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RESEARCH PAPER

Phylogenomic analyses shed light on the relationships of chiton superfamilies and shell-eye evolution

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Abstract

Mollusca is the second-largest animal phylum with over 100,000 extant species representing eight classes. Across 1000 extant species in the class Polyplacophora, chitons have a relatively constrained morphology but with some notable deviations. Several genera possess "shell eyes", i.e., true eyes with a lens and retina that are embedded within the dorsal shells. The phylogeny of the major chiton clades is mostly well established, in a set of superfamily-level and higher level taxa supported by various approaches, including morphological studies, multiple gene markers, mitogenome-phylogeny, and phylotranscriptomic approaches. However, one critical lineage has remained unclear, namely *Schizochiton* which was controversially suggested as being the potential independent origin of chiton shell eyes. Here, with the draft genome sequencing of *Schizochiton incisus* (superfamily Schizochitonoidea) plus assemblies of transcriptome data from other polyplacophorans, we present phylogenetic reconstructions using both mitochondrial genomes and phylogenomic approaches with multiple methods. We found that phylogenetic trees from mitogenomic data are inconsistent, reflecting larger scale confounding factors in molluscan mitogenomes. However, a consistent and robust topology was generated with protein-coding genes using different models and methods. Our results support Schizochitonoidea as the sister group to other Chitonoidea in Chitonina, in agreement with the established classification. Combined with evidence from fossils, our phylogenetic results suggest that the earliest origin of shell eyes is in Schizochitonoidea, and that these structures were also gained secondarily in other genera in Chitonoidea. Our results have generated a holistic review of the internal relationship within Polyplacophora, and a better understanding of the evolution of Polyplacophora.

Keywords Chiton · Mollusca · Phylogenomics · Polyplacophora · Shell eyes

Introduction

Molluscs represent the second most speciose animal phylum with the broadest morphological disparity of body plans. The class Polyplacophora, also known as chitons, includes around 1000 extant species and over 400 fossil species (Stebbins and Eernisse 2009). Chitons are exclusively marine, and their most distinctive feature is eight separate aragonitic valves or plates on their dorsal side (Irisarri et al. 2020; Ladd 1966; Stebbins and Eernisse 2009). They attach to the substratum with a muscular ventral foot and feed with an iron-mineralised radula (Joester and Brooker 2016). They have no head or cephalised senses, and therefore lack conventional eyes. However, the dorsal valves are densely perforated with a complex array of sensory organs called aesthetes. These aesthete pores dot the dorsal shell surface and can have densities over 1000 mm^{-2}.

Special Topic: EvoDevo.

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Aesthetes are present in all chitons, with substantial differences in morphology, arrangement, densities, and presumably also functions, and aesthete pore morphology is often used to discriminate species in taxonomic descriptions (Sirenko 2006). In most cases, the aesthete organs of chitons consist of unpigmented cells (Speiser et al. 2014). In certain species (including the genera *Callochiton* and *Chiton*), some aesthetes contain pigmented cells (Okusu et al. 2003; Sigwart et al. 2013; Sirenko 2006), also referred to as "intrapigmented aesthetes" (Baxter et al. 1990; Haas and Kriesten 1978). In the most elaborate variation of the aesthete organs, in a few genera, some of the larger "megalaesthete" pores have further developed into shell eyes. These are true eyes, embedded in the shell matrix, with a crystalline lens and a pigmented photoreceptive retina (Chappell and Speiser 2023; Li et al. 2015; Sigwart and Sumner-Rooney 2020; Speiser et al. 2011).

Members of two chiton families possess shell eyes: Schizochitonidae and Chitonidae. Schizochitonidae contains two living species in the genus *Schizochiton*, which both have shell eyes. In Chitonidae, the shell eyes are found only in a few genera in the two subfamilies Acanthopleurinae and Toniciinae. The only previous molecular phylogenetic study that included *Schizochiton* was that of Okusu et al. (2003) who reported that five gene fragments resolved *Schizochiton* as sister to all other species in the suborder Chitonina, but also suggested that the phylogenetic position of *S. incisus* was "unstable" and deserved further discussion. Although the question of shell eyes was not discussed by Okusu et al. (2003), the topology and the paraphyly of shell eyes and the unresolved phylogenetic position of *Schizochiton* raised the possibility that shell-eye structures evolved in two separate events. However, in the last 20 years, this hypothesis has not been tested further due to a lack of appropriate *Schizochiton* specimen material for molecular phylogenetic analyses.

Phylogenetic systematics of Polyplacophora has been developed using both morphological and molecular characters (Winston et al. 2020). Extant chitons are divided into three well-resolved orders: Lepidopleurida, Callochitonida, and Chitonida (Giribet and Edgecombe 2020). Lepidopleurida consists of mainly deep-sea species with distinctive morphological synapomorphies including aesthete arrangement, gills, and a specialized sense organ called the Schwabe Organ (Sigwart et al. 2014). The position of *Callochiton* was equivocal in earlier studies but usually resolved as sister to Chitonida (Koch and Lambert 1990; Sigwart et al. 2013) and the single family Callochitonidae is now recognized as comprising a separate order-ranked clade Callochitonida (Giribet and Edgecombe 2020; Irisarri et al. 2014, 2020; Moles et al. 2021; Sigwart et al. 2013). Most living chitons are in the order Chitonida, which is further divided into two suborders, Chitonina (including two superfamilies, Chitonoidea and Schizochitonoidea) and Acanthochitonina (including two superfamilies, Mopalioidea and Cryptoplacoidea). The backbone phylogeny of chitons is well understood, especially at the level of superfamilies, for all clades except for Schizochitonoidea.

Various genomic and transcriptomic data for Polyplacophora are now available on NCBI but were generated independently for several different research purposes (Table 1). There are only two chiton genomes available, *Acanthopleura granulata* (Varney et al. 2021) and *Hanleya hanleyi* (Varney et al. 2022). Meanwhile, two independent phylogenomic studies based on transcriptome sequencing generated data for species and genera that cover all valid superfamilies: *Callochiton*, *Tonicia schrammi*, *Chiton tuberculatus*, *Chiton marmoratus*, *Chaetopleura apiculata*, *Lepidozona mertensii*, *Mopalia muscosa*, *Katharina tunicata*, *Tonicella lineata*, *Nutallochiton* sp., *Cryptoplax japonica* and *Crytoplax larvaeformis* (Varney et al. 2021) and *Lepidopleurus cajetanus* (SRX5063921), *Callochiton septemvalvis*, *Stenoplax bahamensis*, *Cryptoplax japonica* and *Choneplax lata* (Moles et al. 2021). There are also some other studies examining gene expression profiles, which include *Leptochiton cascadiensis* (Halanych and Kocot 2014), *Acanthopleura loochooana* (Liu et al. 2022), *Rhyssoplax olivacea* (Riesgo et al. 2012), *Cryptochiton stelleri* (Nemoto et al. 2019), *Acanthochitona crinita* (De Oliveira et al. 2016), *Acanthochitona rubrolineata* (SRP179406), and *Acanthochitona fascicularis* (SRR13862580).

The growing body of transcriptome data can support a phylogenomic construction with larger taxon coverage. *Schizochiton* occupies an important position for us to better understand chiton evolution, especially chiton shell eyes. Thus, we collected a new sample of *Schizochiton incisus* from the South China Sea (Fig. 1) to sequence and assemble its genome and mitogenome. Combining this with other available chiton data from NCBI and previous studies, we aimed to reconstruct a phylogeny of Polyplacophora at the superfamily level with different phylogenomic inferences and tree reconstruction methods, specifically to test the positions of *S. incisus* and Schizochitonoidea.

