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The elusive genomic signature of tadpole shrimps' ancient morphology

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The tempo of evolutionary change varies widely across the tree of life, with some lineages undergoing extensive morphological diversification while others remain remarkably static. Notostraca, or tadpole shrimps, exemplify the latter, displaying minimal morphological change for hundreds of millions of years. To investigate the molecular basis of this exceptional stasis, we generated high-quality genome assemblies for *Triops granarius* and *Triops simplex*. These genomes, combined with data from 18 additional branchiopod species representing Notostraca and Onychocaudata, were employed for phylogenetic reconstruction and time estimation and support the emergence of Notostraca and their general morphology in the Devonian (approx. 390 Ma). We identified genes with significantly reduced rates of protein evolution in Notostraca compared to their more morphologically diverse sister group, Onychocaudata. Functional annotation linked these genes to morphogenesis and development, but we also detected genes with accelerated protein evolution associated with similar developmental processes. Notably, genes undergoing a decelerated evolution in their protein-coding sequences lack signatures of evolutionary constraints in their non-coding regions. In addition, sequence evolution and gene family expansion/contraction dynamics appear decoupled from the rate of protein evolution, suggesting that genes can undergo reduced evolutionary change in one aspect, but not in others. Our findings reveal a complex interplay between genomic and phenotypic evolution and suggest that morphological stasis is maintained by multiple molecular processes rather than by a single, overarching mechanism.

1. Introduction

The pace of evolutionary change is uneven across the different branches of the tree of life [1] and is affected by several factors, including broad-scale phenomena that can affect multiple lineages co-existing at the same time [2,3]. For example, mass extinctions are frequently followed by pronounced shifts in body size, with surviving taxa generally exhibiting smaller sizes than their predecessors [4]. Nevertheless, evolutionary rate variation is also extensive across contemporary lineages [5–7]. While many examples of uneven rate of change exist for molecular or life history traits [8,9], morphological evolution often provides striking examples of pronounced rate heterogeneity. In certain cases, reduced rates of change regard specific anatomical traits [10]; yet in some instances, stasis pervades multiple aspects. Examples of static morphologies can be found across the tree of life, especially where fossil records

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are available. Horseshoe crabs of the genus *Limulus* [11], coelacanths [12] and *Ginkgo* species [13] are some prominent examples of clades with various degrees of morphological conservatism. They have been appointed several times as 'living fossils', although this status and terminology are debated and considered controversial by many [11,12,14,15]. In some instances, analyses of molecular evolutionary rates in a handful of 'living fossil' lineages have suggested that these clades exhibit slower rates of genomic change, aligning with their phenotypic stasis [16,17].

Among the numerous cases of morphological conservatism, Notostraca—commonly known as tadpole shrimps—stands as an iconic example. Fossils from the Upper Carboniferous (approx. 300 million years ago (Ma)) indicate that the fundamental body plan of Notostraca may have remained virtually unaltered over geological timescales [15,18,19]. Despite exhibiting substitution rates that are comparable to—or only marginally lower than—those of their sister clade Onychocaudata, Notostraca species show a dynamic genome evolution, with a rapid turnover in gene family expansions and contractions, as well as in the transposable elements (TEs) composition [20,21]. This suggests the absence of a molecular counterpart to the morphological stasis often associated with so-called living fossils [22], leaving the genomic basis for Notostraca's striking conservatism elusive. These findings raise a critical question about the mechanisms constraining phenotypic evolution: can conserved morphology be linked to a handful of conserved genes?

Here, we use a phylogenomic framework—augmented with new genomes for *Triops granarius* (Lucas, 1864) and *Triops simplex* (Ghigi, 1921)—to test: (i) whether the subset of genes that exhibit decelerated protein-sequence evolution in Notostraca relative to the sister clade Onychocaudata is enriched for morphogenesis and developmental functions; and (ii) whether these genes also show decelerated changes in complementary metrics, such as conservation in non-coding regions and reduced gene-family turnover. Our findings highlight the complexity of linking genomic dynamics to morphological evolution, and that genes can undergo a slow evolutionary change in one aspect, but not in others.

