight notable differences when applicable.

Consistent with Kocot et al. (2019), Cavibelonia was recovered as non-monophyletic in all analyses with maximal support for all relevant nodes in all analyses (ML bootstrap support [bs]/BI posterior probability [pp]=100/1.00). For further discussion we refer to the clade containing families Proneomeniidae, Epimeniidae and Simrothiellidae as Cavibelonia *sensu stricto*.

In contrast to Kocot et al. (2019), our results support non-monophyly of Pholidoskepia recovering instead three separate clades (100/1.00). In both ML and BI trees, Meiomeniidae falls outside of the clade including the families Dondersiidae and Gymnomeniidae and two undescribed

Table 1
Summary statistics of the various matrices used for phylogenetic analyses.

Matrix	Number of Genes	Number of positions	Missing Data
Complete	949	148,421	22.68 %
Best 100	100	19,174	21.82 %
Best 200	200	35,938	21.63 %
Slowest 100	100	14,780	25.07 %

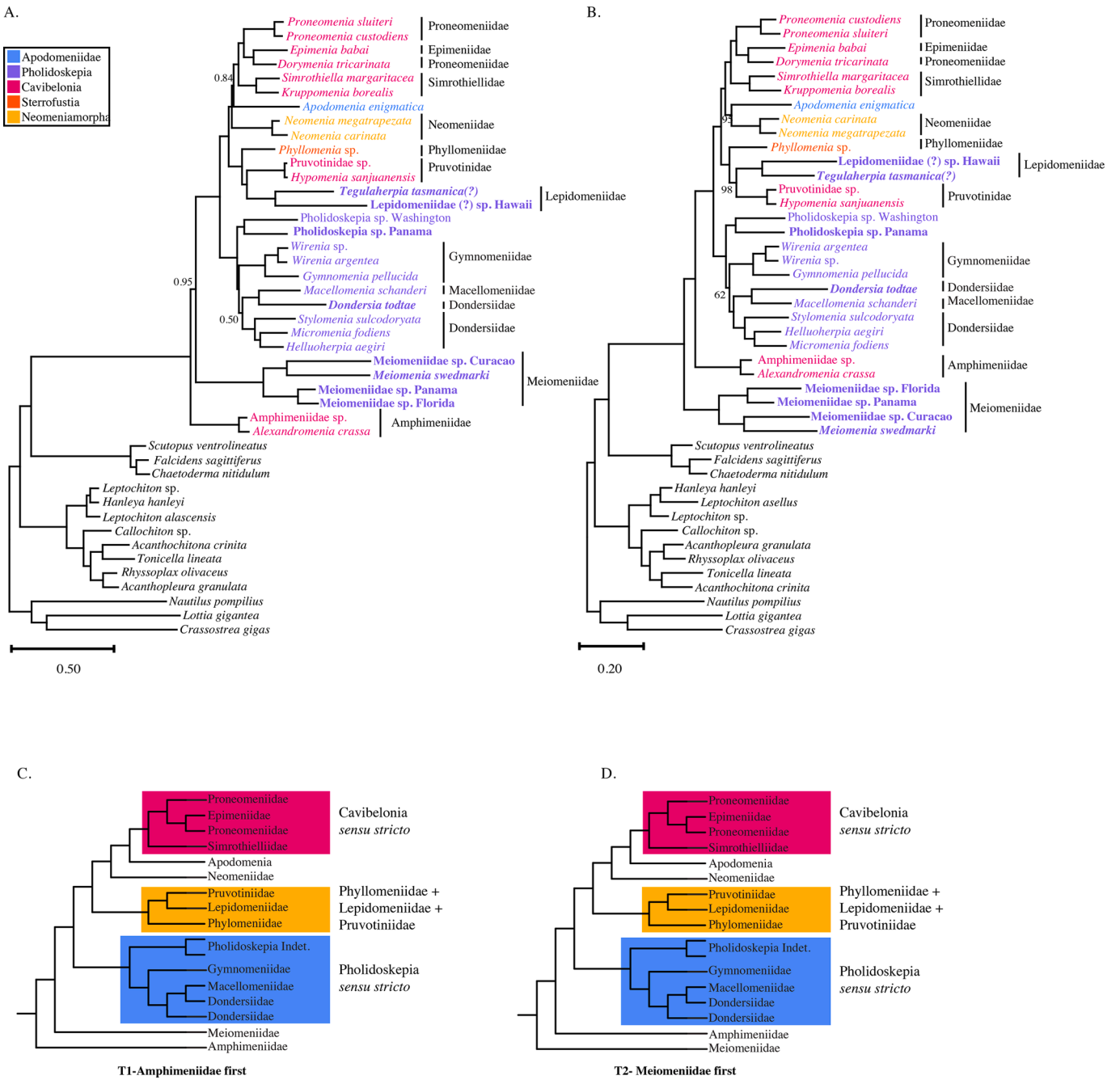

Fig. 1. Comparision of phylogenetic methods used to reconstruct relationships within Solenogastres. Traditional taxonomy based on García-Álvarez and Salvini-Plawen (2007) is indicated by color. A. Bayesian inference tree based the 100 best genes determined by GenesortR. Posterior probabilities <1.0 are show. B. Maxiumum likelihood tree based on complete matrix of 949 genes. Bootstrap support values <100 are shown. C. Summarized topology of Bayesian tree. D. Summarized topology of maximum likelihood tree.

