Tightening the girdle: phylotranscriptomics of Polyplacophora

Juan Moles [1,2,3], Tauana Junqueira Cunha [4], Sarah Lemer [5], David J. Combosch [1,5]
and Gonzalo Giribet [1]

[1]*Museum of Comparative Zoology & Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA;*
[2]*SNSB–Bavarian State Collection of Zoology, 81247 Munich, Germany;*
[3]*Department Biology II, Ludwig-Maximilians-Universität, 82152 Planegg-Martinsried, Germany;*
[4]*Smithsonian Tropical Research Institute, Panama City, 0843-03092, Panama; and*
[5]*Marine Laboratory, University of Guam, Mangilao, GU 96923, USA*

Correspondence: *J. Moles; e-mails:* moles.sanchez@gmail.com, jmoles@g.harvard.edu

(Received 10 November 2020; editorial decision 2 March 2021)

ABSTRACT

The phylogenetic relationships within the molluscan class Polyplacophora (chitons) have been studied using morphology, traditional Sanger markers and mitogenomics, but, to date, no analysis has been carried out using transcriptomic and genome-wide data. Here, we leverage the power of transcriptomes to investigate the chiton phylogeny to test current classification schemes and the position of Callochitonidae, a family whose phylogenetic position and taxonomic assignation are uncertain because of conflicting results from past studies. Using multiple data matrices with different taxon occupancy thresholds and inference methods, including both concatenated and coalescence-based approaches, we find a consistent resolution of the chiton phylogeny. Our results support a system with the orders Lepidopleurida, Chitonida and Callochitonida, with the latter two as sister groups. This resolution is compatible with recent mitogenomic results and rejects the position of *Callochiton* as a member of Chitonina.

INTRODUCTION

Chitons (Polyplacophora) constitute a group of exclusively benthic marine molluscs characterized by a flattened body and eight dorsal articulating shell valves surrounded or entirely covered by a girdle that may present spines, scales or hairs. They attach to hard substrates thanks to a ventral, oval creeping foot that delimits an elongated, pallial cavity with a series of gills. Chitons are ecologically important in the littoral zone, where they are major grazers (Sigwart & Schwabe, 2017). A series of specializations to deeper waters have taken place, especially among Lepidopleurida, which can be found on sunken wood, cold seeps and other abyssal habitats (Sigwart, 2017). A few unrelated species in three genera are predatory, including the western Pacific species *Placiphorella velata* (Carpenter MS, Dall, 1879). Recent research on chitons has focused on novel aspects of their sensory biology, including the anatomy and function of sensory organs (Speiser, Eernisse & Johnsen, 2011; Sigwart *et al.*, 2014; Sumner-Rooney *et al.*, 2014; Li *et al.*, 2015; Sumner-Rooney & Sigwart, 2015).

Extant chitons form a monophyletic group (e.g. Okusu *et al.*, 2003; Stöger *et al.*, 2013; Irisarri *et al.*, 2020) with one subclass, Neoloricata. Until recently, the currently accepted classification, based on a consensus of molecular data and many morphological character sets, divided extant Neoloricata into two orders, Lepidopleurida and Chitonida, with the latter divided into the suborders

Chitonina and Acanthochitonina (Ponder, Lindberg & Ponder, 2020). Despite being previously placed in the suborder Chitonina (Kaas & Van Belle, 1990; Ponder *et al.*, 2020), *Callochiton* had been recovered as the sister group to the order Lepidopleurida in an early five-gene phylogeny (Okusu *et al.*, 2003). This result was questioned by Buckland-Nicks (2008) and subsequent analyses, which used Sanger data, recovered *Callochiton* as the sister group to the remaining Chitonida (Giribet *et al.*, 2006; Wilson, Rouse & Giribet, 2010; Stöger *et al.*, 2013; Irisarri *et al.*, 2020). Because of the impossibility of reconciling current phylogenetic results and the existing classification system, Giribet & Edgecombe (2020: 362) recognized Callochitonida as a newly ranked order within Neoloricata. These changes in classification obviate the use of major groups used in the past, such as Ischnochitonina (e.g. Sirenko, 2006), but correlate well with the evolution of two of the trademark characters of chitons, sperm and egg hull morphology (Buckland-Nicks, Chia & Koss, 1990; Okusu *et al.*, 2003; Buckland-Nicks, 2008).