2. Results and discussion

(a) New genome assemblies, orthology and time tree inference

Currently, genome assemblies are available for eight species within Notostraca: five belonging to the *Lepidurus* genus, while only two to its sister *Triops*. To achieve a more balanced taxonomic representation of the order, we produced new genome assemblies and annotations for *T. granarius* and *T. simplex*. Assembly lengths resulted in 86.69 and 97.86 Mb (electronic supplementary material, table S1), respectively, corresponding to a theoretical genome coverage of 88X and 75X. Assembled genomes were checked for completeness using the arthropods' gene set of BUSCO and resulted in 96.0% and 95.5% of complete genes, respectively (electronic supplementary material, table S1). Gene predictions successfully annotated 11 994 and 12 835 genes, whereas 8.75% and 10.82% of the genomes were composed of repetitive elements (electronic supplementary material, table S2).

The final dataset for orthology inference encompassed the proteomes of 10 Notostraca samples (five species of the genus *Lepidurus* and four species of the genus *Triops*, with *Triops cancriformis* (Bosc, 1801) represented by both a bisexual (ESP) and a parthenogenetic (ITA) lineage; [20,23]), seven Anomophoda (six *Daphnia* species and *Chydorus sphaericus* (O.F. Müller, 1776); [24–29]), two Spinicaudata (*Leptestheria dahalacensis* (Rüppell, 1837) and *Eulimnadia texana* (Packard, 1871); [20,30]) and one Anostraca outgroup (*Artemia franciscana* (Kellog, 1906)). All assemblies included in the dataset have a BUSCO completeness higher than 95% (except *A. franciscana* with 89.6%; electronic supplementary material, table S1).

The orthology inference among the proteomes of the 20 species clustered 93.3% of genes in orthogroups (OGs), of which 5.5% were species-specific. In total, 425 OGs contained single-copy genes for all taxa and reconstructed a maximum likelihood phylogenetic tree with maximum support for all nodes, except for the one leading to the group *Lepidurus apus apus* (Linnaeus, 1758), *Lepidurus cf. couesii* (Packard, 1875) and *Lepidurus arcticus* (Pallas, 1793), which is scored 61. Major phylogenetic relationships within Branchiopoda have been recovered, with Notostraca forming a monophyletic group in a sister relationship to Onychocaudata (figure 1a). Divergence time estimates are coherent with literature and the fossil record ([32]; electronic supplementary material, table S3), and place the emergence of crown Phyllopoda (in this study represented by Notostraca + Onychocaudata) in the Ordovician (approx. 450 Ma), of *L. dahalacensis* + *E. texana* in the Cretaceous (approx. 140 Ma) and of crown Anomopoda (in this study represented by *C. sphaericus* + *Daphnia* spp.) in the Jurassic (approx. 150 Ma; [33–38]).

(b) Protein rate of evolution analysis

Considering that we employed an *ab-initio*-based approach for predictions of CDSs and proteins, without incorporating RNA-seq evidence from the same species, we checked for the possible impact of spurious annotations, which could be relevant and potentially have affected molecular evolutionary analyses [39,40]. Particularly, we checked for: (i) differences in general gene statistics; (ii) differences in BUSCO scores; and (iii) the influence of annotation approaches on predicted protein lengths. We found (i) no consistent deviation of gene, intron and exon lengths between Notostraca and the other species included (electronic supplementary material, figure S1), as well as (ii) no changes in BUSCO statistics at the genome (before annotation) and the proteome levels (after annotation; electronic supplementary material, table S1). For genes included in the molecular evolutionary analyses (see below), we fitted a linear mixed model including OGs (genes) and species (annotation method—Notostraca all share the same *ab-initio* annotation pipeline; see §4) as random effects. Most of the variation in protein length was explained by OGs (variance = 220,154), whereas (iii) the effect of the annotation type was minimal (Notostraca proteins

