# Still waters run deep: Large scale genome rearrangements in the evolution of morphologically conservative Polyplacophora

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## Abstract

**Background:** A major question in animal evolution is how genotypic and phenotypic changes are related, and whether ancient gene order is conserved in living clades. Chitons, the molluscan class Polyplacophora, retain a body plan and general morphology apparently little changed since the Palaeozoic. We present a comparative analysis of five reference quality genomes, including four *de novo* assemblies, covering all major chiton clades, and an updated phylogeny for the phylum.

**Results:** We constructed 20 ancient molluscan linkage groups (MLGs) that are relatively conserved in bivalve karyotypes, but subject to re-ordering, rearrangement, fusion, or partial duplication among chitons, varying even between congeneric species. The largest number of novel fusions is in the most plesiomorphic clade Lepidopleurida, and the chitonid *Liolophura japonica* has a partial genome duplication, extending the occurrence of large-scale gene duplication within Mollusca.

**Conclusions:** The extreme and dynamic genome rearrangements in this class stands in contrast to most other animals, demonstrating that chitons have overcome evolutionary constraints acting on other animal groups. The apparently conservative phenome of chitons belies rapid and extensive changes in genome.

## Introduction

The genomic mechanisms that enable or limit the evolution of major morphological changes remains one of the great questions of evolutionary biology. Living members of Mollusca represent the broadest morphological disparity of any animal phylum: mollusc body plans encompass squid, worms, living rocks and candy-coloured tree snails, as well as diverse intermediate and additional novel forms known from the fossil record. Early work on chromosome numbers in meiotic division used molluscs, and interest in understanding patterns in chromosome numbers across species also led to fundamental insights in animal polyploidy. Despite playing a key role in early advances, this important phylum lags in terms of quality and taxonomic coverage of whole genome sequence data. Reconstructing the evolution of genome architecture through deep time in diverse molluscs remains critical to understand genome evolution in animals. High quality genomic data for the deeply divergent, morphologically constrained chitons, would be expected to offer an opportunity to explore ancient genetic traits and evolutionary mechanisms preserved across the long span of animal evolution.

Prior work has reasonably assumed that rates of intra-chromosomal gene translocation are constant within major groups (Mackintosh et al., 2023). If this is true, syntenic rearrangement could be a clocklike indicator of divergence times. But rates of genomic rearrangement are not well known in molluscs, nor how they might vary across this vast clade, and higher rates of rearrangement confound reconstruction of ancestral states. In molluscs, an excellent fossil record allows an independent record of divergence times that will provide more insight into the variability (and utility) of rates of rearrangement as a measure of divergence time.

Chitons have long been considered the key taxon to understanding the ancestral molluscan body plan (Wanninger and Wollesen, 2019). Around 1000 living chiton

species worldwide all possess an eight-part shell armour that has remained superficially unchanged with relatively little variation since the Carboniferous, over 300 Million years ago (Sigwart, 2017). Chitons are increasingly important for bio-inspired design, which will benefit from genomic tools to understand genetic control of their flexible armour and unique sensory systems (Ampuero et al., 2024; Varney et al., 2021). Chitons are generally conserved, yet the known species richness in chitons is higher than for the more morphologically and behaviourally diverse extant cephalopods. The adaptations that drive speciation in this group remain an open question.

One key issue is whether polyplacophoran molluscs are conservative *per se* or whether their diagnostic body plan adaptations are so distinctive and strange that this has overshadowed full understanding of additional adaptive traits: fully innervated shells, iron mineralised radula, living at almost all depths and latitudes of the world ocean. While the body plan of chitons has persisted for over 300 My, this is a template for remarkable adaptations that have only recently begun to be appreciated.

Chitons are grazers and most are not highly ecologically specialised (Sigwart and Schwabe, 2017). These animals mostly have separate sexes and all are broadcast spawners, are not migratory, cannot reasonably be subject to strong sexual selection, and many similar species co-occur in parapatric or sympatric radiations (Kelly and Eernisse, 2008). Previously published karyotype data shows closely related species with overlapping ranges can have different numbers of chromosomes, such as *Acanthochitona discrepans* (1n=8) and *A. crinita* (1n=9) in the North Atlantic (Certain, 1951). Chromosome rearrangement could be an attractive speculative explanation that could support maintaining species boundaries in this clade.

Here, we sequenced four new reference-quality genomes that cover the three taxonomic orders of living chitons: *Deshayesiella sirenkoi* (Lepidopleurida), *Callochiton*

90 *septemvalvis* (Callochitonida), *Acanthochitona discrepans* and *A. rubrolineata*
91 (Chitonida). Callochitonida is sister to Chitonida (Moles et al., 2021) and together these
92 could be considered the Chitonida *sensu lato*. One aim here was to test the potential
93 genomic differences separating these two orders within Chitonida s.l. We used these
94 four new genomes together with previously published genomic data to reconstruct a
95 phylogeny for the phylum. This allows us to confidently reconstruct ancestral
96 chromosome arrangement of total-group Mollusca, and at different transitions within
97 Polyplacophora.

98

99 **Results**

100 We sequenced chromosome-level genomes of *Deshayesiella sirenkoi*
101 (Lepidopleurida) from the Daikoku vent field, Western Pacific Ocean, *Callochiton*
102 *septemvalvis* (Chitonida s.l.: Callochitonida) and *Acanthochitona discrepans*
103 (Chitonida *sensu stricto*) both from the intertidal of Strangford Lough, N. Ireland, and
104 the congener *A. rubrolineata* from the intertidal of Qingdao, China. These were
105 sequenced with PacBio HiFi and scaffolded using Hi-C, resulting in high quality
106 assemblies with over 97% BUSCO completeness (94% for *Callochiton septemvalvis*)
107 and with numbers of chromosomes that differed among genera from 8-13 (Fig. 1, SI
108 Appendix, Fig. S1, Table S1). These species show high levels of heterozygosity,
109 ranging from almost 1% in *Deshayesiella* to 4.12% in *Callochiton* (Fig. 1, SI Appendix,
110 Fig. S2).