The phylogeny of the order Lepidopleurida has been studied using both anatomy (Sigwart, 2009) and molecular characters (Sigwart *et al.*, 2010), and other subclades of chitons have also been investigated, including Chitonida (Sigwart *et al.*, 2013) and Acanthochitonina (Irisarri, Eernisse & Zardoya, 2014). More recently, the first mitogenomic analysis of the entire Polyplacophora was published (Irisarri *et al.*, 2020). However, no analysis has studied chiton phylogeny using transcriptomes, despite the generalized

Table 1. Assembly statistics of the species included in this study, including the number of reads before and after filtering, and the percentage of completeness evaluated in BUSCO.

Species	Voucher no./ SRA no.	Sampling location	No. of raw reads	No. of reads after filtering	No. of contigs	No. of tran-scripts	N50	No. of unique transcripts	No. of peptides after isoform filter	% BUSCO complete genes	% BUSCO frag-mented genes
Acanthochitonidae											
Acanthochitona crinita (Pennant, 1777)	SRR5110525	France, Atlantic Ocean	35,737,364	28,368,045	150,540	269,342	1,401	141,375	26,295	99.0	0.9
Choneplax lata (Guilding, 1829)	MCZ-384341/ SRR13010088	Belize, Atlantic Ocean	13,841,239	9,476,608	143,264	243,996	515	210,809	11,153	18.9	34.0
Cryptoplacidae											
Cryptoplax japonica Pilsbry, 1901	SRR13010086	Japan, Pacific Ocean	17,800,695	12,798,967	120,707	245,694	492	203,527	10,200	35.8	37.2
Tonicellidae											
Tonicella lineata (Wood, 1815)	SRR6926331	USA, Atlantic Ocean	52,590,364	45,056,892	183,125	257,094	1,199	216,318	57,386	94.5	4.1
Callochitonidae											
Callochiton septemvalvis (Montagu, 1803)	MCZ-378036/ SRR13010089	Spain, Mediter-ranean	26,910,752	20,504,400	112,451	203,179	2,133	147,588	26,028	99.5	0.5
Chitonidae											
Rhyssoplax olivacea (Spengler, 1797)	MCZ-378064/ SRR618506	Spain, Mediter-ranean	23,189,291	11,294,052	161,349	279,802	825	191,249	25,054	57.0	34.9
Ischnochitonidae											
Stenoplax bahamensis Kaas & Van Belle, 1987	MCZ-384368/ SRR13010087	Belize, Atlantic Ocean	38,822,607	29,469,855	137,431	327,012	772	254,950	17,249	45.7	36.3
Leptochitonidae											
Lepidopleurus cajetanus (Poli, 1791)	SRX5063921	Spain, Mediter-ranean	42,198,797	28,421,760	81,028	95,311	343	77,667	7,395	14.1	26.6
Leptochiton rugatus (Carpenter in Pilsbry, 1892)	SRR1611558	USA, Atlantic Ocean	24,835,027	14,476,845	84,281	149,507	1,122	81,431	23,861	69.8	19.1
Solenogastres											
Pholidoskepia sp.	SRR1505105	USA, Pacific Ocean	40,587,186	10,702,010	70,570	127,028	514	91,829	15,014	55.9	30.3

use of phylogenomics to study molluscan phylogeny since a decade ago (Kocot *et al.*, 2011; Smith *et al.*, 2011).

The goal of this paper is to assess the current systematics of Poly-placophora, using a phylotranscriptomic approach, and to specifi-cally investigate the position of *Callochiton*, that is to clarify whether *Callochiton* belongs within Chitonina or is the sister group to ei-ther Chitonida or Lepidopleurida, under the recently recognized Callochitonida.