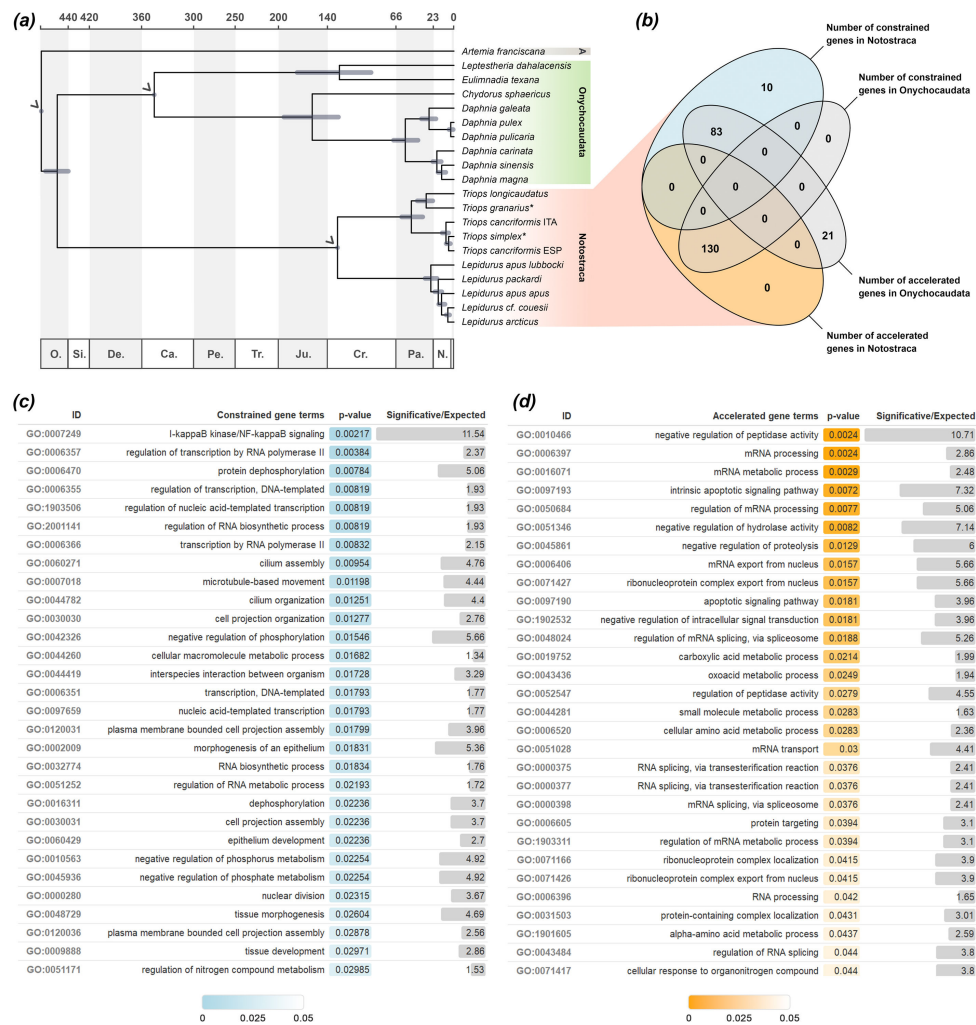


Figure 1. (a) Genome-scale divergence time estimate of the Branchiopoda species analysed in this work. Bootstrap values are 100 for all nodes except for the one leading to *Lepidurus apus apus*, *Lepidurus couesii* and *Lepidurus arcticus* [31]. Age confidence intervals (CIs) as computed by 100 LSD2 replicates are shown for each node; arrowheads indicate calibration nodes. Genomes sequenced in this work are marked with an asterisk next to the tip name. A: Anostraca. (b) Venn graph showing the number and overlaps of genes found to have undergone convergent protein rate of evolution in Notostraca (displaying a more static morphology) and Onychocaudata (displaying a higher morphological disparity). Genes that are constrained in Notostraca (i.e. showing a decelerated rate of protein evolution) are shown in blue, while genes accelerated in Notostraca (i.e. showing a higher rate of protein evolution) are shown in orange. Constrained and accelerated genes in Onychocaudata are shown in grey. The number of overlapping genes in each group is shown in the corresponding intersections. (c,d) Enriched biological-process Gene Ontology (GO) terms associated with genes having convergent protein rate of evolution in Notostraca. Only the top 30 enriched terms are shown. *p*-Values for each term are shown using a colour gradient (colour keys below each panel), while the 'significant/expected' column indicates the ratio between observed and expected terms in our genes of interest. Bars are proportional to ratio values. Colours as in panel (b). A comprehensive list of enriched terms is available in electronic supplementary material, table S5.

are 19.73 amino acids longer, on average) and not significant ($p = 0.199$). Summary statistics of Notostraca were comparable to those of Onychocaudata, indicating that our annotations are reliable despite the lack of transcriptomic evidence. Additionally, considering that comparative molecular evolution analyses were restricted only to the genes that are shared among Notostraca and Onychocaudata (see below), we excluded any potential misprediction that could appear as clone-specific genes and, therefore, with highly divergent sequences [40].