Results

Mitochondrial genome

We assembled the complete mitochondrial genome of *S. incisus*, which was 15,491 bp in length circularized with 13 PCGs, 2 rRNA, and 22 tRNA, a typical mitogenome architecture of bilaterians. Protein-coding genes are coded with normal invertebrate mitochondrial codons, including the start and stop codons. The mitogenome of *S. incisus* follows the proposed hypothetical ancestral gene order for

Table 1 Statistics of chiton genomes and transcriptomes used in this study, including number of contigs and BUSCO scores after filtering

Species	SRA No.	No. of proteins	BUSCO score	Source
Lepidopleurida				
Hanleya hanleyi	SRR11674123	47,786	C:81.7%[S:80.7%,D:1.0%]	Varney, Yap-Chiongco et al. 2022
Lepidopleurus cajetanus	SRX5063921	10,479	C:12.9%[S:12.7%,D:0.2%]	------
Leptochiton asellus	------	**81,610**	**C:94.7%[S:86.9%,D:7.8%]**	**this study**
Leptochiton cascadiensis	SRR1611558	23,030	C:79.4%[S:77.9%,D:1.5%]	Halanych and Kocot 2014
Callochitonida				
Callochiton septemvalvis	SRR13010089	30,618	C:95.9%[S:87.8%,D:8.1%]	Moles, Cunha et al. 2021
Callochiton sp.	SRR11674125	8235	C:28.2%[S:26.1%,D:2.1%]	Varney, Speiser et al. 2021
Chitonida				
Acanthopleura granulata	------	19,621	C:93.8%[S:93.3%,D:0.5%]	Varney, Speiser et al. 2021
Acanthopleura loochooana	------	44,182	C:90.4%[S:85.3%,D:5.1%]	Liu, Liu et al. 2022
Tonicia schrammi	SRR11674132	16,274	C:67.4%[S:67.1%,D:0.3%]	Varney, Speiser et al. 2021
Chiton tuberculatus	SRR11674134	18,002	C:83.2%[S:82.8%,D:0.4%]	Varney, Speiser et al. 2021
Chiton marmoratus	SRR11674135	5848	C:26.5%[S:26.5%,D:0.0%]	Varney, Speiser et al. 2021
Rhyssoplax olivacea	SRR618506	27,356	C:67.1%[S:65.3%,D:1.8%]	Riesgo, Andrade et al. 2012
Chaetopleura apiculata	SRR11674124	18,915	C:79.3%[S:79.1%,D:0.2%]	Varney, Speiser et al. 2021
Lepidozona mertensii	SRR11674130	13,531	C:72.1%[S:71.5%,D:0.6%]	Varney, Speiser et al. 2021
Stenoplax bahamensis	SRR13010087	24,602	C:39.7%[S:39.0%,D:0.7%]	Moles, Cunha et al. 2021
Schizochiton incisus	------	**20,902**	**C:40.9%[S:37.5%,D:3.4%]**	**this study**
Cryptochiton stelleri	DRP005555	19,101	C:82.2%[S:81.7%,D:0.5%]	Nemoto, Ren et al. 2019
Mopalia muscosa	SRR11577121	13,262	C:77.0%[S:76.6%,D:0.4%]	Varney, Speiser et al. 2021
Katharina tunicata	SRR11674131	15,542	C:89.7%[S:88.4%,D:1.3%]	Varney, Speiser et al. 2021
Tonicella lineata	SRR11577222	13,780	C:79.0%[S:77.7%,D:1.3%]	Varney, Speiser et al. 2021
Nutallochiton sp.	SRR11674133	57,110	C:74.3%[S:67.4%,D:6.9%]	Varney, Speiser et al. 2021
Cryptoplax japonica	SRR13010086	14,963	C:34.6%[S:34.3%,D:0.3%]	Moles, Cunha et al. 2021
Crytoplax larvaeformis	SRR11674126	20,128	C:88.1%[S:87.7%,D:0.4%]	Varney, Speiser et al. 2021
Choneplax lata	SRR13010088	16,971	C:14.3%[S:13.4%,D:0.9%]	Moles, Cunha et al. 2021
Acanthochitona rubrolineata	SRP179406	44,221	C:91.8%[S:71.2%,D:20.6%]	------
Acanthochitona crinita	SRR5110525	22,678	C:91.4%[S:91.0%,D:0.4%]	De Oliveira, Wollesen et al. 2016
Acanthochitona fascicularis	SRR13862580	17,427	C:88.9%[S:88.5%,D:0.4%]	------

Polyplacophora (Irisarri et al. 2020), except for an inversion of trnG-trnE, which is also found in Vetigastropoda (Uribe et al. 2017). The mitogenome gene order seems to be relatively conserved in Polyplacophora compared to those in gastropods and bivalves (Irisarri et al. 2020).

Mitochondrial phylogeny

The phylogenetic trees reconstructed with mitogenome data showed significant discordance among different methods and matrices. There were three distinct topologies for the position of *S. incisus*, which were ((Chitonoidea, Schizochitonoidea), Acanthochitonina) (13PCGs with MFP, PB based on modified 3rd codon), ((Acanthochitonina, Schizochitonoidea), Chitonoidea) (13PCGs with C60, PB, PCGs + rRNA with MFP, PCGs + rRNA with PB) and ((Chitonoidea, Acanthochitonina), Schizochitonoidea) (modified 3rd codon), respectively. The statistical support of the *S. incisus* node was lower than 95% in all methods, except for BI, indicating that these nodes were not well supported with mitogenomic data. It is noteworthy that in addition

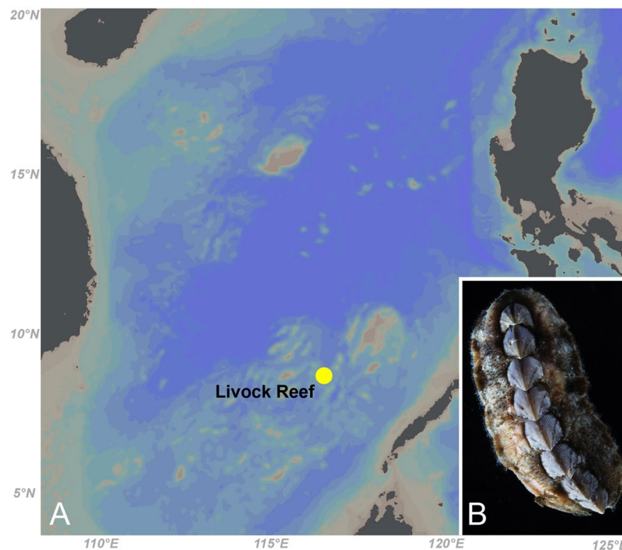

Fig. 1 *Schizochiton incisus*: **A** map indicating the location where the specimen used in this study was collected (yellow dot), **B** the live specimen, photo courtesy of Prof. Xiaoqi Zeng

to *Schizochiton,* the position of *Plaxiphora albida* also varied from one clade to another (Fig. 2). Furthermore, in the representative tree, *Tonicina zschaui* was sister to the rest of the Chitonoidea.

Genome and transcriptome assembly

Genome features of *S. incisus* were estimated with Illumina sequencing reads, which resulted in an estimated genome size of 1.1 Gband genome heterozygosity of 0.93%. Draft genome assembly from MaSuRCA generated a better result [C 73.8% (S: 68.1%, D: 5.7%)] than the Platanus version [C 17.9% (S: 13.7%, D: 4.2%)], and was used for down-stream analyses. After further scaffolding with protein sequences from other chitons with available genomes and removing heterozygous contigs, the final assembly has a BUSCO score of C 73.8%, N50 of 13.2 Kb and an assembled size of 971 Mb.

By collecting the evidence from the *ab initial* method and protein evidence, a total of 23,444 protein-coding genes were predicted in *S. incisus* with a BUSCO score of C: 40.8% (S: 37.0%, D: 3.8%) and F: 19.8%. Although the score is lower than the *Acanthopleura granulata* genome (Varney et al. 2021), 12,419 of the protein-coding genes (52%) can find their reciprocal best hits BLAST in *A. granulata*, suggesting a good coverage for phylogenomic analyses.