MATERIAL AND METHODS

Taxon sampling, library preparation and sequencing

All samples were flash-frozen in liquid nitrogen or fixed in RNA*later*® (Life Technologies, Carlsbad, CA, USA) and stored at −80° C. Total RNA was extracted using TRIzol® (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions and

as described by Lemer *et al.* (2015). mRNA purification, library construction and amplification were carried out using the KAPA mRNA HyperPrep Kit (Illumina, San Diego, CA, USA), follow-ing the manufacturer's protocol. We sequenced the cDNA from four polyplacophorans using an Illumina HiSeq 2500 platform (Table 1). Reads were deposited in the Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under the BioProject ac-cession no. PRJNA674863. These were combined with the six published transcriptomes of *Acanthochitona crinita*, *Rhyssoplax olivacea*, *Lepidopleurus cajetanus*, *Leptochiton rugatus* and *Tonicella lineata*, and a solenogaster species of the order Pholidoskepia, all of which were downloaded from NCBI SRA base using sratoolkit v. 2.7.

Transcriptome assembly

All transcriptomes were assembled *de novo*. The total number of sequencing reads before and after the filtering steps mentioned
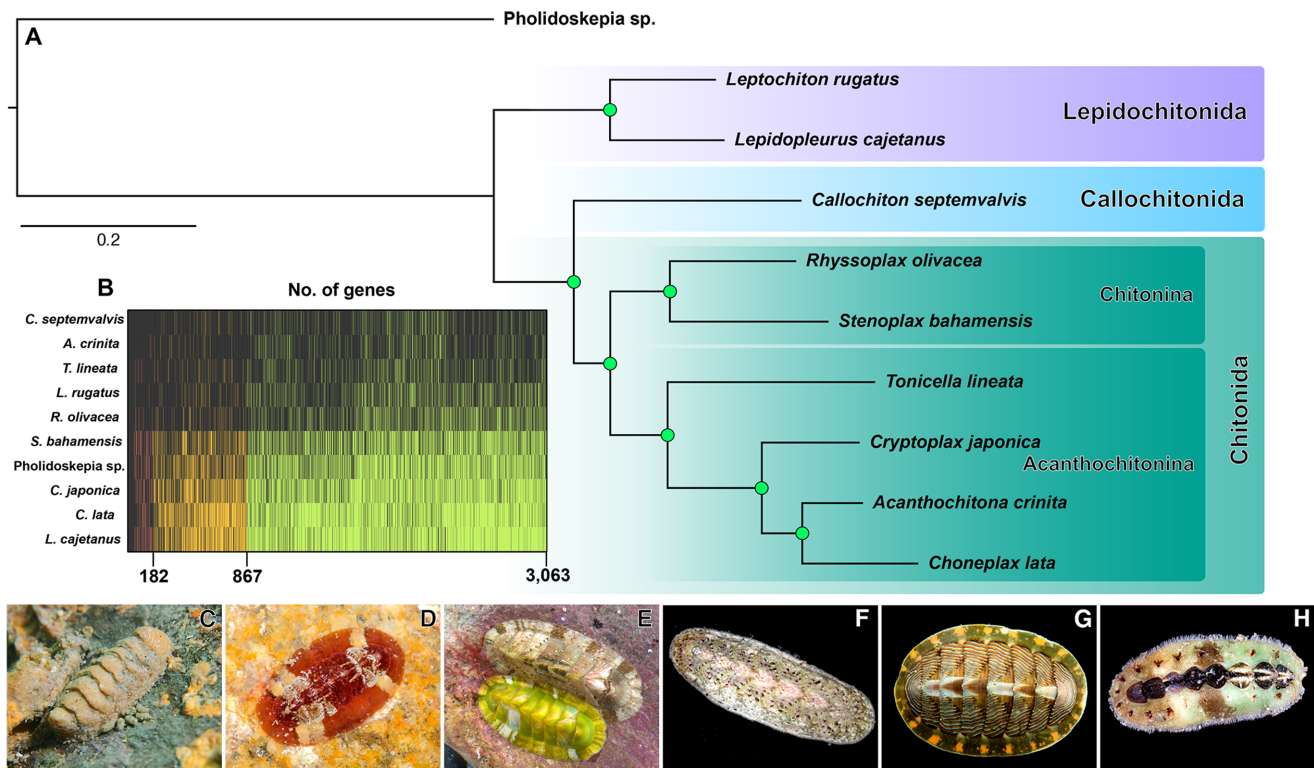
Figure 1. A. The phylogeny of chitons is based on the topology of the ML-cat analysis of the 50% occupancy matrix, which was congruent with all other ML-cat, ML-part, BI and CM analyses. All branches in all analyses received maximal bootstrap support and posterior probability values (denoted by green circles). The tree is rooted with the Solenogastres Pholidoskepia sp. The three polyplacophoran orders, Lepidochitonida, Callochitonida and Chitonida (including Chitonina and Acanthochitonina), are colour coded in different shades of blue. The scale bar indicates substitutions per site. **B.** Taxon occupancy matrices used in the phylogenetic analyses, including the largest matrix with 3,063 genes (50% completeness; in green), a subset with 867 genes (70% completeness; in orange) and one with 182 genes (90% completeness; in red). Genes and species are ordered with the best sampling on the upper left; black cells indicate genes present for each species. **C–H.** Living examples of taxa studied. **C.** *Lepidopleurus cajetanus*. **D.** *Callochiton septemvalvis*. **E.** *Rhyssoplax olivacea*. **F.** *Stenoplax bahamensis*. **G.** *Tonicella lineata*. **H.** *Choneplax lata*. Image credits: **C**, X. Salvador; **D**–**H**, G. Giribet.