species with maximal support (100/1.00). For further discussion, we refer to the clade including most Pholidoskepia taxa but not Meiomeniidae (or Lepidomeniidae; see below) as Pholidoskepia *sensu stricto*. In Pholidoskepia *sensu stricto*, the family Dondersiidae was recovered as non-monophyletic with Macellomeniidae nested within Dondersiidae and sister to *Dondersia todtae* (100/1.00). Non-monophyly of Pholidoskepia is further supported by the recovery of Lepidomeniidae within a clade containing Pruvotinidae and Phyllomeniidae (100/1.00). Our expanded sampling recovers Lepidomeniidae as sister to Pruvotinidae with moderate to maximal support (95/1.00).

ML recovered Neomeniamorpha, represented here by *Neomenia megatrapezata* Salvini-Plawen and Paar-Gausch, 2004 and *Neomenia carinata* Tullberg, 1875, as monophyletic and as the sister taxon of *Apodomenia enigmatica* Kocot, Todt, Mikkelsen and Halanych, 2019. This relationship was not recovered by BI or Kocot et al. (2019), however it is maximally supported in all ML analyses. Interestingly, *A. enigmatica* lacks a radula (or possibly has a highly reduced radula that could not be observed in the type material) and the genus *Neomenia* also lacks a radula (García-Álvarez & Salvini-Plawen, 2007; Kocot et al., 2019).

While ML and BI trees largely support the same topology for higher level solenogaster relationships, the two methods conflict in the recovery of the earliest branching clade. In accordance with the findings of Kocot et al. (2019), BI analysis recovers the family Amphimeniidae (pp=0.95) as the sister group to all other solenogasters. However, for

every matrix investigated, ML analyses recover the family Meiomeniidae as the sister group to all other solenogasters with full support (bs=100, Fig. 1, Supplementary Figures 7–10). We performed hypothesis testing based on both the complete matrix and the matrix with the best 100 genes according to genesortR to assess support for the conflicting Amphimeniidae-first (T1) and Meiomeniidae-first (T2) topologies using the RELL approximation (Kishino et al., 1990) including bootstrap proportion (BP), Kishino-Hasegawa test (Kishino and Hasegawa, 1989), Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999), expected likelihood weights (Strimmer and Rambaut, 2002) and approximately unbiased (AU) test (Shimodaira, 2002). Testing of both matrices using all methods showed that the Amphimeniidae-first topology is not significantly more likely than the Meiomeniidae-first topology (Supplementary Table 4).

Investigation of phylogenetic signal using gene likelihood scores (ΔGLS) demonstrated a higher number of genes in support of the Meiomeniidae-first (T2) hypothesis for both the complete matrix and the best 100 genes matrix (Supplementary Figures 15–16). Distribution of phylogenetic signal between the two topologies suggests that a higher proportion of genes show strong support for Meiomeniidae-first, conflicting with the recovered BI topology.

3.3. Ancestral character state reconstruction

Ancestral character state reconstruction (Fig. 2) based on our maximum likelihood trees supports a meiofaunal ancestor for Solenogastres. The average number of state changes over 10,000 simulations is estimated at 16.59 with the most common change from macrofaunal to meiofaunal (Supplementary Tables 6 and 7). Under this scheme, meiofaunal size in Meiomeniidae is inferred to be ancestral with a shift from meiofaunal to macrofaunal size occurring at the base of the rest of Solenogastres. The larger clade of Pholidoskepia *sensu stricto* including Gymnomeniidae, Macellomeniidae, and Dondersiidae is inferred to have been ancestrally macrofaunal with two independent evolutions of meiofaunal body size. Meiofaunal body size is inferred to have arisen again independently in Lepidomeniidae and Pruvotinidae. These two families form a clade with a species of *Phyllomenia* that is predicted to have evolved from a macrofaunal ancestor. Evolution towards megafaunal body size is inferred to have occurred twice, once in the last common ancestor of Amphimeniidae and again in the last common ancestor of the clade containing Neomeniamorpha, *Apodomenia* and "Cavibelonia".

Ancestral character state reconstruction based on the BI analysis supports a megafaunal ancestor for all Solenogastres (Fig. 2) with the meiofaunal body size of Meiomeniidae inferred to be an independently derived condition. The average number of state changes over 10,000 simulations is estimated to be 17.89 with the most common change from macrofaunal to meiofaunal (Supplementary Tables 5 and 6). Aside from this difference, the analysis inferred the same ancestral states for all other nodes also recovered in the ML trees. At the node representing the ancestor of all solenogasters excluding Meiomeniidae and Amphimeniidae, ancestral character state reconstruction based on both the ML and BI trees supports a macrofaunal ancestor.