111 Our phylogenetic results confirm the placement of chitons as sister to a monophyletic
112 Conchifera, and we recover the expected topology within Polyplacophora consistent
113 with other recent work using genomic and morphological characters (Fig. 2). Within
114 Conchifera, we confirm the topology of other recent studies (Song et al., 2023);

however, our supplementary analyses recovered Scaphopoda sister to Gastropoda but with lower support (SI Appendix, Fig. S3). Comparison with genomes from other molluscan classes shows the molluscan ancestor had a genome composed of 20 linkage groups (Fig. 2), we refer to these as the Molluscan Linkage Groups (MLG) 1-20. Three important fusion events are apparent synapomorphies for Polyplacophora, present in all living chitons and no Conchifera: MLG 4+16+18, MLG 7+10, and MLG 8+9 (Fig. 3, Fig. 4, SI Appendix, Figures S4-S7). There are additional fusions and intra-chromosomal rearrangements that are different in every species sampled.

## Discussion

### *Chiton genomes show frequent and extreme rearrangement*

Four new chiton genomes represent the most complete genomes sequenced for the class, adding to several previously published partial or complete genomes (Hong Kong Biodiversity Genomics Consortium et al., 2024; Liu et al., 2023; Varney et al., 2022, 2021) (SI Appendix, Tables S1-S3). Our comparisons of conchiferan and aculiferan (polyplacophoran) linkage groups confirm previous studies that also predicted a haploid karyotype of 20 for the molluscan ancestor based on other metazoans (Simakov et al., 2022).

Previous work demonstrated the variability in chiton karyotypes; species in the clade Chitonida have reported haploid numbers ranging maximally from 6 to 16 (Odierna et al., 2008) with a mode of 11 (Hallinan and Lindberg, 2011). Our data give the first indication for expected chromosome count in a species in Lepidopleurida (1n=11) within this established range. Using five chromosome-level genome assemblies for chitons, we reconstructed the ancestral karyotype for Polyplacophora (more strictly the taxonomic order Neoloricata), and all intermediate phylogenetic nodes to demonstrate

the stepwise fusion and rearrangement of gene linkage groups during chiton evolution (Fig. 3).

Chitons demonstrate extreme genome rearrangement, even within a single genus. This represents not only gene order differences but syntenic (co-occurrence) changes in genomic architecture. Species of the genus *Acanthochitona* have a relatively short divergence time of maximally ~23 My based on the fossil record (Dell'Angelo et al., 2020). *Acanthochitona discrepans* and *A. rubrolineata* each have 8 haploid chromosomes but these result from two different fusions compared to the reconstructed ancestral karyotype of *Acanthochitona* (Fig. 1, Fig. 4C). Previous karyotype data for *A. discrepans* were actually based on specimens of *A. crinita*; the species are very similar but the correct identification can be determined based on the geographic distributions (Vončina et al., 2023). *Acanthochitona crinita* has 9 haploid chromosomes (Certain, 1951): this is yet another syntenic arrangement within the same genus. There are major changes between congeners in different ocean basins (the Pacific *A. rubrolineata*) but also between two species in the NE Atlantic (*A. discrepans* and *A. crinita*) that are morphologically and ecologically almost indistinguishable.

Living species of Lepidopleurida retain more plesiomorphic morphology and this clade has a deep fossil record extending to the lower Carboniferous (Sigwart, 2017). The largest number of novel fusions among chiton genomes is three, in the lepidopleuran *Deshayesiella* (Fig. 4A). This in particular contrasts with the naive expectation that gene arrangement in most chiton genomes would be relatively conserved; Lepidopleurida retain plesiomorphic conditions in their morphology (Ampuero et al., 2024; Sigwart, 2017) yet this exemplar shows deviations from the reconstructed ancestral karyotype.

165 The remaining living chitons (Chitonida s.l.) comprise two sister clades recognised as
166 separate orders: Chitonida and Callochitonida (Moles et al., 2021). *Callochiton* also
167 has two additional fusion events, and the chitonid *Liolophura japonica* has a partial
168 genome duplication, with two linkage groups fused apparently at first and then
169 duplicated (Fig. 4B). In our reconstruction of ancestral karyotypes, there is no
170 differences in arrangement between the ancestor of Chitonida *sensu stricto* or the
171 ancestor of Chitonida *sensu lato* (Chitonida + Callochitonida). The four species in
172 Chitonida s.l. share a large, fused chromosome (MLG 01+04+16+18) that is notably
173 not well-mixed in *Liolophura japonica* (i.e., Chr01 in *Liolophura japonica*, Chr01 in
174 *Callochiton septemvalvis*, Chr01 in *Acanthochitona rubrolineata*, and Chr03 in *A.*
175 *discrepans*). Part of this pattern has its origin in the ancestral chiton karyotype and is
176 retained in the lepidopleuran *Deshayesiella* (i.e., MLG 04+16+18). This implies a
177 variable rate of intra-chromosomal rearrangement, with several of the MLGs
178 conserved.