below is specified in Table 1. Rcorrector v. 3.0 was used to correct random sequencing errors in Illumina RNA-seq reads (Song & Florea, 2015). The second round of quality threshold filtering was conducted with Trim Galore! v. 3 (Krueger, 2018) setting the quality threshold minimum Phred score to 33 and removing reads <50 bp long. Undesirable ribosomal RNA (rRNA) and mitochondrial sequences were filtered out by mapping reads to downloaded mollusc datasets (SILVA + GenBank) with Bowtie2 v. 2.3.2 (Langmead & Salzberg, 2012). Filtered paired-end reads were *de novo* assembled in Trinity v. 2.4 (Haas *et al.*, 2013). A second round of Bowtie2 filtering was then performed on the assembled transcriptomes to ensure the removal of remaining undesirable rRNA sequences. We used CD-HIT-EST v. 4.6.4 with an identity clustering threshold of 95% to reduce sequence redundancy and improve the performance of downstream analyses (Fu *et al.*, 2012). Afterwards, transcripts for each assembly were scanned for open reading frames and translated into amino acids using TransDecoder v. 3.0 (Haas *et al.*, 2013). Amino acid sequences were then grouped into isoforms and the longest ones were selected for each isoform group using a custom script, *choose_longestMOD_v2.py* (Lemer et al., 2019).

The completeness of our assembled transcriptomes was checked by running them against the BUSCO v. 3.0.2 (Table 1; Simão *et al.*, 2015) Metazoa odb9 dataset (978 total genes). OMA v. 2.2 was used to identify orthologs among peptide assemblies (Altenhoff *et al.*, 2019). The number of ortholog groups per sample before and after matrix selection can be found in Table 1. Ortholog groups were aligned using MAFFT v. 7.407, algorithm L-INS-I (Katoh & Standley, 2013). Alignment ends were trimmed from the alignments using the custom script *trimEnds.sh* (Cunha & Giribet, 2019).

Ortholog matrices accounting for 50%, 70% and 90% taxon occupancy were selected to perform phylogenetic analyses. Trimmed alignments were concatenated using Phyutility v. 3 (Smith & Dunn, 2008). Occupancy matrices were visualized in RStudio v. 1.1.447 (Fig. 1B). Ortholog matrices are available on the Harvard Dataverse (https://doi.org/10.7910/DVN/NUVECG).