Ancestral radular morphology is predicted as distichous under both ML and BI topology with average changes estimated to be 7.023 and 8.47 respectively (Fig. 3, Supplementary Table 7). The most common transition under both topologies was from distichous to monostichous with an estimated 2.06 changed under ML and 2.09 under BI (Supplementary Table 7). Notably, both trees support independent evolution of the polystichous radula at the base of the clade including Proneomeniidae and Epimeniidae and the evolution of a monostichious radula occurring twice at the base of Amphimeniidae and Dondersiidae (Fig. 3).

Ancestral state reconstruction of sclerite morphology recovered scales as the ancestral form for Solenogastres + Caudofoveata under both topologies, however, results differed between the analyses based the ML and BI trees at the node representing the last common ancestor of all Solenogastres (Fig. 3). Under the ML topology, scales were recovered as the ancestral state for Solenogastres with an estimated 10.40 changes between sclerite morphologies while under the BI topology, hollow sclerites were recovered as the ancestral state with an estimated 13.43

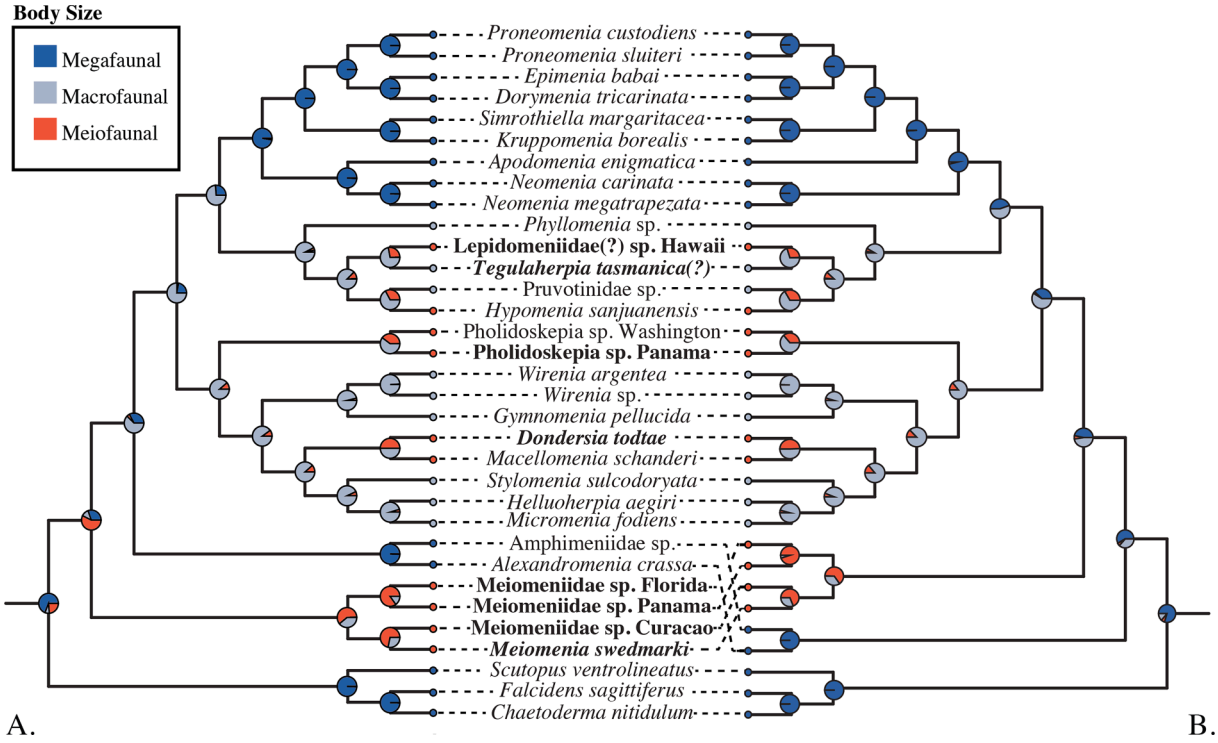

Fig. 2. Ancestral state reconstruction of body size with states as meiofaunal (<2 mm), macrofaunal (<1 cm), or megafuanal (>1 cm) based on maximum likelihood topology (A) and Bayesian inference topology (B). Posterior probabilities of body size are presented at each ancestral node.