179

180 ***Ancestral karyotypes for Mollusca***

181 Chromosome numbers are not strongly conserved in animals. Changes in
182 chromosome numbers are hypothesized to be an important driver of the diversification
183 of Lepidoptera, with a strong correlation shown between rates of chromosome number
184 changes and speciation (de Vos et al., 2020). The instability in lepidopteran
185 chromosome numbers has mainly been studied from karyotype data but recently
186 confirmed in genomic data (Chen et al., 2019), and changes within genera are known
187 to encompass both neutral and adaptive evolution (Lucek, 2018; Vershinina and
188 Lukhtanov, 2017). However, the level of rearrangement may be less than that in
189 Polyplacophora.

190 Even within a single species, chromosome fusions are not uncommon, with
191 Roberstonian translocations occurring in roughly 1 out of every 1000 live human births
192 (Wilch and Morton, 2018). A previous study speculated that chromosome loss in
193 related clades of chitons may be the result of Robertsonian translocation (Odierna et
194 al., 2008). Although we still lack information on the telomere, this simple mechanism
195 is not a sufficient explanation for several more complex intrachromosomal
196 rearrangements (e.g. MLG 7+10: Fig. 4). Chromosome fusion does not present any
197 immediate reproductive barrier, yet chromosome rearrangements between sister
198 species can act as Dobzhansky-Muller incompatibilities and generate reproductive
199 isolation (de Vos et al., 2020). Chitons are broadcast spawners, so such barriers may
200 be speculatively advantageous, but so too are bivalves where these syntenic
201 rearrangements are not found.

202 In order to compare syntenic changes within Polyplacophora to other mollusc clades,
203 we re-analysed all available molluscan genomes in context of the newly identified
204 MLGs. For example, the divergence of the bivalve clade Imparadentia (Lucinida +
205 Venerida) is estimated in the Silurian, ca. 430 Mya (Crouch et al., 2021), or more than
206 150 My deeper than the divergence of crown group Polyplacophora, yet species within
207 Imparadentia have almost no syntenic rearrangement (SI Appendix, Fig. S7, S8).
208 Bivalves in Pteriomorphia, which have an even deeper origin in the lower Ordovician,
209 ca. 485 Mya, show some rearrangement but members of two orders (Arcida and
210 Pectinida) are highly similar, while two species in Ostreida that are intensively
211 cultivated differ from these others and from each other (SI Appendix, Fig. S8).

212 Potential adaptive roles may be connected to contrasting mobility of different MLGs.
213 The smallest two MLGs are the most conserved across the five chiton genomes;
214 MLG20 is the only group that remains separate in all bivalve and chiton taxa. This
215 region is also not duplicated in partial genome duplications in Neogastropoda, but is

duplicated in whole genome duplication in two hermaphroditic terrestrial gastropods (*Achatina fulica* and *Arion vulgaris*) (Fig. 5, SI Appendix, Fig. S5).

Earlier models based on karyotype data predicted three whole genome duplication events in the evolutionary history of living molluscs: in Neogastropoda, Stylommatophora, and coleoid cephalopods . Available genomes of Stylommatophora confirm whole genome duplication, as well as large scale gene duplications in Neogastropoda (SI Appendix, Fig. S6). In coleoid cephalopod genomes, the co-occurrence of loci from MLGs and intensive fusions (SI Appendix, Fig. S6) might not be easily resolved but may be more likely from the chromosome-disrupted processes reported in previous work (Albertin et al., 2023). By contrast, in *Nautilus* the linkage group that contains the conserved *hox* gene sets (Chr11, MLG16) shows no signal of co-occurrence with other MLGs or on other chromosomes (SI Appendix, Fig. S6). New results also show a partial and ancient genome duplication in the chiton *Liolophura* (Fig. 2, Fig. 4). Given that the MLGs are well conserved in all chitons if not their order (SI Appendix, Fig. S4), the event in *Liolophura japonica* is most likely a duplication instead of fission. Whole or partial genome duplication is now known from four molluscan classes, with contrasting patterns of ploidy (e.g. gastropods) or tandem (e.g. scaphopods) duplications (Fig. 5).

Reconstructing genome evolution is naturally more difficult for groups with high rates of intra-chromosomal rearrangement (Farré et al., 2019; Muffato et al., 2023). These patterns of co-occurrence of loci on the same chromosomes (synteny) should also persist for longer in evolutionary time, compared to faster rates of change in gene order (Damas et al., 2022; Simakov et al., 2022). Syntenic changes do not necessarily follow overall rates of translocation, which do not differ obviously among molluscs (SI Appendix, Table S4, S5). Confounding effects of rapid gene order or linkage group changes are not limited to issues of phylogenetic distance, when even closely related

species have significant syntenic differences. Nonetheless a focus on linkage group arrangements is a promising direction for the many unresolved questions of deep molluscan phylogeny. Recent studies have championed the importance of synteny-based studies on genome evolution, as a basis to understand deep divergences and also the general mechanisms that underlie genomic architecture, fusion, fission, and translocation (Mackintosh et al., 2023). A general trend in insect and vertebrates is for increasing chromosome numbers in derived lineages. Fusion events may be more common in more recently derived chitons or connected to specific adaptations.

## Conclusions

The relatively small number of reference quality genomes for most molluscan groups is a temporary limitation to these analyses. High heterozygosity seems to be very common in molluscs, and is clearly a feature of chitons, which causes difficulty for high quality genome assembly. The heterozygosity for *Callochiton* at 4.12% far exceeds the 2.95% genomic heterozygosity reported as “one of the highest” for Lepidoptera (García-Berro et al., 2023). Large scale analyses based on genomics are equivocal about the drivers of genetic diversity , and further data from diverse clades must be included.