A convergent evolutionary rate analysis was used to assess whether genes with significantly decelerated protein evolutionary rates are linked to morphological evolution. This analysis tests, for each OG, whether branches sharing a focal trait—here, Notostraca's morphological stasis—exhibit parallel shifts in their rate of sequence evolution compared to the rest of the tree. TRACCER computes such shifts by firstly normalizing per-branch rates against genome-wide expectations, and then by assessing significance via permutation of trait labels on the tree. Negative scores correspond to convergent rate deceleration (constraint) in the focal clade, while positive scores to convergent acceleration. Of the 7470 OGs analysed, with Notostraca as the focal clade, 222 showed convergent evolutionary rate shifts in the clade. Specifically, 130 were categorized as accelerated and 93 as constrained (figure 1b). We then performed the same analysis using Onychocaudata as the focal clade to assess whether the evolutionary patterns observed in Notostraca were exclusive to the clade or the results of shared life-history traits. This analysis resulted in 234 OGs in Onychocaudata with convergent shifts in the rates of sequence evolution: 104 were accelerated and 130 were constrained. It is interesting to note that most genes found to be accelerated in one clade were found to be constrained in the other, and *vice versa*: that is, 89% of the genes constrained in Notostraca are accelerated in Onychocaudata, and 100% of the genes constrained in Onychocaudata are accelerated in Notostraca (figure 1b). This pattern might reflect

lineage-specific evolutionary paths that shaped the contrasting morphological diversity of the two clades. However, a solid and clear explanation would require a more in-depth knowledge of the identities and functions of genes in Branchiopoda.

Notostraca constrained OGs are enriched for several terms, with some of them associated with morphological development (e.g. epithelium development (GO:0048856) and tissue morphogenesis (GO:0048729); figure 1c; electronic supplementary material, table S5). Conversely, accelerated OGs lack any evident connection to development and morphological conservatism, as they are associated with the regulation of transcription, and the metabolism of amino acids and proteins (figure 1d; electronic supplementary material, table S5).

A more specific characterization of the genes associated with the Gene Ontology (GO) term 'developmental process' (GO:0032502) found 822 genes among those analysed, of which nine were constrained and 12 accelerated in Notostraca. Interestingly, a functional characterization of both constrained and accelerated genes revealed similar patterns, with homologues of genes involved in key roles in morphological development, despite their contrasting evolutionary rates (electronic supplementary material, table S5).

(c) Whole genome alignment and prediction of discrete conserved elements

To understand whether Notostraca genes identified as constrained at the protein level are also characterized by a higher conservation of their non-coding regions (introns and UTRs) compared to accelerated genes, we performed a whole genome alignment (WGA) of all Notostraca genomes and built a null model of sequences evolution using 489 582 bases corresponding to ancestral repeats, similarly to [41]. We then used this model to identify discrete conserved elements (CEs) on the chromosome-scale genome of *Lepidurus packardii*, focusing on genomic regions on which at least six species could align, thus ensuring to consider at least one species from both *Lepidurus* and *Triops*. Overall, between 45% and 65% of Notostraca genomes were successfully aligned with Progressive Cactus on *L. packardii* scaffolds, with higher rates on congeneric species, as expected (electronic supplementary material, table S6). Of the 7470 OGs considered in evolutionary rate analyses, 6524 have an orthologue gene in *L. packardii*. Of these, 95% of their exons, 81% of their introns, 62% of their 3', and 75% of 5'-UTR regions are covered for at least 75% of their length by cross-genus alignable regions, and were therefore considered for CEs comparison. Interestingly, we found that the different rates of protein evolution identified by TRACCER are also reflected at the nucleotide level, with constrained genes showing a higher percentage of CEs compared to the accelerated ones along their exons (two tails Wilcoxon rank sum test; $p = 0.01471$; figure 2a). On the other hand, despite the presence of CEs in both introns and UTR regions—approximated to 250 bp gene-flanking regions—we did not find any trace of higher nucleotide conservation (two tails Wilcoxon rank sum test; $p > 0.05$; figure 2a). The relatively high fragmentation did not allow us to properly test for the degree of conservation along enhancers and promoters. Highly conserved regulatory sequences are frequently associated with genes that play key roles in development, and with genes exhibiting slow rates of evolution in their protein-coding regions (PCRs; [42–44]). However, changes in enhancer and promoter sequences can evolve rapidly and lead to significant regulatory variation, even when the corresponding PCRs remain conserved [45–47].