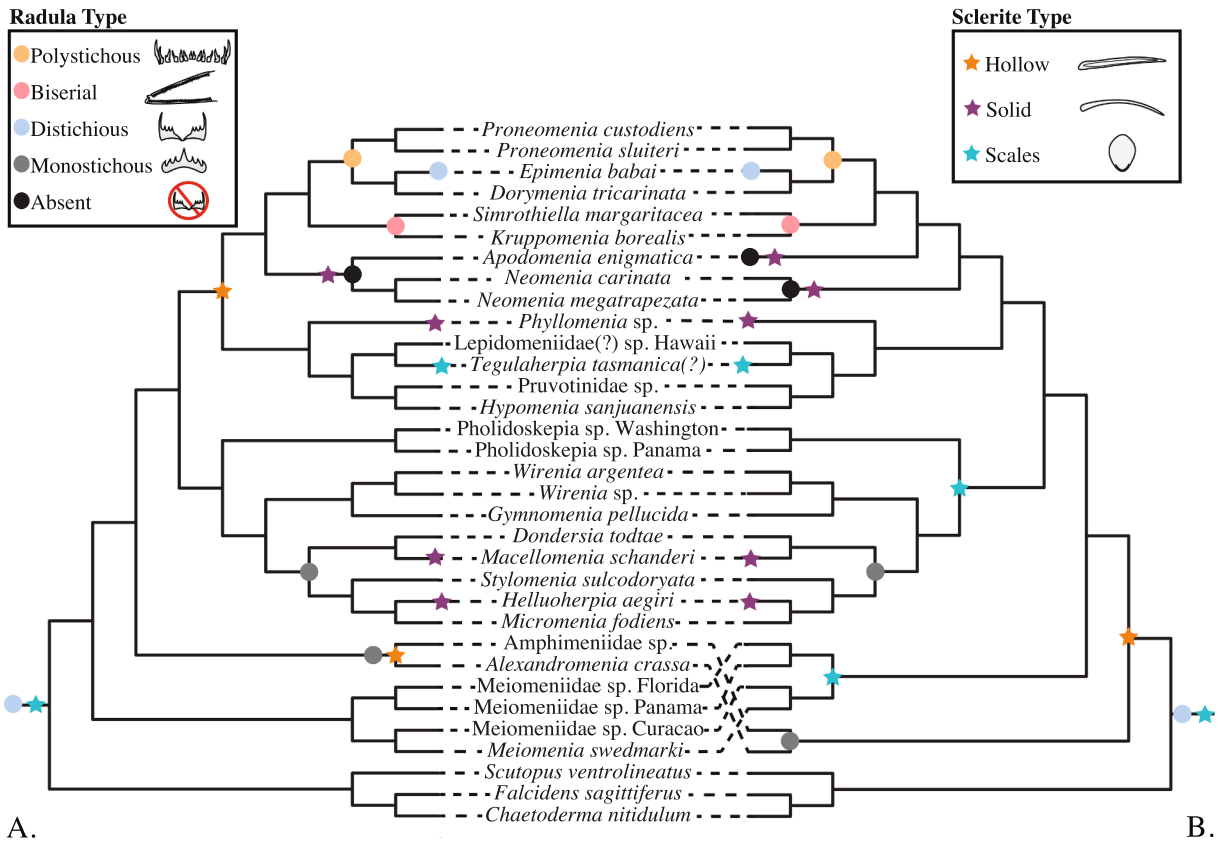

Fig. 3. Ancestral state reconstruction of radular (circles) and sclerite (stars) morphology based on maximum likelihood topology (A) and Bayesian inference topology (B). Colored shapes correspond with inferred ancestral for each indicated node.

state changes (Supplementary Table 8). Under the ML topology, hollow sclerites evolved twice, once in Amphimeniidae and once at the base of the clade including Cavibelonia *sensu stricto*, Neomeniamorpha and Phyllomeniidae + Lepidomeniidae + Pruvotinidae. In contrast, under the BI topology, hollow sclerites are ancestral to all solenogasters with three independent evolutions to scales in Meiomeniidae, Pholidoskepia *sensu stricto* and *Tegulaherpia tasmanica* (Fig. 3). Under both topologies, solid acicular sclerites were inferred to have independently evolved in Macellomeniidae, the dondersiid species *Helluoherpia aegiri*, and either the last common ancestor of the clade containing Neomeniamorpha + Apodomeniidae (ML) or independently in Neomeniamorpha and Apodomeniidae (BI).

Phylogenetic uncertainty about the relative placement of the large-bodied Amphimeniidae and meiofaunal Meiomeniidae at the base of the tree clouds our interpretation of the ancestral state of all Solenogastres, however in both analyses we find clear evidence for a macrofaunal ancestor of the clade containing the majority of solenogasters (90%).

4. Discussion

4.1. Inferring ancestral body size

Earlier views on solenogaster evolution placed Pholidoskepia as the sister taxon to all other solenogasters based on their relatively small body size and presence of scale-like sclerites similar to those of caudofoveates (Haszprunar, 1992, 2000; Salvini-Plawen, 1980). These interpretations were based on the hypothesis that the relative simplicity of Solenogastres in comparison to other molluscs is plesiomorphic. However, based on recent studies in phylogenomics, developmental biology, and the fossil record, the current leading hypothesis is that aplacophorans evolved from a chiton-like ancestor (Kocot, 2013; Vinther, 2014; Vinther et al., 2012; Wanninger and Wollesen, 2019), consistent with the Aculifera hypothesis put forth by Scheltema (1993, 2014). Therefore, we can now take the arguments made for a small-bodied (=macrofaunal) ancestor of Solenogastres (e.g., Haszprunar, 1992, 2000; Salvini-Plawen, 1980; Salvini-Plawen, 2003) and view them through the lens of the current understanding of molluscan phylogeny.