The concept of "diverse groups" is mostly based on perceived variability, typically manifested via morphological, ecological, or genetic differences. As one interesting comparison, Lepidoptera is commonly regarded as super-diverse, but also represent a recognisably constrained form, with variation in striking difference in colours, patterns and wing shapes. While lepidopteran variability is visually conspicuous, chitons are apparently equally variable, as demonstrated through their genetic rearrangement. Chitons, despite exceeding 1000 species in diverse ecological niches, with a wide

267 range of morphological adaptations, are considered a “minor” and “neglected” clade.
268 The group is taxonomically challenging – on the one hand, because of the supposed
269 morphological stasis as a group, on the other, because of their high interspecific
270 variability. Our comparative analyses suggest chromosomal-level changes are a pattern
271 throughout much of chiton evolution, since chromosomal rearrangements are found when
272 comparing congeneric species (*Acanthochitona* spp., Fig 4C) and also across orders (Fig
273 4A, 4B).

274 Chitons exhibit chromosome rearrangements at an almost unprecedented level. This
275 is clear in the present study, the first comparative genomic study for Polyplacophora,
276 compared to orders of magnitude more data for better studied groups. All this poses a
277 more general question on how we define variability, how we perceive it, and also, if we
278 actually understand it at all? Recognition of variability in the genome and phenome is
279 crucial in understanding and re-evaluating unbiased measures of diversity in
280 overlooked groups of organisms. The relationship revealed between the unstable
281 arrangement of chiton genomes and species diversity provides new insights into
282 potential mechanisms for speciation and broader diversification within Mollusca.

283

284 **Methods**

285 Specimens were collected alive and flash frozen in liquid nitrogen (*A. rubrolineata*) or
286 frozen at -80°C and stored at -80°C. High molecular weight genomic DNA was
287 extracted from the foot of an individual specimen of each species, following the
288 guidelines of the SDS method, and used for PacBio high fidelity (HiFi) sequencing and
289 Hi-C library preparation. The PacBio Sequel II / IIe instrument was used for sequencing
290 in CCS mode. For transcriptome sequencing, five separate tissues were dissected
291 from the same specimen as DNA extraction, and preserved in RNAlater: foot (F),
292 perinotum (P), radula sac (R), shell edge (S), and visceral mass (V).

293

294 Adapters in the reads were checked and removed using HiFiAdapterFilt version 2.0.
295 The genome survey was finished using jellyfish v2.2.10 and Genomescope v2.0
296 (Ranallo-Benavidez et al., 2020), implemented 21- and 23-mer, which produced the
297 estimated genome size and heterozygosity level for each species (SI Appendix, Fig.
298 S2). The four genomes were assembled de novo based on qualified HiFi reads using
299 hifiasm v0.16.1 (Cheng et al., 2021), with some additional curation steps for *C.*
300 *septemvalvis* because of the high heterozygosity (SI Appendix). Potential
301 contamination in the genome was detected and removed using blobtools v1.1.1
302 (Laetsch & Blaxter, 2017). Duplicated haplotigs and overlaps were removed for *D.*
303 *sirenkoi*, *A. discrepans* and *A. rubrolineata* using Purge_Dups v1.2.6 (Guan et al.,
304 2020).

305

306 The aligner STAR version 2.7.10a or STAR v2.7.3a was employed to map RNA-seq
307 data into the working genome data (Dobin et al., 2012). The resulting alignment file
308 served as an important support in prediction methods. *Ab initio* gene prediction was
309 performed on the repeat-masked assembly in comparison with other published
310 genomes (SI Appendix). Summary information on assembly, gene model, and
311 annotation is provided in Table S2.

312

313 We identified putative orthologous sequences shared among genomes or
314 transcriptomes for 58 molluscs and 4 additional metazoans to generate an alignment
315 for phylogenetic analysis using VEHoP (Li et al., 2024). The alignments were removed
316 if the overlap among them was less than 20 amino acids and there were less than 75%
317 of taxa sampled, then, each alignment was used to construct “approximately maximum
318 likelihood” tree using FastTree version 2.1.11 (Price et al., 2010). Phylogenetic
319 relationships were investigated using IQ-TREE version 2.1.3 (Minh et al., 2020) with

320 the "-MFP" model to compute the best-fit model of each partition and 1000
321 ultrabootstraps to test the topological support. To test the impact on conchiferan
322 topology we ran a second analysis excluding Solemyida, the earliest diverging clade
323 in Bivalvia (SI Appendix, Fig. S3).
324
325 The method for identifying ancient linkage groups followed previously published works
326 . We resconstructed the linkages of the orthologues in molluscs, with the demonstrated
327 commands, and conserved and representative proteins and their presumptive
328 locations (https://github.com/ylify/MLGs). In detail, the ancient and conserved linkage
329 groups were inferred from 7 chromosome-level mollusk assembles, including 1
330 gastropod (*Gibbula magus*), 1 bivalve (*Mizuhopecten yessonensis*), and the 5 chitons
331 (*A. rubrolineata*, *A. discrepans*, *C. septemvalvis*, *D. sirenkoi*, and *Liolophura japonica*).
332 The obtained gene set consisted of 4729 homologs, which could be detected in all 7
333 assembles and located in 20 linkage groups. The ancestral states of nodes were
334 predicted to trace the karyotype evolutionary route in Polyplacophora, from the
335 common ancestor of Mollusca to the genus *Acanthochitona*. Similarly, the nodes with
336 more than two chromosome-level genomes within Mollusca were also investigated for
337 the chromosome number of their common ancestor. We also compared the
338 translocation rates across Mollusca, based on the non-syntenic rate of change divided
339 by estimated divergence time. In this study, 25 genomes from four classes were
340 selected to calculated the translocation rate at the inter-chromosome level, including 2
341 scaphopods, 5 chitons, 8 bivalves, and 10 gastropods.
342
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## Declarations