The transcriptome of *Leptochiton asellus* generated from five tissues was assembled into 390,724 contigs with an N50 value of 1.68 Kb, and a BUSCO score of C 94.5% (S: 83.1%,D: 11.4%). For the remaining transcriptome assembly of the publicly available data, the BUSCO completeness ranged from 12.9% (*Lepidopleurus*

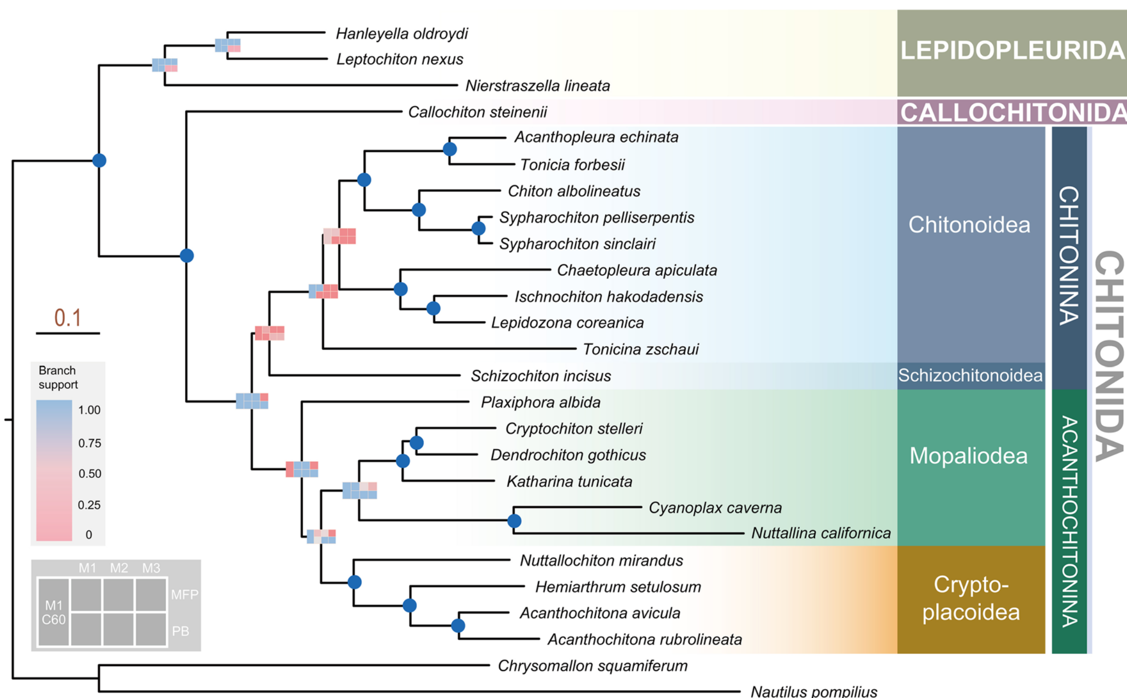

Fig. 2 Mitogenome phylogeny of Polyplacophora, reconstructed with multiple methods. M1–M3 indicate Matrix1–Matrix3; C60 and MFP represent the corresponding models implemented in IQ-Tree; PB, PhyloBayes

cajetanus) to 95.8% (*Callochiton septemvalvis*) (for the list of species and their corresponding BUSCO scores, see Table 1).

Phylogenomics

The phylogenomic analysis was based on a combination of transcriptome and genome data, covering all extant superfamilies in Polyplacophora (Table 1). There were four matrices generated by genesortR forming seven distinct phylogenetic signals. Minimum occupancy for all matrices was set to 50%. The sites contained in the four matrices are 696,897 (3593 genes, all genes, Matrix 1), 194,356 (best 800 genes, Matrix 2), 299,710 (best 1300 genes, Matrix 3), and 554,857 (best 2700 genes, Matrix 4), respectively (Fig. 3).

The phylogenetic trees reconstructed from nuclear data, including coalescent approach results, showed a high degree of consistency for the position of *S. incisus* as sister to Chitonoidea (Fig. 4). Support for this Schizochitonoidea + Chitonoidea clade retrieved node support of 100% in all analyses except for PMSF-C20 of Matrix1 (which was 59), showing a relatively stable topology. The support for all superfamily-level groups and their arrangement was consistently high. However, the positions of some tips are unstable, e.g., *Chaetopleura apiculata*, *Lepidozona mertensii*, and *Stenoplax bahamensis* were placed in variable positions within the superfamilies. The relationship of *Choneplax* relative to the members of genus *Acanthochitona* was also variable.

Topology test

We performed AU-tests on two topologies based on Matrix1 to determine the best-supported tree topology. And the given results by AU-test with *P*-value lower than 0.05 will be rejected. The results showed that the first tree topology ((Chitonoidea, Schizochitonoidea), Acanthochitonina) was accepted with a *P*-value of 0.952, and the second topology [(Acanthochitonina, Schizochitonoidea), Chitonoidea] was rejected with a *P*-value of 0.0476.

Discussion

The phylogenetic relationships of chitons at the order and superfamily levels are relatively stable and well resolved. Based on a consensus of molecular phylogenetic analyses and morpho-anatomical evidence, Polyplacophora is divided into three orders, Lepidopleurida, Callochitonida, and Chitonida (Irisarri et al. 2020; Moles et al. 2021), which is also recovered in the present analyses. At the superfamily level, previous molecular studies lacked data to test the position of Schizochitonoidea, and our results support the sister relationship of Chitonoidea + Schizochitonoidea in a monophyletic suborder Chitonina, as proposed from integrated morphological and anatomical evidence (Sirenko 2006).

Phylogeny based on mitochondrial data is considered a powerful tool to resolve relationships within Molluscs (Wang et al. 2023). But here, the mitochondrial data were much less informative than nuclear transcriptomic and