(d) Gene copy number dynamics

Genes undergoing accelerated or constrained rates of protein sequence evolution were also tested to assess whether they show similar patterns in their copy number dynamics. The evolution of gene copy numbers in our dataset is best described by the CAFE model implementing two different gene family turnover levels (λ), one for Notostraca and one for all the other species (likelihood ratio of -142 ; electronic supplementary material, figure S2). Particularly, the rate of gene family turnover appears 1.2 times higher in Notostraca ($\lambda = 1.00501 \times 10^{-3}$) than in other branchiopods ($\lambda = 8.04632 \times 10^{-4}$), confirming previous observations [20]. Theoretical models in evolutionary genomics suggest that genes experiencing lower sequence constraints (evolving at faster rates) should also be subject to fewer constraints on copy number. Empirical studies have consistently supported this hypothesis across diverse taxa [48–51]. However, our findings in Notostraca do not align with this broadly observed pattern (figure 2b), as no significant difference in the net gene copy number variation was detected between accelerated and decelerated genes. Hence, a strong decoupling of gene copy number dynamics from coding sequences evolution rates is observed in both groups (figure 2b).

3. Conclusions

Our study provides new insights into the molecular basis of morphological conservatism in Notostraca and reveals a complex interplay between genomic and phenotypic evolutionary rates. We identified developmental genes with markedly decelerated rates of protein evolution that likely contribute to the morphological stability of this lineage. Yet, we also found that other genes involved in similar developmental processes evolve rapidly, suggesting that stasis and change can coexist within the same developmental framework. The decoupling between morphological and molecular evolution observed in Notostraca aligns with contemporary views on so-called 'living fossils' [52]. While these species appear to have remained morphologically and ecologically static over time [18,19], their development and physiology evolved at a different pace [53,54]. Furthermore, the very notion of morphological stasis in 'living fossils' has been repeatedly challenged (e.g. [15,55]). Although direct genotype–phenotype associations have been demonstrated for certain traits in specific systems (e.g. [56]), such clear correspondences are often absent in phenotypes shaped by extensive networks of interacting genes [57,58]. Our results therefore suggest that the

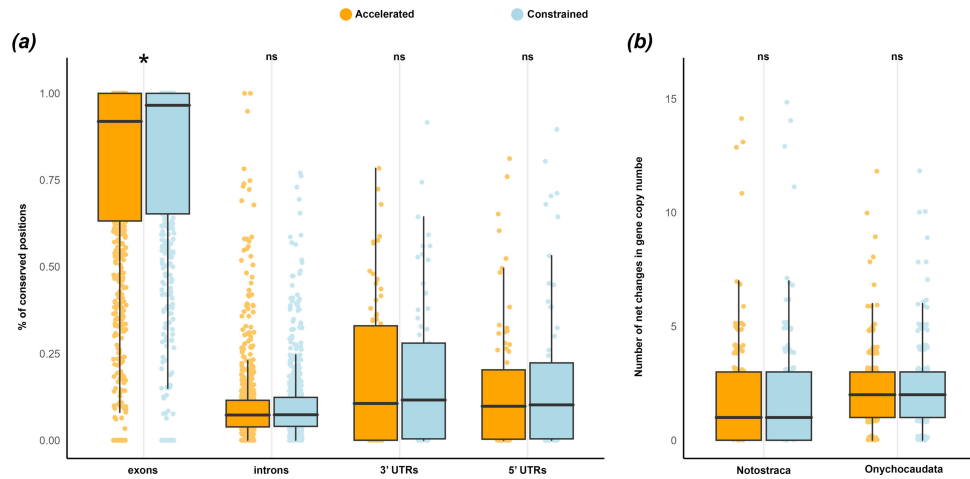


Figure 2. (a) Percentage of conserved nucleotide positions in exons, introns and UTR regions of *Lepidurus packardii* orthologues identified as accelerated and constrained during Notostraca evolution. (b) Number of net changes in gene copy number between accelerated and constrained genes in Notostraca and Onychocaudata. Significant Wilcoxon test results are shown at the top of the plot (* $p < 0.05$). Colours as in figure 1.

morphological conservatism of Notostraca stems from the interplay of multiple processes, rather than a single overarching mechanism.