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**Availability of data and materials**

The four chiton genomes projects have been deposited with the NCBI BioProject, with *Acanthochitona discrepans* in PRJNA1114954, *A. rubrolineata* in PRJNA1114370, *Callochiton septemvalvis* in PRJNA1114372, *Deshayesiella sirenkoi* in PRJNA1114373. In each project, the whole-genome sequencing data, Hi-C data, RNA-seq data have been affiliated. The genome assembly and gene-model predictions are deposited at figshare (10.6084/m9.figshare.27894189). The constructed Molluscan Linkage Groups (MLGs) are available, with the conserved and representative sequences and their predictive corresponding locations (https://github.com/ylify/MLGs/).

The commands used in this study have been deposited on GitHub (https://github.com/ylify/MLGs/), including the genome assembly, repeats and coding regions prediction, phylogenomic inference, and mutual best hits and ancient linkage groups detections.

**Author contributions**

JDS and JS conceived the study and collected the samples. YL and ZC performed the analyses. JDS and KV led the biological interpretation of the results and review of comparative information from other studies. JDS drafted the manuscript. All authors contributed to interpreting the results and writing the manuscript.

## Competing interests

The authors declare they have no competing interests.

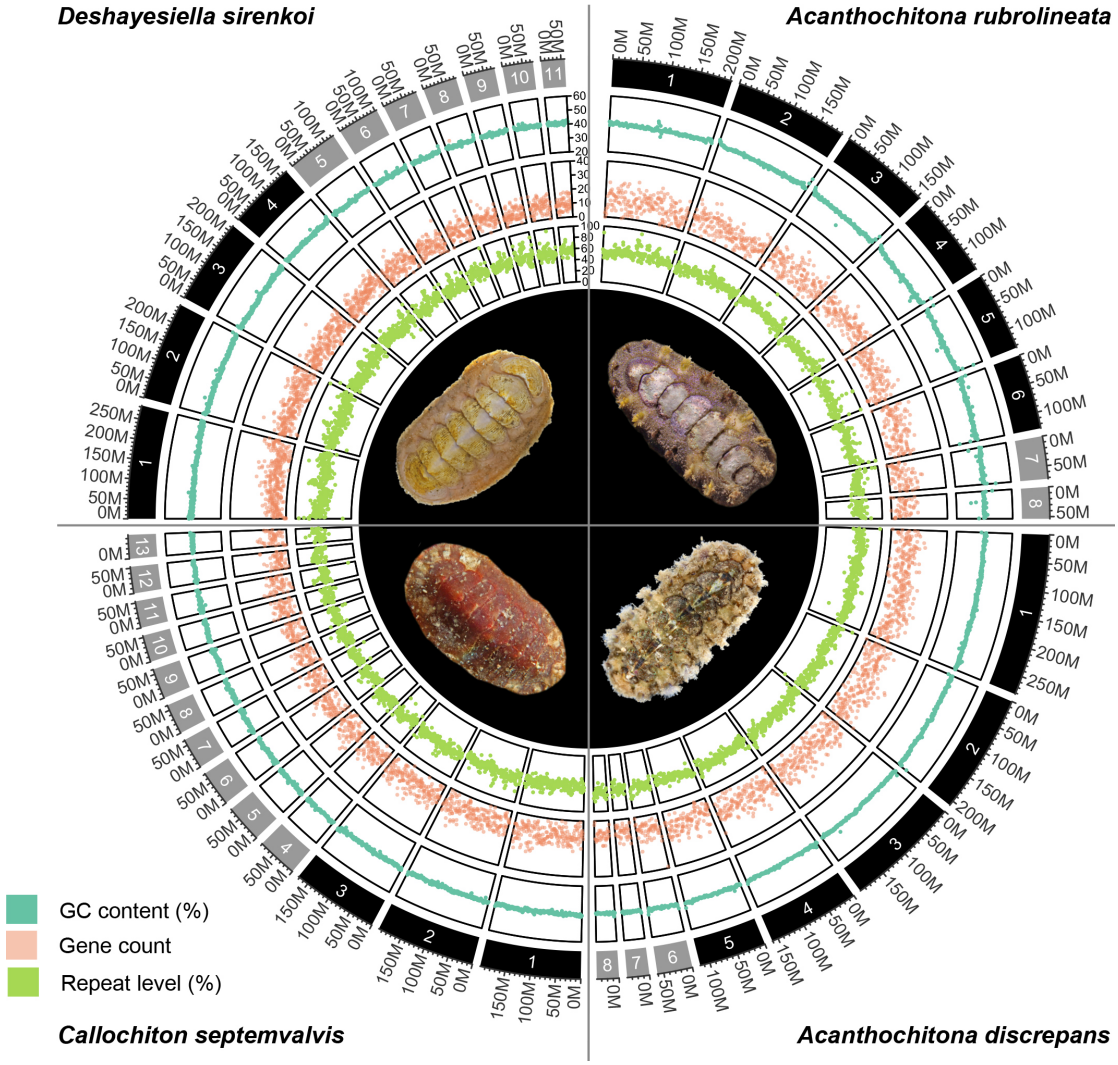
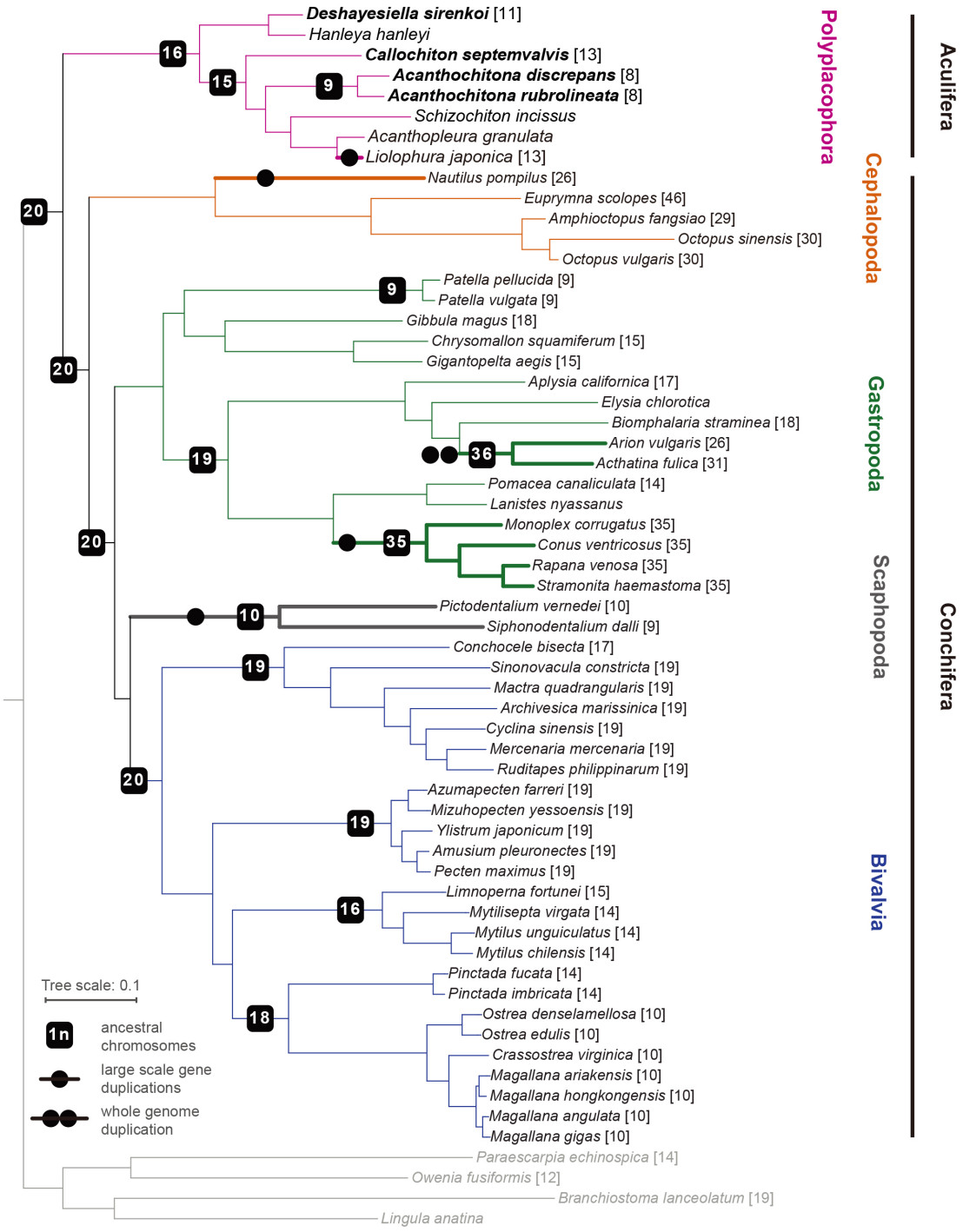