Phylogenetic analyses

Species trees were inferred using maximum likelihood (ML) implemented in IQ-TREE-MPI v. 1.5.5 (Nguyen *et al.*, 2015). We used (1) a model search including the C10 to C60 profile mixture models (Quang, Gascuel & Lartillot, 2008), ML variants of the Bayesian CAT model (Lartillot & Philippe, 2004; hereafter ML-cat), and (2) ModelFinder (Kalyaanamoorthy *et al.*, 2017) to set a model for each partition (hereafter ML-part). The top 10% partition merging schemes of ML-part were examined via a relaxed hierarchical clustering algorithm to reduce computational burden (Lanfear *et al.*, 2014). Branch support was estimated via ultrafast bootstraps (1,500 replicates). Bayesian inference (BI) was executed in ExaBayes 1.5 (Aberer, Kobert & Stamatakis, 2014) using the GTRGAMMA model on the unpartitioned datasets, with three coupled Markov chain Monte Carlo (MCMC) runs, each with 1 million generations and sampling every 500 generations. The first 25% of trees were discarded as burn-in for each MCMC run. Convergence was considered acceptable when effective sample size was >200 and potential scale reduction factor was ≈1. Convergence was confirmed by assessing whether the maximum and average standard deviation of split frequencies were <0.001. Topological robustness was assessed

using posterior probabilities. A coalescent method (CM) based on gene trees for the reconstruction of species trees was performed using ASTRAL-III (Zhang *et al.*, 2018), with initial gene trees for each orthogroup analysed in RAxML v. 8.2.11 (Stamatakis, 2014). Trees were visualized in FigTree v. 1.4.4 (Rambaut, 2014) and edited in Inkscape v. 1 (Inkscape Project, 2020) (Fig. 1A).

RESULTS AND DISCUSSION

All analyses found a basal divergence between Lepidopleurida and the remaining taxa, with *Callochiton septemvalvis* being the sister group to all other Chitonida included in this analysis (Fig. 1A), and thus supporting the division of Polyplacophora into three main lineages, Lepidopleurida, Callochitonida and Chitonida. Our phylogeny agrees with previous Sanger sequence-based analyses, which included numerous chitons (Wilson *et al.*, 2010; Stöger *et al.*, 2013; Irisarri *et al.*, 2014), as well as with recently published mitogenomic analyses (Irisarri *et al.*, 2020). We also found that Chitonida is divided into Chitonina and Acanthochitonina, the latter containing Mopalioidea and Cryptoplacoidea; again, this being consistent with published studies (e.g. Sirenko, 2006; Sigwart *et al.*, 2013; Irisarri *et al.*, 2020). While other classifications include Callochitonidae in Chitonida (Ponder *et al.*, 2020), they are unable to accommodate the unique position of this taxon. For example, maintaining the division of Chitonida into Chitonina and Acanthochitonina—a system currently followed by Ponder *et al.* (2020), which lists Callochitonidae as a family in the suborder Chitonina (Chitonoidea: Chitonida)—would result in paraphyly of these three taxa. Likewise, the topology of Irisarri *et al.*'s (2020) phylogeny recognized Lepidopleurida and Chitonida but left Callochitonidae as a family outside both these orders. A sister-group relationship between Callochitonidae and Acanthochitonina has also been proposed based on a handful of standard Sanger markers (Sigwart *et al.*, 2013), but the rooting of the trees with Lepidopleurida did not test this position with an outgroup outside of Polyplacophora.

Our results are consistent and strongly supported, and support the classification of Giribet & Edgecombe (2020), who recognized three orders of chitons. Callochitonida is, thus, recognized as an order that shares a combination of characters with both Lepidopleurida and Chitonida. These characters are an egg hull similar to that of Lepidopleurida, and therefore possibly plesiomorphic, and sperm with the filamentous nuclear extension and reduced acrosome typical of Chitonida (Buckland-Nicks & Hodgson, 2000; Buckland-Nicks & Reunov, 2010). Callochitonida's valve insertion plates and lateral gill placement are additional characters that are shared with Chitonida (Sirenko, 2006).