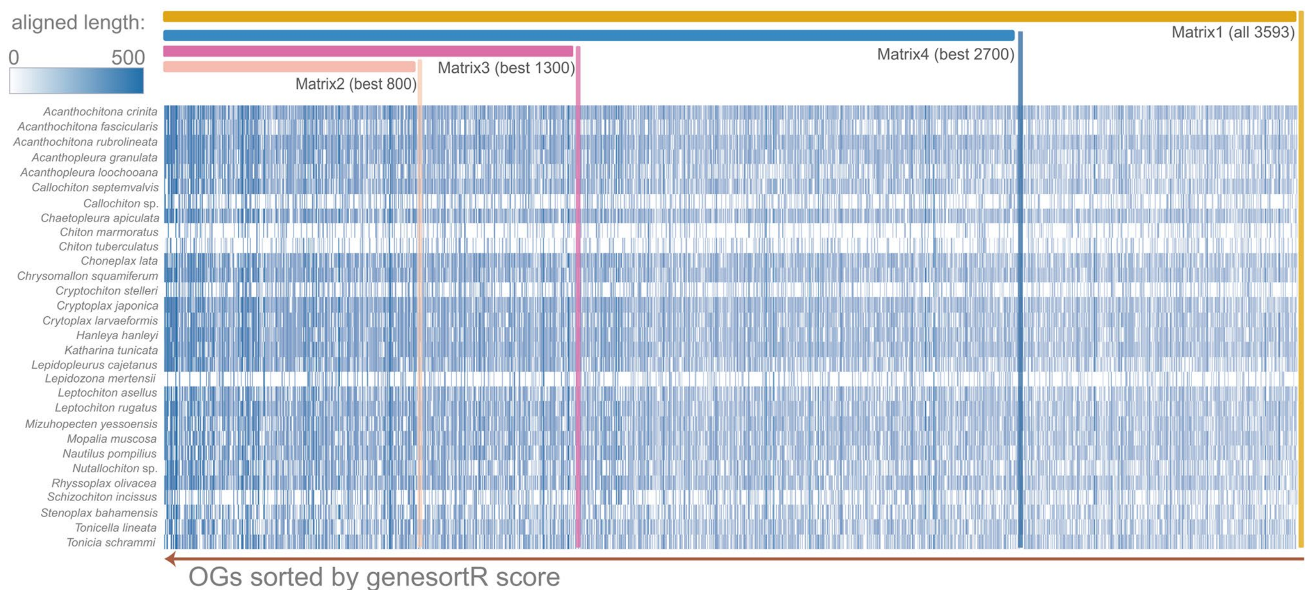

Fig. 3 The occupancy of the four matrices generated by genesortR

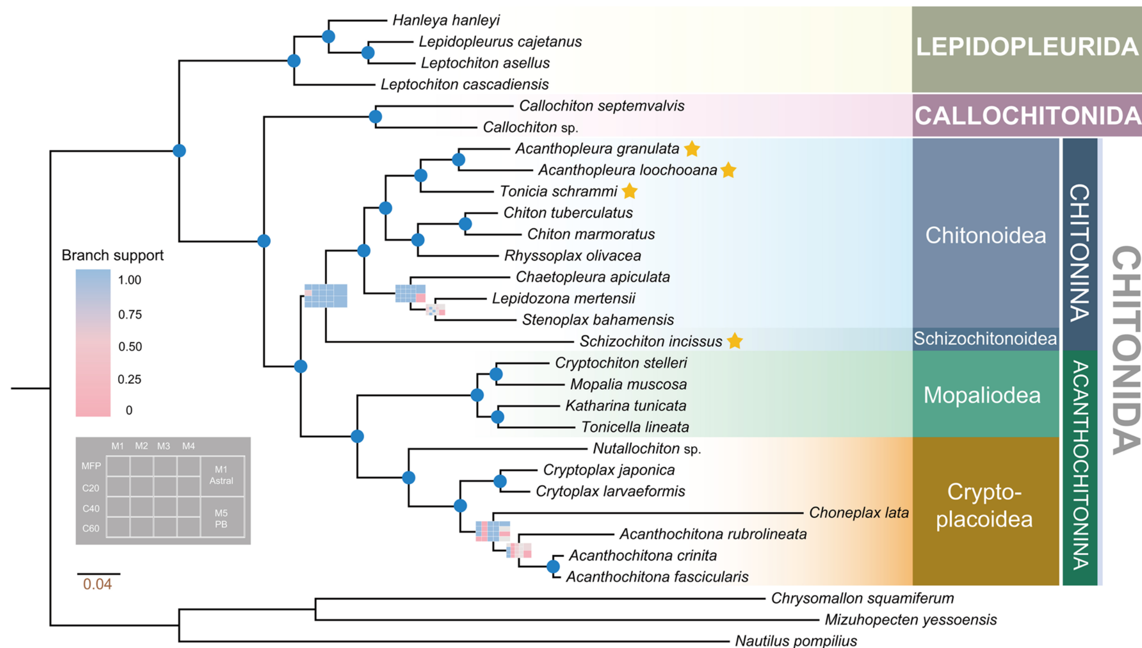

Fig. 4 Phylogeny of chitons, based on a phylogenomic approach using different methods. Node supported values are transferred to matrices colored with a gradient indicating a continuous scale from 0 (pink) to 100% (blue). Blue indicates 100% support and pink indicates that the topology is not supported by the representative tree. Nodes with blue dots indicate full support in all methods. Abbreviations: M1–M5, matrix 1–5; MFP, IQ-Tree MFP model; C20–C60, profile mixture models C20–C60; M1 Astral, coalescent analysis based on Matrix1; M5 PB, PhyloBayes analysis based on Matrix5. Species that possess shell eyes are marked with yellow stars

genomic data. We used mitogenome data of available chitons to reconstruct phylogenetic trees with different approaches, including ML and Bayesian inference, but the results for taxa below the rank of superfamily are unstable. For example, in the representative tree selected for mitochondrial analyses, *Tonicina zschaui* formed a sister group to other remaining Chitonoidea, whereas current systematics would predict a placement for *Tonicina* within the small clade formed by the genera *Lepidozona, Ischnochiton* and *Chaetopleura*. The topology we illustrated is not supported by four of seven trees reconstructed by corresponding methods, so this placement should be taken as unresolved. As already suggested in the previous mitogenome phylogeny of chitons (Irisarri et al. 2020), this could be a result of poor taxon sampling, but the robustness of the phylogeny was not improved by including a few additional taxa in the present study. Indeed, this issue of low phylogenetic signal in mitogenome phylogeny has also been raised in data from other molluscan classes, including Monoplacophora (Stoger et al. 2016), Gastropoda (Uribe et al. 2019), Cephalopoda (Uribe and Zardoya 2017), and Bivalvia (Doucet-Beaupré et al. 2010), and confounding features occur in many molluscan mitogenomes (Ghiselli et al. 2021).

Interestingly, *Schizochiton* possesses a unique mitogenome gene order, differing from all other chitons with available mitogenomes, which might imply relatively fast evolution of this genus. Mitogenome phylogenies are currently not reliable for reconstructing detailed phylogenies for Polyplacophora and potentially other molluscan clades. This may be improved with better taxon sampling or may be a fundamental problem of insufficient phylogenetic signal. It is clear that improved phylogenomic approaches are needed to reconstruct the phylogeny of chitons at or below superfamily-level resolution.

All the phylogenomic results for the main lineage in this study shared the same topology with strong nodal support except for Matrix1-C20. The topology is consistent with, what is by now, a well-established backbone phylogeny for Polyplacophora and is also concordant at superfamily and higher level with the mitogenome phylogeny (Irisarri et al. 2020; Moles et al. 2021; Sigwart et al. 2013). Lepidopleurida is sister to the remaining Polyplacophora. *Callochiton*, representing the order Callochitonida, is sister to Chitonida. This latter order is divided into two distinct clades representing the suborders Chitonina and Acanthochitonina.

Building on the existing body of knowledge of previous molecular studies of chitons (Irisarri et al. 2020; Moles et al. 2021; Okusu et al. 2003; Sigwart et al. 2013), our phylogeny based on a phylogenomic approach offers new insights by providing broader taxon sampling and more orthologs used for tree reconstruction, and robustly resolves

the relationships among the main lineages of chitons. The genus- and family-level arrangement of taxa in this study are largely concordant with the established taxonomy and with other molecular studies based on smaller data matrices. Within Lepidopleurida, the family Leptochitonidae s.s. is restricted to NE Atlantic species, represented here by *Leptochiton asellus* and *Lepidopleurus cajetaus*, with the Pacific *Leptochiton cascadiensis* outside this clade, as reported in the previous molecular studies using Sanger sequencing (Sigwart 2016; Sigwart et al. 2011).

Acanthochitonina is divided into two clades that can be easily separated based on the morphology of egg hulls. One of these clades, Mopalioidea, includes *Cryptochiton*, *Mopalia*, *Katharina*, and *Tonicella* (Okusu et al. 2003) which is also well supported by every other molecular phylogeny and modern phylogenetic systematics (Sigwart et al. 2013). Due to limited taxon sampling, we cannot comment on family-level systematics in Mopalioidea, but these four genera tend to form a clade that is separate from another clade composed of *Nuttallina* and *Cyanoplax*, which is consistent with the findings of Irisarri et al. (2020). In the other superfamily Cryptoplacoidea, *Nuttallochiton* is sister to the rest of Cryptoplacoidea, in accordance with previous molecular studies (Irisarri et al. 2020; Okusu et al. 2003; Sigwart et al. 2013). However, the position of *Plaxiphora* within Acanthochitonina is equivocal and this has been a persistent problem in every molecular phylogeny of chitons, although multiple morphological characters unite *Plaxiphora* with the family Mopaliidae (Sirenko 2006).

Schizochiton resolved as sister to Chitonoidea, forming a monophyletic suborder Chitonina with full support in all analyses except for the Matrix1-C20 method where it is sister to the order Chitonida. The only previous molecular analysis to include *Schizochiton* also recovered it as sister to the remaining Chitonina in one version of their analyses but concluded that its phylogenetic position was effectively unresolved (Fig. 5 in Okusu et al. 2003). The position of *Schizochiton* was controversial because of an unusual combination of morphological characters and the balance of evidence placed this genus in the suborder Chitonina (Sirenko 2006). *Schizochiton* possess a caudal sinus in tail valve that is similar to others in Mopalioidea as well as egg