**Fig. 1.** CIRCOS plots for 4 new chitons genome assemblies, clockwise from top left: one species in the order Lepidopleurida *Deshayesiella sirenkoi*, and three species in the clade Chitonida *sensu lato*: *Acanthochiton rubrolineata*, *A. discrepans*, and *Callochiton septemvalvis*. Each quarter circle shows the pseudochromosome content for each species, in order of size, with concentric rings indicating GC content, gene count, percent repeat content, and a photograph of the respective species.




527

528 **Fig. 2.** Phylogeny of Mollusca, with new genomes noted in bold type, and the
529 chromosome number in square brackets for each species where known. Lineages
530 with known whole (double circle) or partial genome (single circle) duplication are in
531 thicker lines for emphasis; boxes on branches show the reconstructed ancestral 1n
532 chromosome number for the respective clade.

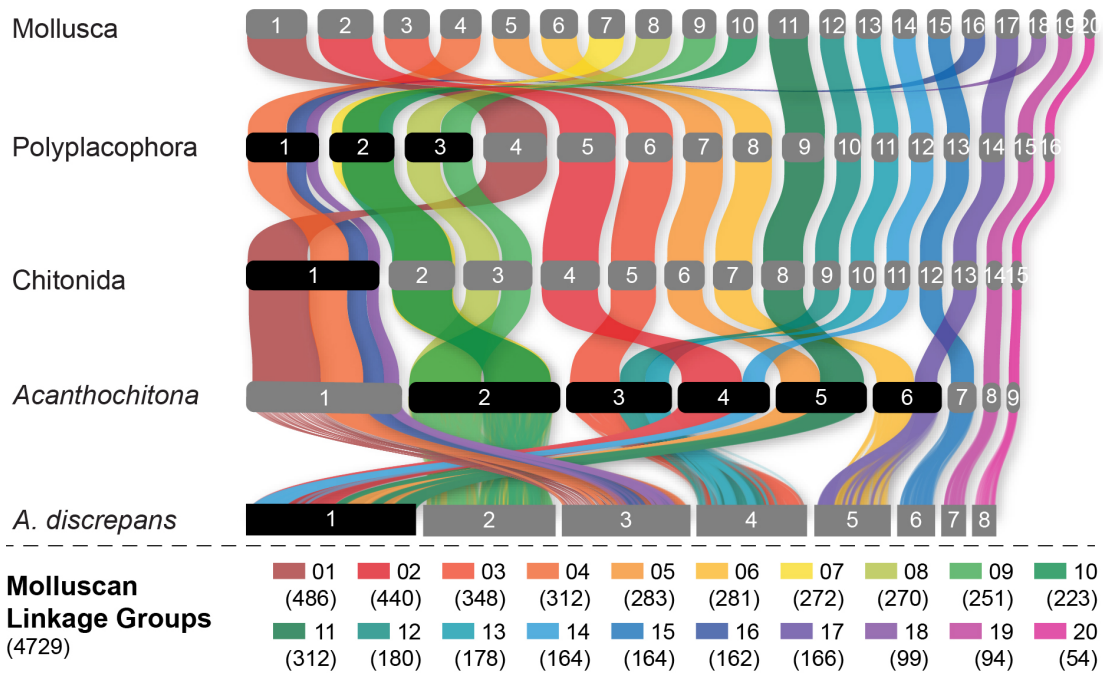



**Fig. 3.** Evolution of the ancestral molluscan linkage groups (MLGs) within Polyplacophora using the lineage leading to *Acanthochitona discrepans* as an example. MLGs are distinguished by colours, at the top of the diagram and in the key at bottom showing the number of orthologs. Each row is the reconstructed karyotype of the ancestor of living Polyplacophora, the order Chitonida *sensu lato*, and the genus *Acanthochitona*. Reconstructed chromosomes on each row are numbered in order of size from largest (left) to smallest (right); chromosome fusions are highlighted with chromosome numbers in black boxes. This presentation highlights the extent of shifts especially in comparison to the molluscan or polyplacophoran ancestor.