Placement of Amphimeniidae and Meiomeniidae influence our interpretation of body size evolution in Solenogastres with a megafaunal ancestor favored by BI and a meiofaunal to macrofaunal ancestor favored by ML. This uncertainty may be a result of taxon sampling. While we increased the number of meiofaunal and macrofaunal solenogasters in our data set and our taxon sampling is relatively balanced with respect to the known higher-level diversity of the group, our overall taxon sampling represents just 30 of ~300 described species and some family-level taxa from which no RNA-viable material is available are lacking. Moreover, meiofaunal solenogasters are arguably understudied and easily overlooked. It is possible, if not likely, that additional lineages of meiofaunal solenogasters remain to be discovered. Notably, the analyses for this study were re-done multiple times as we continued to expand our taxon sampling through field work. Even with the addition of these taxa, our analysis is still biased towards macrofaunal (or "large-bodied") individuals in other lineages on the tree. For instance, increased sampling of Amphimeniidae specifically may help to resolve this uncertainty as we only have two particularly large-bodied species represented out of the 28 in the family, whose members can range in size from 8 mm to 9 cm (Salvini-Plawen & Schwabe, 2012).

Under this unclear ancestral body size, we can infer two potential modes of evolution under the topologies recovered (T1 and T2; Fig. 1C and 1D): T1 supports evolution from a megafaunal, or large-bodied, ancestor that underwent a reduction in body size to either meiofaunal (in the last common ancestor of Meiomeniidae) or macrofaunal ("small"; in the last common ancestor of Pholidoskepia *sensu stricto*,

Phyllomeniidae+Lepidomeniidae+Pruvotinidae, Neomeniamorpha, *Apodomenia*, and Cavibelonia *sensu stricto*). Reduction to a meiofaunal ("minute") size occurred three to four times in Pholidoskepia *sensu stricto* and twice in Phyllomeniidae+Lepidomeniidae+Pruvotinidae. Megafaunal ("large") body size secondarily evolved in the last common ancestor of the clade containing Neomeniamorpha, *Apodomenia* and Cavibelonia *sensu stricto*. On the other hand, T2 supports evolution from a meiofaunal to macrofaunal ancestor. Here we see two transitions to large (macrofaunal) body size: in Amphimeniidae and in the last common ancestor of the clade containing Neomeniamorpha, *Apodomenia* and Cavibelonia *sensu stricto*. At the base of all Solenogastres (minus Meiomeniidae) a macrofaunal ancestor is favored with transitions to meiofaunal size three-four times in Pholidoskepia *sensu stricto* and twice in Phyllomeniidae+Lepidomeniidae+Pruvotinidae.

Independent from the first two branching lineages (either Amphimeniidae or Meiomeniidae as the sister taxon to all other Solenogastres), our analyses support a macrofaunal (small-bodied) ancestor for the clade containing all remaining Solenogastres.

4.2. Evolution from a macrofaunal (Small-bodied) ancestor

Haszprunar (1992) hypothesized that molluscs evolved from a small-bodied (defined by Haszprunar as 1–3 mm and falling within our definition of meiofaunal) aplacophoran-like ancestor with plesiomorphic molluscan characters including ciliary gliding with a mucus gland, a posterior position of the mantle cavity, a simple through gut and a simple radula (Haszprunar, 1992). As stated by Haszprunar, these inferences were based mainly on the conditions found in the aplacophoran groups, which were generally considered to be the earliest offshoots of the molluscan line at that time (e.g., Salvini-Plawen, 1985). While phylogenomic studies have shown that Solenogastres is not the sister taxon to all other molluscs (reviewed by Kocot, 2013; Kocot et al., 2011, 2020; Smith et al., 2011), the evidence put forward by Haszprunar (1992) still provides key insights concerning body size evolution within Aplacophora. Our phylogenomic analyses resulted in conflicting topologies with BI placing Amphimeniidae, one of the largest-bodied, megafaunal families of Solenogastres as the sister taxon to all others and ML placing one of the most minute, meiofaunal families as the first-branching lineage. Although this hinders confident inference of the body size of the last common ancestor of Solenogastres, ancestral state reconstruction analyses based on both our ML and BI trees supports a macrofaunal (small-bodied) ancestor for the grouping of all solenogasters except Meiomeniidae and Amphimeniidae.