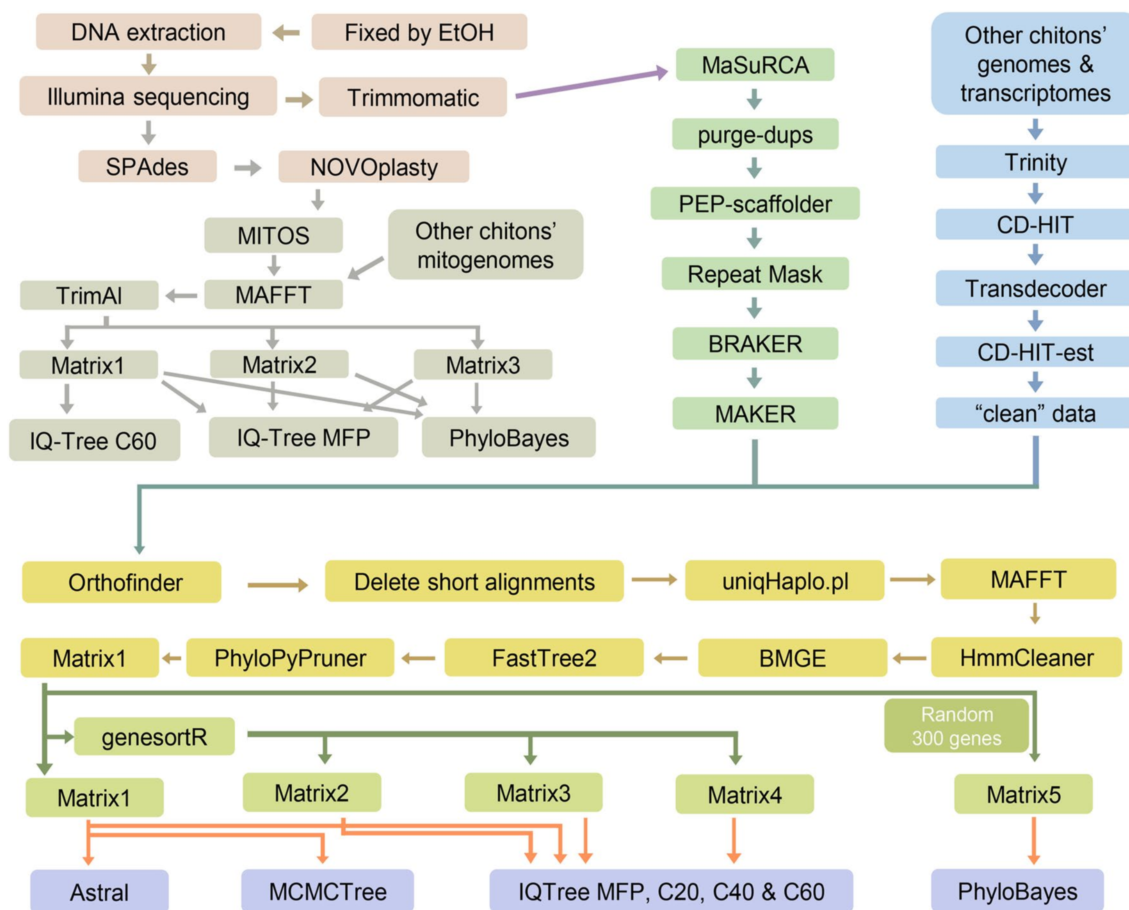

Fig. 5 The analytical pipeline used in this study, including: mitogenome assembly, annotation and phylogeny; genome assembly and annotation; transcriptome filtration; orthologue inference; and phylogenomic analyses

hulls with cupules that are simpler but comparable to other Mopalioidea. Based on the new phylogenetic tree, we can infer these features may be plesiomorphic for the order Chitonida.

Evolution of shell eyes

One important morphological feature of *Schizochiton* that differs from almost all other chitons is their shell eyes. Shell eyes were first described by Moseley (1884) from specimens of *Schizochiton incisus*, and were immediately recognized as modifications of the chiton aesthete system (Moseley 1885). All chitons possess aesthetes in their shell plates, and some are photosensitive (Boyle 1974; Fischer 1978; Sigwart et al. 2014). But shells eyes are restricted to only a few genera, in two families: Schizochitonidae and Chitonidae.

Although discovered more than 130 years ago, chiton shell eyes were long neglected as a topic of research until a number of recent studies revealed additional important details and stimulated further interest in terms of material properties, growth, and behavior of chitons (e.g., Chappell and Speiser 2023; Li et al. 2015; Sigwart and Sumner-Rooney 2020; Speiser et al. 2011, 2014). All recent work on understanding shell eyes has used species of the family Chitonidae, and mainly of the genus *Acanthopleura* (e.g., Chappell and Speiser 2023; Liu et al. 2022). Chiton shell eyes are increasingly included as a model for distributed visual systems (Buschbeck and Bok 2023). The difficulty in accessing specimens of *Schizochiton* has hampered the key questions in systematics that are addressed in the present study, and likewise means the eyes have not been studied in detail. Although clearly equivalent structures, it is so far unclear how similar to or different from *Schizochiton* fare eyed species of Chitonidae in terms of the detailed morphology of the eyes and of visually mediated behavior.

As shell eyes are naturally features of the shell, the chiton fossil record can give us some insight into the minimum age of these features in evolutionary history (Puchalski et al. 2008). In the Schizochitonidae, eyes appear early and are a consistent feature in the fossil history of the family. The oldest known shell eyes are from the fossil genus *Incissiochiton* from the lower Palaeocene (61–66 Mya), which is a member of the family Schizochitonidae, the only family in the superfamily Schizochitonoidea (Sirenko 2006, 2013). This genus *Incissiochiton* is not strongly morphologically distinguishable from *Schizochiton* (Sirenko 2013). Material of the recently described species *Incissiochiton pustulifer* from the lower Paleocene preserves the oldest known shell eyes (Sirenko and Dell'Angelo 2023).

Those genera in the family Chitonidae with shell eyes form a monophyletic group and have a fossil record dating back to the Middle Eocene (ca. 47.8–38 Mya) (Puchalski et al. 2008). The presence of eyes in fossil material of *Tonicia* spp. is inferred from similar fossils available in the Senckenberg collections, but the precise age has not been confirmed. Most morphological descriptions of chitons do not explicitly refer to the shell eyes. The origin of the family Chitonidae dates to at least the Cretaceous (> 66 Mya) based on both the fossil record (Pulchalski 2008; Sirenko 2006) and molecular dating (Ibáñez et al. 2019). There are older chitonid fossil species that would date back to the Cretaceous split between Chitonidae + Schizochitonidae, but to date no evidence has been shown that any of these had shell eyes.

These fossil dates provide a minimum age for the origin of shell eyes in these two separate linages. The chiton fossil record is constrained by limited sampling, but nonetheless is sufficiently robust to show important patterns of diversification over time (Puchalski et al. 2008). It is noteworthy that the earliest fossils of these two eye-bearing lineages are separated by over 13 million years, and that the order of occurrence matches the branching pattern recovered by independent phylogenomic analyses here. Phylogenetic and fossil evidence suggests that shell eyes evolved first in Schizochitonidae and again a second time more recently in Chitonidae.

Addressing the question of whether there were one or two origins of shell eyes within living Polyplacophora depends on the topology of the phylogenetic trees of both living and fossil species. Limited taxon sampling in phylogenomic approaches means that this cannot yet be tested quantitatively in a meaningful way, but we can combine the patterns in our trees and the fossil record, to identify the most parsimonious scenario. The topology recovered presents two possible evolutionary explanations: either one origin of eyes, or two separate origins, one in Schizochitonidae and a second in the clade containing *Tonicia* + *Acanthopleura* (eyed Chitonidae). The eyed Chitonidae consistently resolve as a single clade in other studies with higher taxon sampling (e.g., Alnashiri et al. 2023).

A single origin of eyes in Polyplacophora would need to have occurred in an ancestor of the clade Schizochitonidae + Chitonidae, a scenario that also necessitates at least two additional separate loss events in the other subclades within Chitonidae. Alternatively, this could be seen as a single loss in the stem of Chitonidae and a secondary gain in the ancestry of eyed Chitonidae. A single loss-and-regain scenario is effectively equivalent to two separate origins of eyes. The more materially interesting questions, therefore, are on the functional genomics underlying the expression of shell eyes, as well as the potential overlap in genomic mechanisms controlling the eyes and the resulting morphology in both clades. Sufficient data are not yet available to allow a detailed comparison of the morphology and innervation of the eyes in the two clades, although it is known that the eyes of *Schizochiton* are larger than those of other genera (Moseley 1885).

The new information here presents an important step in developing further tools to investigate the genes involved in eye development and evolution within Polyplacophora.

Recognizing *Schizochiton* as a member of the superfamily Schizochitonoidea, and as sister to Chitonoidea, confirms the relationship predicted by morphological systematics and provides a more stable phylogeny than earlier preliminary results (Irisarri et al. 2020; Okusu et al. 2003; Sigwart et al. 2013). This also supports the assertion that the multiple lines of evidence from morphological, anatomical, and gamete characters previously recognized in chitons provide a robust basis for phylogenetic systematics.

Materials and methods

The whole pipeline used in this study can be found in Fig. 5, including sample preparation, sequencing, mitogenome and genome assembly and annotation, ortholog inference, and phylogenetic analyses.