Our resolution of internal relationships within Chitonida is compatible with that of most recent analyses of this group, with Chitonida divided into Chitonina and Acanthochitonina, the latter containing Mopalioidea and Cryptoplacoidea (e.g. Sirenko, 2006; Sigwart *et al.*, 2013; Irisarri *et al.*, 2020). Our phylotranscriptomics-based approach agrees well with earlier work on chiton phylogeny and, despite the small number of taxa used, has yielded consistent and well-supported relationships. Furthermore, the novel transcriptomic resources provided here will be able to inform probe design in enrichment-based phylogenomic studies using high-throughput sequencing of hundreds of genes (e.g. Faircloth *et al.*, 2012; Abdelkrim *et al.*, 2018; Quattrini *et al.*, 2018; Moles & Giribet, 2021).

ACKNOWLEDGEMENTS

REFERENCES

ABDELKRIM, J., FEDOSOV, A., KANTOR, Y., LOZOUET, P., PHUONG, M., ZAHARIAS, P. & PUILLANDRE, N. 2018. Exon-capture based phylogeny and diversification of the venomous gastropods (Neogastropoda, Conoidea). *Molecular Biology and Evolution*, **35**: 2355–2374.

ABERER, A.J., KOBERT, K. & STAMATAKIS, A. 2014. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. *Molecular Biology and Evolution*, **31**: 2553–2556.

ALTENHOFF, A.M., LEVY, J., ZAROWIECKI, M., TOMICZEK, B., VESZTROCY, A.W., DALQUEN, D.A., MÜLLER, S., TELFORD, M.J., GLOVER, N.M., DYLUS, D. & DESSIMOZ, C. 2019. OMA standalone: orthology inference among public and custom genomes and transcriptomes. *Genome Research*, **29**: 1152–1163.

BUCKLAND-NICKS, J. 2008. Fertilization biology and the evolution of chitons. *American Malacological Bulletin*, **25**: 97–111.

BUCKLAND-NICKS, J., CHIA, F-S. & KOSS, R. 1990. Spermiogenesis in Polyplacophora, with special reference to acrosome formation (Mollusca). *Zoomorphology*, **109**: 179–188.

BUCKLAND-NICKS, J. & HODGSON, A.N. 2000. Fertilization in *Callochiton castaneus* (Mollusca). *Biological Bulletin*, **199**: 59–67.

BUCKLAND-NICKS, J. & REUNOV, A.A. 2010. Egg hull formation in *Callochiton dentatus* (Mollusca, Polyplacophora): the contribution of microapocrine secretion. *Invertebrate Biology*, **129**: 319–327.

CUNHA, T.J. & GIRIBET, G. 2019. A congruent topology for deep gastropod relationships. *Proceedings of the Royal Society B*, **286**: 20182776.

FAIRCLOTH, B.C., McCORMACK, J.E., CRAWFORD, N.G., HARVEY, M.G., BRUMFIELD, R.T. & GLENN, T.C. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, **61**: 717–726.

FU, L., NIU, B., ZHU, Z., WU, S. & LI, W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics*, **28**: 3150–3152.

GIRIBET, G. & EDGECOMBE, G.D. 2020. *The invertebrate tree of life*. Princeton University Press, Princeton, NJ.

GIRIBET, G., OKUSU, A., LINDGREN, A.R., HUFF, S.W., SCHRÖDL, M. & NISHIGUCHI, M.K. 2006. Evidence for a clade composed of molluscs with serially repeated structures: monoplacophorans are related to chitons. *Proceedings of the National Academy of Sciences of the USA*, **103**: 7723–7728.

HAAS, B.J., PAPANICOLAOU, A., YASSOUR, M., GRABHERR, M., BLOOD, P.D., BOWDEN, J., COUGER, M.B., ECCLES, D., LI, B., LIEBER, M., MacMANES, M.D., OTT, M., ORVIS, J., POCHET, N., STROZZI, F., WEEKS, N., WESTERMAN, R., WILLIAM, T., DEWEY, C.N., HENSCHEL, R., LEDUC, R.D., FRIEDMAN, N. & REGEV, A. 2013. *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, **8**: 1494–1512.

INKSCAPE PROJECT. 2020. Inkscape. Available at: https://inkscape.org. Accessed 3 November 2020.