Available morphological and molecular data support the last common ancestor of the clade containing the majority of Solenogastres as macrofaunal with meiofaunal and megafaunal taxa in this clade having evolved secondarily. As available evidence suggests aplacophorans evolved from a relatively large-bodied (macrofaunal to megafaunal), chiton-like ancestor (Wanninger & Wollesen, 2019), we hypothesize that Solenogastres have undergone a sort of morphological bottleneck where simplification and morphological novelty are a result of reduction toward a comparatively smaller body size.

Ciliary gliding is a form of locomotion in which an animal is propelled by the beating of cilia on a secreted layer of mucus (Martin, 1978; Satir & Sleigh, 1990.) Ciliary gliding is a common form of locomotion for small-bodied and minute organisms, but the effectiveness of ciliary activity decreases as the size of an organism increases beyond 1 mm (Chia et al., 1984). In Solenogastres, the foot is restricted to a so-called pedal groove with musculature connected to the folds for retraction. Movement is performed by compound cilia of the foot with mucosal secretions originating from the anterior pedal gland and sole glands distributed along the foot (Haszprunar, 1992, 2000). As reported by Salvini-Plawen (1968), movement in even relatively large species of Solenogastres does not involve muscular activity. This differs from ciliary gliding in other molluscs such as the gastropods *Lymnaea* and *Helix*, where movement takes place through a combination of ciliary gliding and movement of the underlying smooth muscle (Pavlova, 2019). It has been shown that cilia alone are used for slow gliding in these gastropods, however any gliding above that rate requires smooth muscle for quick movement (Pavlova, 2019). This may be due to the inefficiency of cilia to propel larger-bodied animals at higher rates (Chia et al., 1984). Locomotion reliant solely on ciliary gliding in solenogasters therefore may be an evolutionary consequence of an ancestor in which small (macrofaunal) body size allowed for effective movement across (or within) substrate without the aid of musculature.

Solenogastres lack respiratory organs in the form of the ctenidia present in all other molluscan classes. This loss of true ctenidia may provide evidence for a small-bodied ancestor of Solenogastres whose relatively high surface area to volume ratio may have made adequate gas exchange possible without a need for specialized structures (Graham, 1988). The mantle cavity of Solenogastres serves as an outlet for the gonoducts and anus while the ventilation of the mantle cavity through ciliary action allows for gas exchange without a specialized structure in small-bodied and minute solenogasters (Haszprunar, 1992; Salvini-Plawen, 1985a). Under this scenario, as a consequence of the relative reduction in surface area for gas exchange, increased ventilation of the mantle cavity is necessary in solenogasters that secondarily evolved large (macrofaunal) body size (Haszprunar, 1992), consistent with the preponderance of respiratory papillae and folds in mantle cavity and epidermal papillae in macrofaunal and megafaunal solenogaster lineages.

While caudofoveates have a digestive system with an esophagus with a paired glandular pouch, a stomach with paired digestive glands and an intestine, the relatively simple gut found in Solenogastres has been traditionally viewed as ancestral for molluscs (Haszprunar, 1992; Scheltema, 1981; Salvini-Plawen, 1981, 1985a, 1988; Salvini-Plawen, 2003). There is no distinction in the stomach but complex glands associated with the foregut are present and differ between groups (Handl & Todt, 2005; Todt, 2006). Apparent simplification of the digestive tract is thought to be related to an ancestral specification to cnidarian prey (Bergmeier et al., 2021; Haszprunar, 1992; Salvini-Plawen, 2003). Haszprunar (2000) suggested that the radular morphology of solenogasters in combination with an undifferentiated digestive tract serves as evidence for adaptation for micro-carnivory by a small ancestor. Four basic radular morphologies occur in Solenogastres, which are distinguished based on number of teeth per row: biserial (two denticulated plates per row), distichous (two hook-shaped teeth per row), monostichous (one tooth per row), and polystichous (many teeth per row) (Scheltema, 2014; Scheltema et al., 2003). Traditional taxonomic hypotheses suggest an ancestor with a distichous radula (Scheltema, 2014; Salvini-Plawen, 2003), which is supported by our ancestral character state reconstruction of radular morphology. Barcoding of solenogasters and their prey reveal a high level of food specialization within species (Bergmeier et al., 2021). Comparing food source and morphology, Bergmeier et al. (2021) inferred an ancestral hydrozoan food source with specialization on non-cnidarian prey as a secondary adaptation that co-occurs with modifications in the digestive tract (Bergmeier et al., 2021). In our analyses, the ancestral state of the radula is inferred to be distichous, which could support specialization for cnidarian prey as proposed by Haszprunar (1992), Salvini-Plawen (2003), and Bergmeier et al. (2021), although foregut gland morphology and its association with body size, radular type and prey type should be further investigated.