4. Material and methods

(a) Genome sequencing and annotation

For the two species *T. granarius* (collected in Mongolia, Lat.: 47.072500N; Lon.: 105.841111E; [59]) and *T. simplex* (collected in Tunisia, Lat.: 35.799165N; Lon.: 10.147774E; [60]), total DNA was extracted from individual specimens using the E.Z.N.A.® Insect DNA Isolation Kit (Omega Bio-Tek) following gut removal. Whole genome sequencing was carried out on an Illumina platform (paired-end, 2×150 bp; insert size = 350 bp). Reads were deposited under bioprojects PRJNA1227130 (*T. granarius*) and PRJNA1227002 (*T. simplex*). Quality trimming and Illumina adapter removal were conducted using Trimmomatic v.0.39 [61], requiring a minimum quality score of 20 and a minimum read length of 30 bp. Assembly was performed using ABySS v.1.5.8 [31] with default parameters; optimal k-mer sizes (32–128 bp) were selected based on N50 statistics, considering only scaffolds longer than 1000 bp. Redundans v.0.14a [62] was used for redundancy reduction, short-read scaffolding and gap closing. BlobTools2 [63] assessed contaminants through BLAST against the NCBI nt database and taxonomic annotation at the phylum level. Minimap2 [64] aligned short reads to the assembly to calculate coverage and mapping statistics, with contaminant reads removed and re-assembled. Genome completeness was evaluated using BUSCO v.5 [65] on the Arthropoda orthologue set ($n = 1,013$) both on the genome assembly and on the predicted proteomes.

TEs were mined from the 10 Notostraca genomes using RepeatModeler2 [66] with the LTR-pipeline extension. The raw repeat libraries were then subjected to automatic refinement via MCHelper [67] in fully automatic mode. Each resulting TE library was then re-classified using the RepeatClassifier module of RepeatModeler2 based on DFAM (Version) and RepBase (Version) reference sequences. TEs were annotated in each genome with its respective TE library with RepeatMasker [68] in sensitive mode. After repeat masking, protein-coding genes were predicted using Augustus v.2.5 [69] as described in [21,23], with gene models from *Lepidurus apus lubbocki* (Brauer, 1873) and *L. arcticus*. Briefly, to generate reliable training sets, initial gene loci were predicted using the existing Augustus gene model for *Daphnia magna* (Straus, 1820) [23]. The resulting sequences were then aligned against the *D. magna* proteome using BLASTP (e-value $< 1e-4$) to identify high-confidence homologues ($> 90\%$ identity and $> 70\%$ coverage) for both sequences. From these, 500 non-redundant loci (similarity $< 30\%$) were selected per species to train Augustus, yielding optimized *Lepidurus*-specific models for final *ab-initio* gene prediction [23].

(b) Orthology and divergence time estimation

Protein sequences for each species were used as input for OrthoFinder v.2.4.1 [70] to reconstruct OGs. OGs were aligned using MAFFT v.7.520 [71] with default parameters and trimmed using trimAl v.1.4.rev15 [72] with the heuristic selection of trimming method (–automated1). OrthoFinder single-copy OGs were used to infer the species tree with branch length estimations. IQ-TREE v.2.2.6 [73] was employed to run three independent maximum likelihood partitioned analyses on trimmed alignment, with automatic model selection as implemented by ModelFinder [74] and partition merging. Branch statistical supports were obtained with 1000 ultrafast bootstrap replicates [75]. The timetree was calibrated using LSD2 as implemented in IQTREE [76]. Calibration points are available in electronic supplementary material, table S2.

(c) Paralog pruning and protein rate of evolution

OG trees were inferred using IQ-TREE as described before, and multi-copy OGs were decomposed to single-copy using DISCO [77]. We then optimized the branch lengths on the species tree for each decomposed OG using IQ-TREE. Convergent rate analysis was performed with TRACER [78] on the 20 Branchiopoda species. Gene trees were discarded if they had fewer than three species with or without morphological stasis, or six total, as they were unlikely to be informative. Initially, all Notostraca species were flagged as having the trait of interest (morphological stasis). Subsequently, all Onychocaudata species were flagged as having the trait of interest (morphological disparity). For OGs included in comparative genomic analyses, we tested whether Notostraca have a significant different protein length that might be due to our annotation strategy, fitting a linear mixed model using OGs and species as random effect with the *lmer* function of the lme4 R package [79]. The *p*-value for the fixed effect was computed with the lmerTest R package [80] using the Satterthwaite approach to approximate the number of degrees of freedom.