Sample collection

All genomes and transcriptomes used in this study are listed in Table 1. To increase taxon sampling, we newly sequenced an individual of *Schizochiton incisus* and also of *Leptochiton asellus. Schizochiton incisus* was collected from a rock on Livcock coral reef (10°10′ N, 115°19′ E) at a depth of 80 m in the South China Sea on July 11, 2020, using a fishing net (Fig. 1). A whole individual of *S. incisus* was preserved in 95% EtOH, which was later stored at room temperature, and a small piece of girdle tissue was removed for DNA extraction. The *S. incisus* sample was deposited in the malacology collections at the Senckenberg Museum, Frankfurt with catalogue number SMF 386201. *Leptochiton asellus* was collected on the rocky shore at Ballyhenry Island, Strangford Lough, Portaferry, N. Ireland, in September 2019. For *L. asellus*, five tissues, including foot, perinotum, aesthetes, viscera, and shell edge were dissected and fixed in RNAlater (ThermoFisher) at 4 °C and transferred to a – 80 °C freezer for storage.

Genome and RNA sequencing

Total genomic DNA of *S. incisus* was extracted with a DNeasy Blood & Tissue Kit (QIAGEN, Germantown, Maryland), which was further sequenced for 150 bp paired-end Illumina sequencing to generate approximately 40 Gb of raw data on NovaSeq 6000 platform at Novogene (Beijing).

RNA of *Leptochiton asellus* was extracted using Trizol (ThermoFisher) and sent to Novogene (Beijing) for Eukaryotic type transcriptome library preparation and further sequenced on a NovaSeq 6000 platform. Approximately 6 Gb of raw reads were generated for each tissue.

Mitogenome analysis

Mitogenome assembly and annotation

The raw data were trimmed using Trimmomatic v.0.39 (Bolger et al. 2014) with strict filtering settings (ILLUMINACLIP: adapters.fa:2:30:10 LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:140) to remove low-quality reads and adapter-contaminated reads. The resultant clean reads were initially assembled by SPAdes v.3.15.3 (Prjibelski et al. 2020) with default settings. The partial COI sequence of *S. incisus* was then extracted from the assembled contigs, which was later used as the "seed input" in NOVOplasty v.4.2 (Dierckxsens et al. 2017) to obtain the complete mitogenome of *S. incisus*. The mitogenome was then annotated using the MITOS web server (Donath et al. 2019) with the invertebrate genetic code and the rest default settings, followed by a manual mitogenome annotation confirmation by comparing with other chiton mitogenomes (Irisarri et al. 2020).

Matrix construction

All *gb* files of chiton mitogenomes available on NCBI were downloaded and imported into Phylosuite v.1.2.2 (Zhang et al. 2020), which is an application that allows users to perform phylogenetic analyses on relatively small datasets. All procedures of mitogenome phylogenetic analyses, except for tree constructing and visualization, were carried out through Phylosuite built-in plugins. In brief, 13 protein-coding genes and two rRNA genes were extracted from the chiton mitogenomes. Afterward, MAFFT v. 7.471 was used to align sequences, followed by trimAL v. 1.2rev57 with the "automated1" option to remove spurious sequences and misaligned regions. Trimmed sequences were concatenated, generating three different matrices. Amino acid sequences of 13 protein-coding genes (PCGs) were extracted and concatenated in Matrix1. All nucleotides of 13 PCGs and two rRNA were concatenated in Matrix2. To avoid phylogenetic signal saturation on the third codon, the third codons of 13 PCGs were replaced by degenerate bases (A, G replaced by R and C, T replaced by Y), then these modified sequences were concatenated in Matrix3. The generated gene matrices and the corresponding partition files were later used for maximum-likelihood (ML) and Bayesian inference (BI) tree construction.

Mitogenome phylogeny

For the ML framework, IQ-Tree v.2.1.3 (Minh et al. 2020) was implemented using -MFP to select the best-fit model for each partition. An additional empirical profile mixture model, C60, was also carried out on the AA matrix (Matrix1). All ML analyses were performed with 1000 replicates of ultrafast bootstrapping (-bb 1000).

BI was carried out using PhyloBayes MPI (abbreviated as PB) v.1.8c (Lartillot et al. 2013) with CAT-GTR + Γ4 models. For each matrix, four independent Monte Carlo Markov chains (MCMC) were run simultaneously and convergence was checked with the bpcomp program. A consensus tree was obtained after discarding the first 10% cycles as a burn-in. All trees obtained were visualized with Figtree (http://tree.bio.ed.ac.uk/software/figtree/).

Genome analysis

Genome assembly and annotation

The Illumina raw data were filtered with Trimmomatic v.0.39 (Bolger et al. 2014) with settings of "PE ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:15 MINLEN:40". Genome features were calculated using jellyfish v.2.3.0 (Marçais and Kingsford 2011) (19mer) and GenomeScope2 (Vurture et al. 2017). A benchmark of commonly used assemblers for Illumina data, including Platanus v.1.2.4 (Kajitani et al. 2014) and MaSuRCA v.4.0.3 (Zimin et al. 2013), was performed based on BUSCO v.5.1.2 scores by searching against the metazoan odb10 database. Purge-dups v.1.2.5 (Guan et al. 2020) was used to remove redundant contigs, and the resultant contigs were further scaffolded using PEP-scaffolder (Zhu et al. 2016) with the help of protein sequences from the concatenation of the genome of *Acanthopleura granulata* (Varney et al. 2021).

A custom repeat library of *S. incisus* was de novo generated by RepeatModeler v.2.0.2a (Flynn et al. 2020). RepeatMasker v.4.1.0 (Tarailo-Graovac and Chen 2009) was performed with the species-specific repeat library mentioned above, followed by a second round of RepeatMasker but with Repbase library 2018 (https://www.girinst.org/repbase/). BRAKER v.2.1.6 (Hoff et al. 2019) was run to train an ab initio gene predictor Augustus v.3.4.0 (Stanke et al. 2006) with the ODB10 v.1 database downloaded from OrthoDB (Kriventseva et al. 2018). This generated a config file of *S. incisus*, which was used as one piece of evidence while running the genome annotator MAKER v.3.01.04 (Holt and Yandell 2011). Because no transcript evidence was available, all Mollusca proteins on NCBI were downloaded (Date: Jan 20, 2022), and redundancy was removed with CD-HIT v.4.8.1 with the "-c 0.9" setting. These protein sequences were regarded as the protein homology evidence in MAKER. The proteins generated from MAKER were used for further phylogenetic analyses.

Transcriptome assembly and filtration

The protein-coding genes of *Acanthopleura granulata*, *A. loochooana* (Liu et al. 2022), and all other available transcriptomes were downloaded from the NCBI SRA database. For transcriptome SRA datasets and the transcriptome sequencing of *L. asellus*, the raw reads were de novo assembled in Trinity v.2.13.2 or v.2.14.0 (Haas et al. 2013), using the "trimmomatic" setting, followed by one round of CD-HIT v.4.8.1 (Fu et al. 2012) with the strictest threshold (-c 0.8) to remove redundant sequences. CD-HIT was run multiple times and was continuously monitored by BUSCO5 with the aim of obtaining the highest "S" score and the lowest "D" (duplicated BUSCO) score. Transdecoder v.5.5.0 (Douglas 2018) was performed to search for open-reading frames with the "single_best_only" option. The generated peptide files were filtered using CD-HIT with the "-c 0.8" option to ensure that the "D" score did not drop any further This step aimed to remove as many heterozygous and transcript isoforms as possible, so that they would not mislead orthology inference.

Orthology inference and matrix construction

Orthology inference was accomplished with a pipeline that was generated in previous studies (Kocot et al. 2017; Sun et al. 2021) with slight modifications. We ran Orthofinder v.2.5.4 (Emms and Kelly 2019) to search for orthologues within selected taxa. Then, in the "Orthogroup_Sequences" directory of the Orthofinder output, OG heads were fixed with a custom shell script to ensure that the orthology inference pipeline did not include any errors. PREQUAL v.1.02 (Whelan et al. 2018) was used to detect and mask non-homologous characters. Sequences shorter than 100 amino acids were deleted. Occupancy was set to 50%, and redundant sequences were removed with another custom shell script named uniqHaplo.pl. The leftover.*fasta* files were aligned using MAFFT v.7.490 (Katoh and Standley 2013) with default settings. HmmCleaner (Di Franco et al. 2019) was used to remove misaligned regions. Alignments were trimmed with BMGE v.1.12 (Criscuolo and Gribaldo 2010). FastTree2 (Price et al. 2010) was used to construct fast-ML trees for each remaining OG. PhyloPyPruner v.1.2.4 (https://pypi.org/project/phylo-pypruner) was performed to identify putative orthology sequences based on the former FastTree2 results, giving an initial matrix containing 3593 OGs.