Our ancestral state reconstruction supports scales as the ancestral form of the scleritome in the last common ancestor of Solenogastres + Caudofoveata (Fig. 2). As briefly discussed above, García-Álvarez et al. (2000) viewed scale-like sclerites as a potential adaptation to aid in movement within the interstitial environment. While many meio- and macrofaunal solenogasters have scale-like sclerites, the correlation between body size, sclerite type, and habitat is still unclear. For example, the interstitial species *Biserramenia psammobionta* Salvini-Plawen, 1967 have a combination of hollow, needle-like and knife-like sclerites and

the meiofaunal members of the family Macellomeniidae have solid, nail-like sclerites. For simplicity's sake, our reconstruction of sclerite morphology is limited to three categories. A likely productive future direction of research, especially in light of broader taxonomic sampling, would be an expanded analysis including more detailed classifications of sclerites to further clarify the potential adaptive potential of different sclerite types.

A pedal commissural sac is found within some minute (meiofaunal) and small-bodied (macrofaunal) species of Pholidoskepia and within *Scheltemaia mimus* Scheltema and Schander, 2000, a species of pruvotinid. This structure is thought to be a convergently evolved gravity-sensing organ as ultrastructural differences indicate that the structure is not homologous to the molluscan statocyst (Hazprunar, 1986; Bergmeier et al., 2016). The homology of the pedal commissural sac within Solenogastres is unknown, but it may be either an ancestral character that has been secondarily lost in some lineages or an independently evolved structure that is selected for in small-bodied lineages that allowed for further facilitation into interstitial habitats through the sense of gravity and movement (see Bergmeier et al., 2016 for a more detailed discussion). Because of its minute size, the pedal commissural sac may be present in more (perhaps many more) species than it is currently known from but it has been overlooked.

4.3. Progenesis revisited under the Meiomeniidae-first hypothesis

Under the Meiomeniidae-first hypothesis, ancestral state reconstruction supports a meiofaunal ancestor for Solenogastres. Progenesis, the evolutionary process by which an organism retains ancestral characteristics through the acceleration of sexual maturity of juvenile or larval stages of an ancestor, has been shown to be a common theme in meiofaunal animal evolution (Gould, 1977; Westheide, 1987). Scheltema (1993, 2014) argued a scheme in which some aplacophoran structures are retained from the larval form of a chiton-like ancestor and that derived morphologies in this group are indicative of progenesis (see below and Martynov et al., 2020 for discussion on the use of the term progenesis). She argued that the small size and worm-like body shape of aplacophorans evolved as a result of elongation from an embryological form (as observed in the development of *Neomenia carinata* Tullberg, 1875, *Epimenia babai* Salvini-Plawen, 1997 and *Wirenia argentea* Odner, 1921) (Thompson, 1959; Okusu, 2002; Scheltema, 2014; Todt & Wanninger, 2010). She interpreted the reduction and/or loss of morphological characters (such as pedal glands, radular morphology, paired gonads and musculature) in solenogasters as a result of progenetic origins from a chiton-like ancestor.

The pedal glands of solenogasters supply mucus to the pedal pit for ciliary gliding. A pedal gland is present in larval chitons as an attachment mechanism but this structure is lost shortly after metamorphosis (Scheltema, 1993). Scheltema argued that the presence of pedal glands and the lack of musculature within the foot are evidence of retained larval characteristics in adult Solenogastres (Scheltema, 1993). She furthers her argument with the morphology of the radula. Solenogasters lack the central rhachidian tooth in the radula that is found in most other molluscs. It has been shown that during radular development in chitons, the rhachidian tooth does not form until the formation of one to many rows of lateral teeth (Scheltema, 2003, 2014). The lack of rhachidian tooth in solenogasters, therefore, may be a consequence of progenesis. Further retention of larval characteristics is proposed by Scheltema in the gonopericardial system. In solenogasters the gonad and pericardium are connected (=gono-pericardioducts) and therefore they lack true gonoducts (Eernisse and Kerth, 1988; Scheltema, 1993, 2014; Salvini-Plawen, 2003 but see investigations by Salvini-Plawen, 1970 on *Phyllomenia austrina* for an exception). During chiton development the gonads differentiate from an anlage of the pericardium forming a connection between the structures that is later lost (Higley & Heath, 1912). Thus, the connection observed in solenogasters reflects the early ontogeny of the gonopericardial system in chiton development (Scheltema, 1993, 2014).