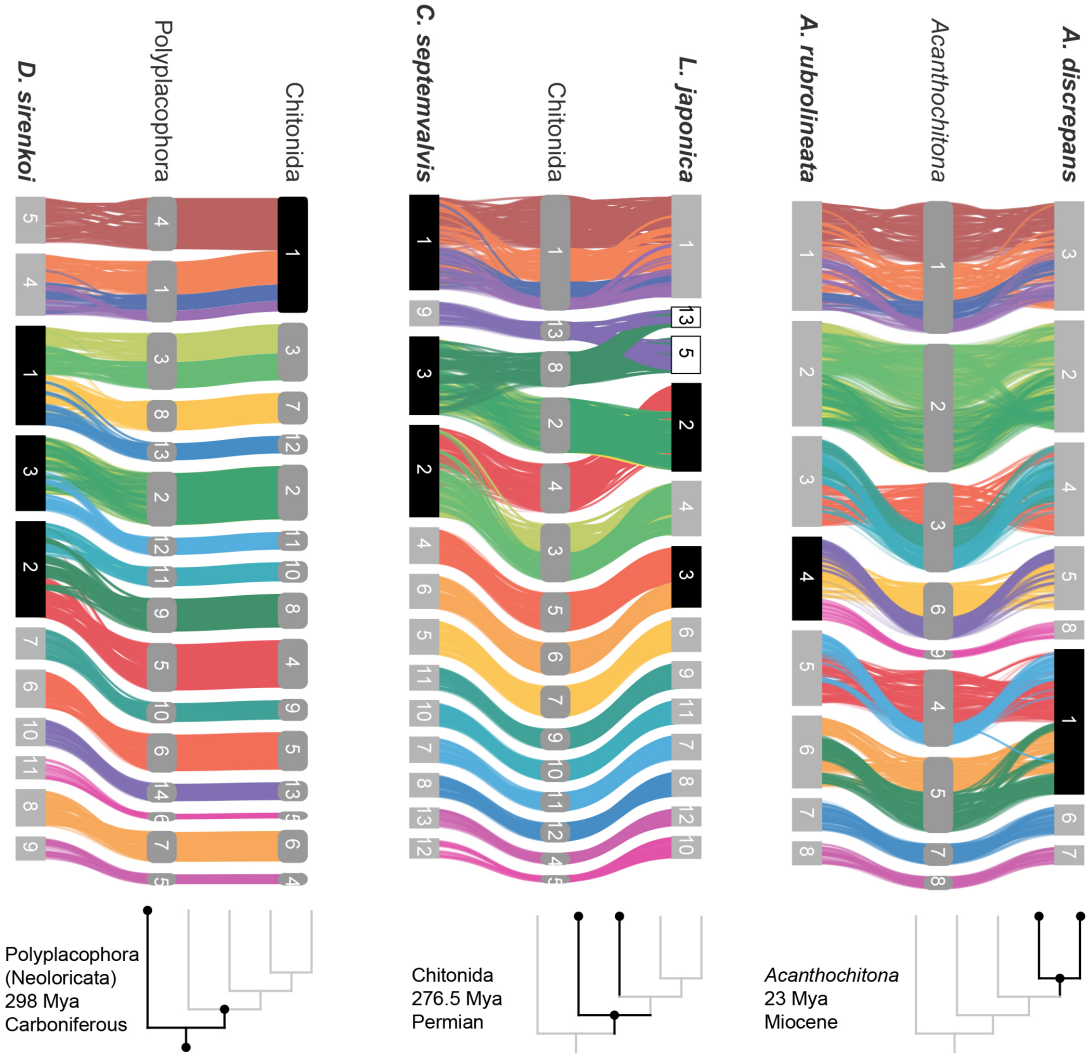



**Fig. 4.** Syntenic rearrangements of MLGs within the evolution of Polyplacophora. Each part shows the reconstructed karyotypes of an ancestor (middle) and two descendent lineages, with a schematic cladogram for orientation. From left to right, the divergence of A) ancestor of Polyplacophora, leading to the lepidopleuran species *Deshayesiella sirenkoi* (left) and ancestor of Chitonida (right), B), ancestor of Chitonida s.l. leading to the callochitonid *Callochitona septemvalvis* (left) and chitonid *Liolophura japonica* (right) and C) ancestor of the genus *Acanthochitona* leading to the two congeneric species *A. rubrolineata* (left) and *A. discreapans* (right). Colours and presentation are as in Figure 3, and chromosome numbers indicate the sequence in terms of size from largest (1) to smallest. Here, the chromosomes are not in order of size but