We performed genesortR (Mongiardino Koch 2021) to sort and select "best" OGs based on seven commonly

used phylogenetic gene properties. Thus, genes with best phylogenetic signals could be used for down streaming analysis. An ML tree for the initial matrix was constructed with the IQ-Tree "-MFP" model as input. ML trees for each gene were also constructed in IQ-Tree with the same settings. Four matrices, including an initial matrix (Matrix1), the best 800 genes matrix (Matrix2), the best 1300 genes matrix (Matrix3), and the best 2700 genes matrix (Matrix4) generated by genesortR, were prepared for phylogenetic analyses.

Phylogenomics

ML phylogenetic analysis was performed using IQ-Tree 2 (Minh et al. 2020) on the four matrices generated above. The ML approach was carried out using the best-fitting model for each partition (-m MFP). Using the *.contree* file generated by the MFP model as the guide tree, The PMSF model was performed in IQ-Tree 2 with site-specific frequency models (C20, C40 and C60). All ML analyses were carried out with 1000 ultrafast bootstrap.

For BI analysis, all matrices mentioned above were too large to run in PhyloBayes MPI v.1.8c. Therefore a fifth matrix, produced by 300 random genes from Matrix1, was generated. Four independent chains were run simultaneously until convergent with the CAT-GTR + Γ4 model. In contrast to concatenated-based phylogenetic analyses, a coalescent approach was adopted to evaluate evolutionary relationships in Polyplacophora with ASTRAL v.5.7.1 (Sayyari and Mirarab 2016). An AU-test was performed with IQ-tree 2 on two topologies, which were ((Chitonoidea, Schizochitonoidea), Acanthochitonina) and ((Acanthochitonina, Schizochitonoidea), Chitonoidea), respectively.

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Author contributions JS conceived and guided the study. JDS identified the species. XL conducted the research and wrote the manuscript. JS and JDS revised and edited the manuscript. All the authors read and approved the final version of the manuscript.

Data availability The raw Illumina sequencing data were deposited in the NCBI SRA database with accession number PRJNA909482. The assembled mitogenome was deposited in the NCBI nucleotide database with accession number OP994082. The assembled genomic contigs, predicted gene models can be accessed via FigShare with the URL https://doi.org/10.6084/m9.figshare.21709742.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement The authors declare that all applicable international, national, and or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed and all necessary approvals have been obtained.

References

Alnashiri H, Thomas L, Thaikkottathil M, Philip S, Kutty R, Sivanpillai S, Abo Rasain AM (2023) Phylogenetic affinities of Acanthopleurinae Dall, 1889 chitons (Mollusca: Polyplacophora: Chitonidae) from Jazan coast in the Red Sea and western Indo-Pacific. J Mar Sci Eng 11:158

Baxter JM, Sturrock MG, Jones AM (1990) The structure of the intrapigmented aesthetes and the properiostracum layer in *Callochiton achatinus* (Mollusca: Polyplacophora). J Zool 220:447–468

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

Boyle PR (1974) The aesthetes of chitons. Cell Tissue Res 153:383–398

Buschbeck E, Bok M (2023) Distributed vision: from simple sensors to sophisticated combination eyes. Springer, Cham Switzerland

Chappell DR, Speiser DI (2023) Polarization sensitivity and decentralized visual processing in an animal with a distributed visual system. J Exp Biol 226:jeb244710

Criscuolo A, Gribaldo S (2010) BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evol Biol 10:210

De Oliveira A, Wollesen T, Kristof A, Scherholz M, Redl E, Todt C, Bleidorn C, Wanninger A (2016) Comparative transcriptomics enlarges the toolkit of known developmental genes in mollusks. BMC Genomics 17:1–23

Di Franco A, Poujol R, Baurain D, Philippe H (2019) Evaluating the usefulness of alignment filtering methods to reduce the impact of errors on evolutionary inferences. BMC Evol Biol 19:21

Dierckxsens N, Mardulyn P, Smits G (2017) NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res 45:e18

Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M (2019) Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. Nucleic Acids Res 47:10543–10552

Doucet-Beaupré H, Breton S, Chapman EG, Blier PU, Bogan AE, Stewart DT, Hoeh WR (2010) Mitochondrial phylogenomics of the Bivalvia (Mollusca): searching for the origin and mitogenomic correlates of doubly uniparental inheritance of mtDNA. BMC Evol Biol 10:50

Douglas (2018) TransDecoder/TransDecoder. GitHub. Available from: https://github.com/TransDecoder/TransDecoder (accessed March 23, 2020).

Emms DM, Kelly S (2019) OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 20:238

Fischer FP (1978) Photoreceptor cells in chiton aesthetes. Spixiana 1:209–213

Flynn JM, Hubley R, Goubert C, Rosen J, Clark AG, Feschotte C, Smit AF (2020) RepeatModeler2 for automated genomic discovery of transposable element families. Proc Natl Acad Sci USA 117:9451–9457

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

Ghiselli F, Gomes-Dos-Santos A, Adema CM, Lopes-Lima M, Sharbrough J, Boore JL (2021) Molluscan mitochondrial genomes break the rules. Philos Trans R Soc Lond B Biol Sci 376:20200159

Giribet G, Edgecombe GD (2020) The invertebrate tree of life. Princeton University Press, Princeton

Guan D, McCarthy SA, Wood J, Howe K, Wang Y, Durbin R (2020) Identifying and removing haplotypic duplication in primary genome assemblies. Bioinformatics 36:2896–2898

Haas W, Kriesten K (1978) Aesthetes with intrapigmental eye in the shell of *Chiton marmoratus* L. (Mollusca, Placophora). Zoomorphologie 90:253–268

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel R et al (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc 8:1494–1512

Halanych KM, Kocot KM (2014) Repurposed transcriptomic data facilitate discovery of innate immunity toll-like receptor (TLR) genes across Lophotrochozoa. Biol Bull 227:201–209

Hoff KJ, Lomsadze A, Borodovsky M, Stanke M (2019) Whole-genome annotation with BRAKER. In: Kollmar M (ed) Gene prediction. Methods in molecular biology. Humana, New York, pp 65–95

Holt C, Yandell M (2011) MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. BMC Bioinformatics 12:491

Ibáñez CM, Eernisse DJ, Méndez MA, Valladares M, Sellanes J, Sirenko BI, Pardo-Gandarillas MC (2019) Phylogeny, divergence times and species delimitation of Tonicia (Polyplacophora: Chitonidae) from the eastern Pacific Ocean. Zool J Linn Soc 186:915–933

Irisarri I, Eernisse DJ, Zardoya R (2014) Molecular phylogeny of Acanthochitonina (Mollusca: Polyplacophora: Chitonida): three new mitochondrial genomes, rearranged gene orders and systematics. J Nat Hist 48:2825–2853

Irisarri I, Uribe JE, Eernisse DJ, Zardoya R (2020) A mitogenomic phylogeny of chitons (Mollusca: Polyplacophora). BMC Evol Biol 20:22

Joester D, Brooker LR (2016) The chiton radula: a model system for versatile use of iron oxides*. In: Faivre D (ed) Iron oxides. Wiley, New York, pp 177–206

Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, Kohara Y, Fujiyama A, Hayashi T, Itoh T (2014) Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 24:1384–1395

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

Koch RA, Lambert CC (1990) Ultrastructure of sperm, spermiogenesis, and sperm-egg interactions in selected invertebrates and lower vertebrates which use external fertilization. J Electron Microsc Tech 16:115–154

Kocot KM, Struck TH, Merkel J, Waits DS, Todt C, Brannock PM, Weese DA, Cannon JT, Moroz LL, Lieb B, Halanych KM (2017) Phylogenomics of Lophotrochozoa with consideration of systematic error. Syst Biol 66:256–282