IRISARRI, I., EERNISSE, D.J. & ZARDOYA, R. 2014. Molecular phylogeny of Acanthochitonina (Mollusca: Polyplacophora: Chitonida): three new mitochondrial genomes, rearranged gene orders and systematics. *Journal of Natural History*, **48**: 2825–2853.

IRISARRI, I., URIBE, J.E., EERNISSE, D.J. & ZARDOYA, R. 2020. A mitogenomic phylogeny of chitons (Mollusca: Polyplacophora). *BMC Evolutionary Biology*, **20**: 1–15.

KAAS, P. & VAN BELLE, R.A. 1990. *Monograph of living chitons (Mollusca: Polyplacophora)*. E.J. Brill, Leiden.

KALYAANAMOORTHY, S., MINH, B.Q., WONG, T.K.F., von HAESELER, A. & JERMIIN, L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**: 587–589.

KATOH, K. & STANDLEY, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**: 772–780.

KOCOT, K.M., CANNON, J.T., TODT, C., CITARELLA, M.R., KOHN, A.B., MEYER, A., SANTOS, S.R., SCHANDER, C., MOROZ, L.L., LIEB, B. & HALANYCH, K.M. 2011. Phylogenomics reveals deep molluscan relationships. *Nature*, **477**: 452–456.

KRUEGER, F. 2018. Trim Galore. Available at: https://github.com/FelixKrueger/TrimGalore. Accessed 22 June 2019.

LANFEAR, R., CALCOTT, B., KAINER, D., MAYER, C. & STAMATAKIS, A. 2014. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology*, **14**: 1–14.

LANGMEAD, B. & SALZBERG, S.L. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods*, **9**: 357–359.

LARTILLOT, N. & PHILIPPE, H. 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution*, **21**: 1095–1109.

LEMER, S., BIELER, R. & GIRIBET, G. 2019. Resolving the relationships of clams and cockles: dense transcriptome sampling drastically improves the bivalve tree of life. *Proceedings of the Royal Society B*, **286**: 20182684.

LEMER, S., KAWAUCHI, G.Y., ANDRADE, S.C.S., GONZÁLEZ, V.L., BOYLE, M.J. & GIRIBET, G. 2015. Re-evaluating the phylogeny of Sipuncula through transcriptomics. *Molecular Phylogenetics and Evolution*, **83**: 174–183.

LI, L., CONNORS, M.J., KOLLE, M., ENGLAND, G.T., SPEISER, D.I., XIAO, X., AIZENBERG, J. & ORTIZ, C. 2015. Multifunctionality of chiton biomineralized armor with an integrated visual system. *Science*, **350**: 952–956.

MOLES, J. & GIRIBET, G. 2021. A polyvalent and universal tool for genomic studies in gastropod molluscs (Heterobranchia). *Molecular Phylogenetics and Evolution*, **155**: 106746.

NGUYEN, L.T., SCHMIDT, H.A., von HAESELER, A. & MINH, B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**: 268–274.

OKUSU, A., SCHWABE, E., EERNISSE, D.J. & GIRIBET, G. 2003. Towards a phylogeny of chitons (Mollusca, Polyplacophora) based on combined analysis of five molecular loci. *Organisms Diversity & Evolution*, **3**: 281–302.

PONDER, W.F., LINDBERG, D.R. & PONDER, J.M. 2020. *Biology and evolution of the Mollusca*. Vol. 2. CRC Press, Boca Raton, FL.

QUANG, L.S., GASCUEL, O. & LARTILLOT, N. 2008. Empirical profile mixture models for phylogenetic reconstruction. *Bioinformatics*, **24**: 2317–2323.

QUATTRINI, A.M., FAIRCLOTH, B.C., DUEÑAS, L.F., BRIDGE, T.C.L., BRUGLER, M.R., CALIXTO-BOTÍA, I.F., DELEO, D.M., FORÊT, S., HERRERA, S., LEE, S.M.Y., MILLER, D.J., PRADA, C., RÁDIS-BAPTISTA, G., RAMÍREZ-PORTILLA, C., SÁNCHEZ, J.A., RODRÍGUEZ, E. & McFADDEN, C.S. 2018. Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: new approaches to long-standing problems. *Molecular Ecology Resources*, **18**: 281–295.