(d) Gene Ontology-term enrichment analysis

For each OG, GO terms were assigned to the *L. packardii* gene using OMA [81]. When a sequence of that species was missing, another random Notostraca sequence was used. Enrichment analyses were conducted separately for accelerated and decelerated genes using the TopGO R package in Bioconductor [82]. The analyses utilized the Biological Processes ontology and Fisher's exact test with the classic algorithm. GO terms were considered significantly enriched if the *p*-value was less than 0.01. Furthermore, genes with a decelerated rate of protein evolution in Notostraca were further characterized by manually blasting OGs to the NCBI nr dataset. Additionally, we retrieved all genes associated with one of the 5480 GO terms child to 'developmental process' (GO:0032502) and manually assessed how many were either accelerated or decelerated.

(e) Whole-genome alignment and conserved element prediction

Soft-masked genomes resulting from repeat annotation were used to perform a WGA with Progressive Cactus [83], using the previously inferred Notostraca species tree and the long-read-based chromosome-scale genome of *L. packardii* as the reference. Alignment coverage information for each of the six longest scaffolds of *L. packardii*, representing its putative six pseudo-chromosomes [84], was extracted along with a MAF representation of the WGA using cactus-hal2maf. Per-base alignment coverage information was extracted with the halAlignmentDepth program.

A neutral model of evolution was trained with 'halPhyloPTrain.py' on ancestral transposons annotated with RepeatMasker on the reconstructed ancestral genome of Notostraca, using all previously obtained repeat libraries. From this initial neutral model, we separately estimated a conserved and non-conserved model of sequence evolution for each *L. packardii* chromosome using phastCons [44,85] with the parameters: --target-coverage 0.28 --expected-length 30 --estimate-trees. Per-chromosome conserved and non-conserved models were then averaged with PhyloBoot to obtain genome-wide conserved and non-conserved models. These were used in a final PhastCons run with the same target coverage and expected length parameters to obtain discrete CE predictions. Only results obtained from the six *L. packardii* pseudo-chromosomes were used in following analyses. CEs were filtered to remove unalignable genomic regions, defined as genomic positions where fewer than six other Notostraca species could align to the reference genome of *L. packardii*. We excluded all predicted elements in which more than 25% of the positions were covered by unalignable genomic regions.

(f) Gene family expansion and contraction

To calculate the rate of gene family turnover (λ) we used CAFE (v5.1.0; [86]) on the OG counts as inferred by OrthoFinder. Then, after having implemented the CAFE estimation of the gene-family error model, we calculated (i) a global λ for the entire tree, and (ii) two different λ s for Notostraca and all the other branchiopods. To test which model better fitted the data, we simulated 100 OG datasets with a λ of 8.56591×10^{-4} and 6,643 simulated families. Then we estimated again a global λ and two different λ s for each simulated dataset, in order to compute a distribution of likelihood ratios between the two models. Each CAFE analysis, including simulations, was run for 1000 iterations to ensure convergence of likelihood values. To test whether OGs returned by TRACER as either accelerated or decelerated in Notostraca also underwent an expansion or contraction of their copy numbers, we employed two different metrics, calculated across all the internal and terminal nodes of Notostraca: (i) the number of significant CAFE expansions and contractions per OG; (ii) the sum of all gene gains and losses per OG. The same metrics were also obtained for accelerated and decelerated genes in Onychocaudata, considering all the internal and terminal nodes of the clade.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Data and code used for the analyses, as well as intermediate results and a description of provided files, are made available as electronic supplementary materials. Assembled genomes have been submitted to NCBI and are still under processing by the system (submission numbers: SUB15106804, SUB15106571). Genome annotations, together with intermediate results of the analysis and scripts to generate plots, are available from the Dryad Digital Repository [87].

Supplementary material is available online [88].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. G.F.: conceptualization, data curation, formal analysis, visualization, writing—original draft, writing—review and editing; F.N.: conceptualization, data curation, formal analysis, writing—review and editing; J.M.: conceptualization, data curation, formal analysis,

writing—review and editing; C.S.: data curation, formal analysis, writing—review and editing; S.C.: formal analysis, writing—review and editing; F.M.: resources, writing—review and editing; A.L.: funding acquisition, resources, supervision, validation, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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