Kriventseva EV, Kuznetsov D, Tegenfeldt F, Manni M, Dias R, Simão FA, Zdobnov EM (2018) OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. Nucleic Acids Res 47:D807–D811

Ladd HS (1966) Chitons and gastropods (Haliotidae through Adeorbidae) from the western Pacific islands. US Government Printing Office, Washington DC

Lartillot N, Rodrigue N, Stubbs D, Richer J (2013) PhyloBayes MPI: phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst Biol 62:611–615

Li L, Connors MJ, Kolle M, England GT, Speiser DI, Xiao X, Aizenberg J, Ortiz C (2015) Multifunctionality of chiton biomineralized armor with an integrated visual system. Science 350:952–956

Liu C, Liu H, Huang J, Ji X (2022) Optimized sensory units integrated in the chiton shell. Mar Biotechnol 24:380–392

Marçais G, Kingsford C (2011) A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics 27:764–770

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534

Moles J, Cunha TJ, Lemer S, Combosch DJ, Giribet G (2021) Tightening the girdle: phylotranscriptomics of Polyplacophora. J Molluscan Stud 87:eyab019

Mongiardino Koch N (2021) Phylogenomic subsampling and the search for phylogenetically reliable loci. Mol Biol Evol 38:4025–4038

Moseley HN (1884) XIX.—On the presence of eyes and other sense-organs in the shells of the Chitonidae. Ann Mag Nat His; Zool Bot, Geol 14:141–147

Moseley HN (1885) Memoirs: on the presence of eyes in the shells of certain Chitonidæ, and on the structure of these organs. J Cell Sci 2:37–60

Nemoto M, Ren D, Herrera S, Pan S, Tamura T, Inagaki K, Kisailus D (2019) Integrated transcriptomic and proteomic analyses of a molecular mechanism of radular teeth biomineralization in *Cryptochiton stelleri*. Sci Rep 9:1–10

Okusu A, Schwabe E, Eernisse DJ, Giribet G (2003) Towards a phylogeny of chitons (Mollusca, Polyplacophora) based on combined analysis of five molecular loci. Org Divers Evol 3:281–302

Price MN, Dehal PS, Arkin AP (2010) FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS ONE 5:e9490

Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A (2020) Using SPAdes de novo assembler. Curr Protoc Bioinf 70:e102

Puchalski SS, Eernisse DJ, Johnson CC (2008) The effect of sampling bias on the fossil record of chitons (Mollusca, Polyplacophora). Am Malacol Bull 25:87–95

Riesgo A, Andrade S, Sharma PP, Novo M, Pérez-Porro AR, Vahtera V, González VL, Kawauchi GY, Giribet G (2012) Comparative description of ten transcriptomes of newly sequenced invertebrates and efficiency estimation of genomic sampling in non-model taxa. Front Zool 9:1–24

Sayyari E, Mirarab S (2016) Fast coalescent-based computation of local branch support from quartet frequencies. Mol Biol Evol 33:1654–1668

Sigwart JD (2016) Deep trees: woodfall biodiversity dynamics in present and past oceans. Deep Sea Res II Top Stud Oceanogr 137:282–287

Sigwart JD, Sumner-Rooney L (2020) Continuous and regular expansion of a distributed visual system in the eyed chiton. Biol Bull 240:23–33

Sigwart JD, Schwabe E, Saito H, Samadi S, Giribet G (2011) Evolution in the deep sea: a combined analysis of the earliest diverging

living chitons (Mollusca: Polyplacophora: Lepidopleurida). Invertebr Syst 24:560–572
Sigwart JD, Stoeger I, Knebelsberger T, Schwabe E (2013) Chiton phylogeny (Mollusca: Polyplacophora) and the placement of the enigmatic species *Choriplax grayi* (H. Adams & Angas). Invertebr Syst 27:603–621
Sigwart JD, Sumner-Rooney LH, Schwabe E, Heß M, Brennan GP, Schrödl M (2014) A new sensory organ in "primitive" molluscs (Polyplacophora: Lepidopleurida), and its context in the nervous system of chitons. Front Zool 11:7
Sirenko B (2006) New outlook on the system of chitons (Mollusca: Polyplacophora). Venus 65:27–49
Sirenko B (2013) Four new species and one new genus of Jurassic chitons (Mollusca: Polyplacophora: Lepidopleurida) from the Middle Russian Sea. Proc Zool Inst RAS 317:30–44
Sirenko B, Dell'Angelo B (2023) Paleocene genus *Incisiochiton* Van Belle, 1985 (Mollusca: Polyplacophora) with the description of a new species from Ukraine. Proc Zool Inst RAS 327:128–134
Speiser D, Demartini D, Oakley T (2014) The shell-eyes of the chiton *Acanthopleura granulata* (Mollusca, Polyplacophora) use pheomelanin as a screening pigment. J Nat Hist 48:1–13
Speiser Daniel I, Eernisse Douglas J, Johnsen S (2011) A chiton uses aragonite lenses to form images. Curr Biol 21:665–670
Stanke M, Keller O, Gunduz I, Hayes A, Waack S, Morgenstern B (2006) AUGUSTUS: ab initio prediction of alternative transcripts. Nucleic Acids Res 34:W435–W439
Stebbins TD, Eernisse DJ (2009) Chitons (Mollusca: Polyplacophora) known from benthic monitoring programs in the Southern California Bight. Festivus 41:53–100
Stoger I, Kocot KM, Poustka AJ, Wilson NG, Ivanov D, Halanych KM, Schrodl M (2016) Monoplacophoran mitochondrial genomes: convergent gene arrangements and little phylogenetic signal. BMC Evol Biol 16:274
Sun J, Li R, Chen C, Sigwart JD, Kocot KM (2021) Benchmarking Oxford Nanopore read assemblers for high-quality molluscan genomes. Philos Trans R Soc Lond B Biol Sci 376:20200160
Tarailo-Graovac M, Chen N (2009) Using RepeatMasker to identify repetitive elements in genomic sequences. Curr Protoc Bioinf 25:4.10.1–4.10.14
Uribe JE, Zardoya R (2017) Revisiting the phylogeny of Cephalopoda using complete mitochondrial genomes. J Molluscan Stud 83:133–144
Uribe JE, Williams ST, Templado J, Abalde S, Zardoya R (2017) Denser mitogenomic sampling improves resolution of the phylogeny of the superfamily Trochoidea (Gastropoda: Vetigastropoda). J Molluscan Stud 83:111–118
Uribe JE, Irisarri I, Templado J, Zardoya R (2019) New patellogastropod mitogenomes help counteracting long-branch attraction in the deep phylogeny of gastropod mollusks. Mol Phylogenet Evol 133:12–23
Varney RM, Speiser DI, McDougall C, Degnan BM, Kocot KM (2021) The iron-responsive genome of the chiton *Acanthopleura granulata*. Genome Biol Evol 13:evaa263
Varney RM, Yap-Chiongco MK, Mikkelsen NT, Kocot KM (2022) Genome of the lepidopleurid chiton *Hanleya hanleyi* (Mollusca, Polyplacophora). F1000Res 11:555
Vurture GW, Sedlazeck FJ, Nattestad M, Underwood CJ, Fang H, Gurtowski J, Schatz MC (2017) GenomeScope: fast reference-free genome profiling from short reads. Bioinformatics 33:2202–2204
Wang Y, Yang Y, Kong L, Sasaki T, Li Q (2023) Phylogenomic resolution of Imparidentia (Mollusca: Bivalvia) diversification through mitochondrial genomes. Mar Life Sci Technol 5:326–336
Whelan S, Irisarri I, Burki F (2018) PREQUAL: detecting non-homologous characters in sets of unaligned homologous sequences. Bioinformatics 34:3929–3930
Winston FP, David RL, Juliet MP (2020) Biology and evolution of the Mollusca, vol 2. CRC Press, Boca Raton
Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour 20:348–355
Zhu BH, Song YN, Xue W, Xu GC, Xiao J, Sun MY, Sun XW, Li JT (2016) PEP_scaffolder: using (homologous) proteins to scaffold genomes. Bioinformatics 32:3193–3195
Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA (2013) The MaSuRCA genome assembler. Bioinformatics 29:2669–2677

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