RAMBAUT, A. 2014. *FigTree, a graphical viewer of phylogenetic trees*. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.

SIGWART, J.D. 2009. Morphological cladistic analysis as a model for character evaluation in "primitive" living chitons (Polyplacophora, Lepidopleurina). *American Malacological Bulletin*, **27**: 95–104.

SIGWART, J.D. 2017. Deep trees: woodfall biodiversity dynamics in present and past oceans. *Deep Sea Research Part II: Topical Studies in Oceanography*, **137**: 282–287.

SIGWART, J.D. & SCHWABE, E. 2017. Anatomy of the many feeding types in polyplacophoran molluscs. *Invertebrate Zoology*, **14**: 205–216.

SIGWART, J.D., SCHWABE, E., SAITO, H., SAMADI, S. & GIRIBET, G. 2010. Evolution in the deep sea: a combined analysis of the earliest diverging living chitons (Mollusca: Polyplacophora: Lepidopleurida). *Invertebrate Systematics*, **24**: 560–572.

SIGWART, J.D., STOEGER, I., KNEBELSBERGER, T. & SCHWABE, E. 2013. Chiton phylogeny (Mollusca: Polyplacophora) and the placement of the enigmatic species *Choriplax grayi* (H. Adams & Angas). *Invertebrate Systematics*, **27**: 603–621.

SIGWART, J.D., SUMNER-ROONEY, L.H., SCHWABE, E., HESS, M., BRENNAN, G.P. & SCHRÖDL, M. 2014. A new sensory organ in "primitive" molluscs (Polyplacophora: Lepidopleurida), and its context in the nervous system of chitons. *Frontiers in Zoology*, **1**: 17.

SIMÃO, F.A., WATERHOUSE, R.M., IOANNIDIS, P, KRIVENTSEVA, E.V. & ZDOBNOV, E.M. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, **31**: 3210–3212.

SIRENKO, B.I. 2006. New outlook on the system of chitons (Mollusca: Polyplacophora). *Venus*, **65**: 27–49.

SMITH, S.A. & DUNN, C.W. 2008. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics*, **24**: 715–716.

SMITH, S.A., WILSON, N.G., GOETZ, F.E., FEEHERY, C., ANDRADE, S.C.S., ROUSE, G.W., GIRIBET, G. & DUNN, C.W. 2011. Resolving the evolutionary relationships of molluscs with phylogenomic tools. *Nature*, **480**: 364–367.

SONG, L. & FLOREA, L. 2015. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. *GigaScience*, **4**: 1–8.

SPEISER, D.I., EERNISSE, D.J. & JOHNSEN, S. 2011. A chiton uses aragonite lenses to form images. *Current Biology*, **21**: 665–670.

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**: 1312–1313.

STÖGER, I., SIGWART, J.D., KANO, Y., KNEBELSBERGER, T., MARSHALL, B.A., SCHWABE, E. & SCHRÖDL, M. 2013. The continuing debate on deep molluscan phylogeny: evidence for Serialia (Mollusca, Monoplacophora + Polyplacophora). *BioMed Research International*, **2013**: 1.

SUMNER-ROONEY, L.H., MURRAY, J.A., CAIN, S.A. & SIGWART, J.D. 2014. Do chitons have a compass? Evidence for magnetic sensitivity in Polyplacophora. *Journal of Natural History*, **48**: 3033–3045.

SUMNER-ROONEY, L.H. & SIGWART, J.D. 2015. Is the Schwabe organ a retained larval eye? Anatomical and behavioural studies of a novel sense organ in adult *Leptochiton asellus* (Mollusca, Polyplacophora) indicate links to larval photoreceptors. *PLoS One*, **10**: e0137119.

WILSON, N.G., ROUSE, G.W. & GIRIBET, G. 2010. Assessing the molluscan hypothesis Serialia (Monoplacophora + Polyplacophora) using novel molecular data. *Molecular Phylogenetics and Evolution*, **54**: 187–193.

ZHANG, C., RABIEE, M., SAYYARI, E. & MIRARAB, S. 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics*, **19**: 15–30.