Comparison of myogenesis between the chiton *Leptochiton assellus* Gmelin, 1791 and solenogaster *Wirenia argentea* Odhner, 1920 showed that the larval musculature of solenogasters is nearly identical to that of chitons including enrolling and ventromedian muscles and a seven-fold arrangement of dorsoventral muscles (Scherholz et al., 2013, 2015). Scheltema (2014) interpreted the findings of Scherholz et al (2013) as evidence for progenesis due to the offset in timing of musculature development and the simplification of the muscle groups throughout solenogaster ontogeny. During later stages of development, the larval musculature of *W. argentea* is remodeled to form a three-layered body wall not unlike the arrangement in other vermiform animals (Scherholz et al., 2015). Seven sets of inner dorsoventral muscles form simultaneously during early development and undergo multiplication after metamorphosis (Scherholz et al., 2015). Seven-fold seriality is also present in chitons, where the eight dorsoventral muscles fuse to form seven paired shell muscle units. The eighth set of shell muscle units forms much later in development along with the final shell plate (Scherholz et al., 2015). The presence of seven-fold seriality may be an ancestral condition of Aculifera with the addition of the eighth set of muscles being a derived condition of polyplacophorans (Scherholz et al., 2013, 2015). Scherholz et al (2013) further posited that the polyplacophoran-like arrangement is plesiomorphic with a reduction to just seven-fold seriality occurring in Solenogastres is an equally probable scenario. This study also concluded that the adult enrolling muscle of solenogasters was not homologous to the enrolling muscle in adult chitons but rather to the ventrolateral muscle found in chiton larvae (Scherholz et al., 2015). Remodeling of larval musculature from a chiton-like ancestor to the simplified three-layered body wall is a derived condition of solenogasters. Further data investigating myogenesis in Caudofoveata is required to continue to untangle the aculiferan condition in order to further speculate on musculature as evidence in Solenogastres.

Scheltema (1993, 2014) argued for progenesis in Solenogastres due morphological similarities to larval and juvenile chitons, however the use of the term is problematic (Martynov et al., 2020; Worsaae et al., 2023). An underdeveloped phenotype (paedomorphosis) can be achieved through alterations in developmental rate or timing (heterochrony) through processes such as neoteny (decelerated rate of development), progenesis (early offset of development), and post-displacement (late onset of development) (Worsaae et al., 2023). However, the definitions of these processes have been used inconsistently in the literature and in themselves do not necessarily reflect the evolutionary and genetically controlled processes that affect heterochrony (see Martynov et al., 2020; Worsaae et al., 2023). Further, it is difficult to determine which process is responsible for developmental offset as paedomorphic taxa often display a mosaic of delayed and accelerated growth characters (Martynov et al., 2020). Therefore, we are restrained to use the encompassing term paedomorphosis to refer to taxa with retained ancestral larval characteristics and in our discussion of the origin of Solenogastres.

As paedomorphic taxa are common among meiofaunal lineages, it is possible that under the Meiomeniidae-first hypothesis the evidence provided by Scheltema (1993, 2014) is indicative of a paedomorphic ancestor for Solenogastres. Although questions about when and how miniaturization and secondary evolution of large body size took place during solenogaster evolution, this group presents an interesting framework in which to study body size evolution. Comparative genetic study is possible at an interspecific level in families with both meiofaunal and large-bodied species or between chitons and solenogasters. Investigation into differences in lineages of solenogasters and between aculiferans will aid in our understanding of the mechanisms of miniaturization in this group.

5. Conclusions

Although our maximum likelihood and Bayesian inference analyses provide conflicting results regarding the earliest branching lineage (megafaunal Amphimeniidae or meiofaunal Meiomeniidae first) under, our results show the majority of Solenogastres evolved from a macrofaunal ancestor with scale-like sclerites and a distichous radula. Increased taxon sampling would likely help further clarify these relationships and improve our understanding of the early evolution of Solenogastres. Under these differing topologies we can infer two general hypotheses of solenogaster evolution: 1) A large, amphimeniid-like ancestor that underwent a secondary reduction in size to macrofaunal ancestor for the majority of Solenogastres, with meiofaunal and large body size as independent evolutions and 2) A meiofaunal, meiomeniid-like ancestor that underwent secondary enlargement to a macrofaunal for the majority of Solenogasters with megafaunal and meiofaunal body size independently evolved.

Haszprunar (1992) provided evidence for an epibenthic, 1–3 mm sized ancestor of Mollusca through interpretation of contemporary Solenogastres. Under our current understanding of aplacophoran evolution from a chiton-like ancestor, we use these arguments as further support for a small-bodied (within our definition of macrofaunal as 3 mm-1 cm) ancestor of Solenogastres rather than Mollusca as a whole. Further, we re-visit arguments by Scheltema (1993, 2014) for progenetic origins of Aplacophora and instead suggest heterochrony as a potential evolutionary force leading to paedomorphosis in the last common ancestor of Solenogastres (under the Meiomeniidae-first hypothesis) or in meiofaunal taxa. Parsing out this relationship of early solenogaster evolution will further aid in our understanding of aplacophoran evolution and may provide an interesting framework in which to study evolutionary processes such as miniaturization and pedomorphosis.

CRediT authorship contribution statement

Meghan K. Yap-Chiongco: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Resources, Visualization. **Franziska S. Bergmeier:** Conceptualization, Resources, Writing – review & editing, Investigation. **Nickellaus G. Roberts:** Data curation, Formal analysis, Software, Visualization, Writing – review & editing, Investigation. **Katharina M. Jörger:** Conceptualization, Resources, Writing – review & editing, Investigation. **Kevin M. Kocot:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing, Investigation.

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.

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Appendix A. Supplementary material

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