reordered such that each transition from the nearest ancestor is visible in more detail. Chromosome fusions are highlighted with chromosome numbers in black boxes, and duplications in white boxes.

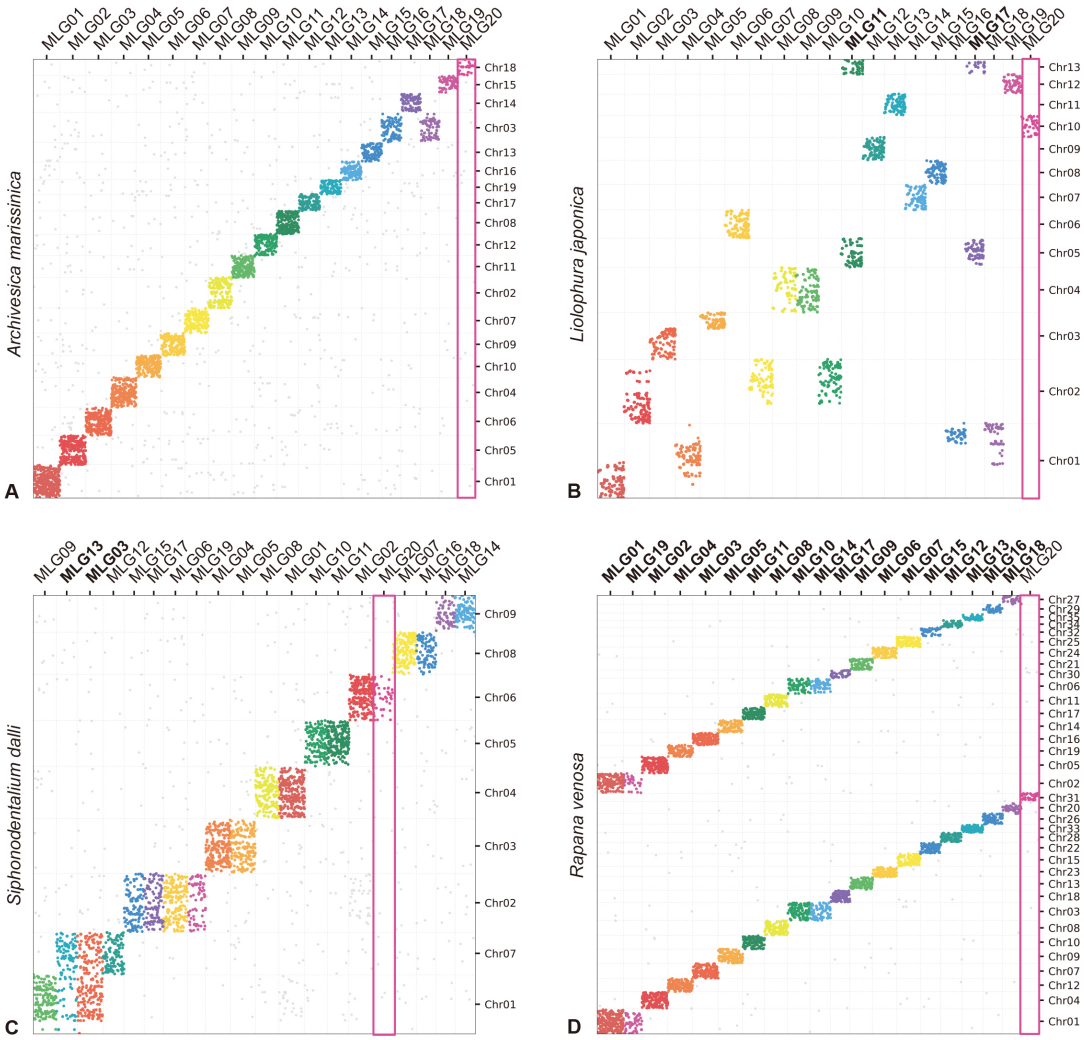



**Fig. 5.** Oxford plots comparing gene occupancy of species from four different classes of molluscs (vertical) to the ancestral molluscan linkage groups (MLG, horizontal): A) the bivalve *Archivesica marisinica* retains a plesiomorphic karyotype reflecting the 20 MLGs, B) the chiton *Liolophura japonica* has large scale gene duplication in MLG11 and MLG17 on separate pseudochromosomes, C) the scaphopod *Siphonodetalium dalli* has large scale gene duplication of MLG13 and MLG03 on separate pseudochromosomes, D) the gastropod *Rapana venosa* demonstrates a nearly whole genome duplication with MLG20 not duplicated. Colours follow the presentation in Figure 3.