



Time calibrated phylogenomic analysis of cyclostome divergence, diversity and biogeography

Evan James Christensen^{*1}, Douglas S. Fudge², David C. Plachetzki¹

^{*}Presenter ¹Department of Molecular, Cellular, and Biomedical Sciences, University of New Hampshire, Durham, NH, USA
²Schmid College of Science and Technology, Chapman University, Orange, CA, USA



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Cyclostomes

- The only surviving lineage of jawless vertebrates (agnatha)
- Superclass made up of and lampreys (Petromyzontida) and hagfishes (Myxini)
- Once believed to be paraphyletic, molecular data has supported monophyly
- Phylogenomic analyses of species relationships, biogeography, and timing of diversification are lacking outside of lampreys^{1,2}
- Origin of Galápagos hagfish biodiversity an open question³

Methods

Data collection

- Novel RNA-seq data derived from three hagfish genera: *Myxine*, *Eptatretus*, and *Neomyxine*
- Additional genome, RNA-seq, and mitochondrial (12S, 16S, COI, CYTB) data gathered from GenBank⁴
- Missing mtDNA markers retrieved from RNA-seq data using Nucleotide BLAST⁵

Ortholog filtering (Orthofinder⁶) and sequence alignment (MAFFT⁷, MACSE⁸)

- Identified orthologs across genome/RNA-seq taxa using Orthofinder⁶
- Filtered to retain orthologs present in ≥75% of taxa (392 orthologs retained)
- Aligned orthologs and non-coding mtDNA (12S, 16S) sequences using MAFFT⁷
- Aligned coding mtDNA (COI, CYTB) sequences using MACSE⁸

Phylogenomic tree (IQ-TREE⁹)

- Tree estimated from data matrix of 392 filtered ortholog alignments used as separate partitions under MFP+MERGE using IQ-TREE⁹
- Resulting topology received high support for all nodes; used as a constraint for further analysis.

Mitochondrial tree (IQ-TREE⁹)

- mtDNA partition data (12S, 16S, COI, CYTB) and preceding constraint tree analyzed under MFP+MERGE using IQ-TREE⁹

Time calibration (MCMCtree¹⁰)

- Calibrated select nodes using literature-based priors (gathered using TimeTree¹¹)
- 20,000 samples from the posterior (freq=10) summarized in time-calibrated phylogeny

Biogeographic reconstruction (BioGeoBEARS¹²)

- Modern ranges of taxa determined using distribution information gathered on FishBase¹³
- Reconstructed ancestral ranges of taxa using realms of the Marine Ecoregions of the World¹⁴
- Filtered out low-confidence reconstructions (<50%)

Figure 1. Workflow for phylogenetic data collection and analysis

Phylogenomic analysis clarifies cyclostome relationships

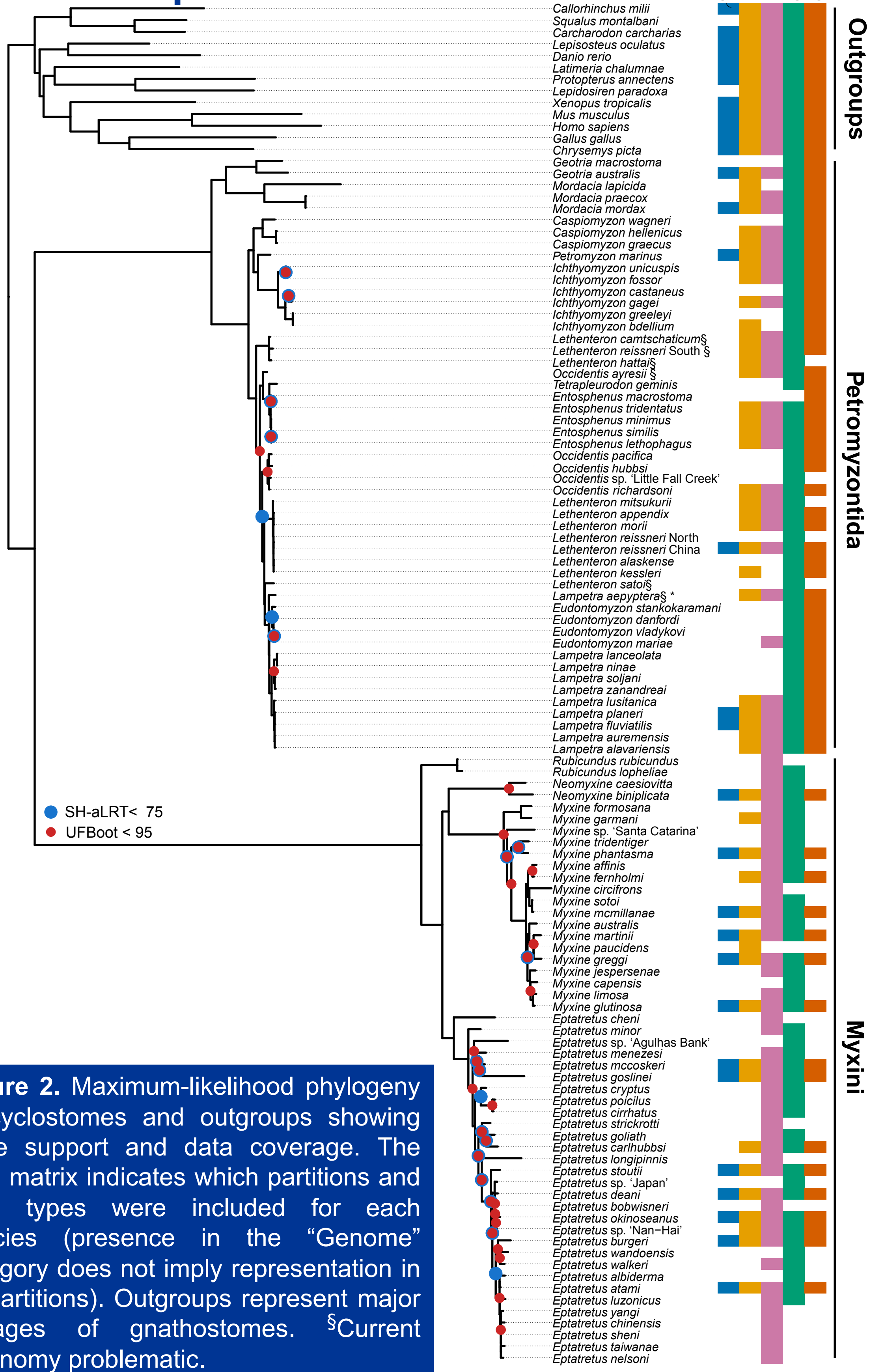


Figure 2. Maximum-likelihood phylogeny of cyclostomes and outgroups showing node support and data coverage. The data matrix indicates which partitions and data types were included for each species (presence in the “Genome” category does not imply representation in all partitions). Outgroups represent major lineages of gnathostomes. ⁵Current taxonomy problematic.

Cyclostome time-tree reveals an ancient split, but more recent diversification of hagfish and lamprey

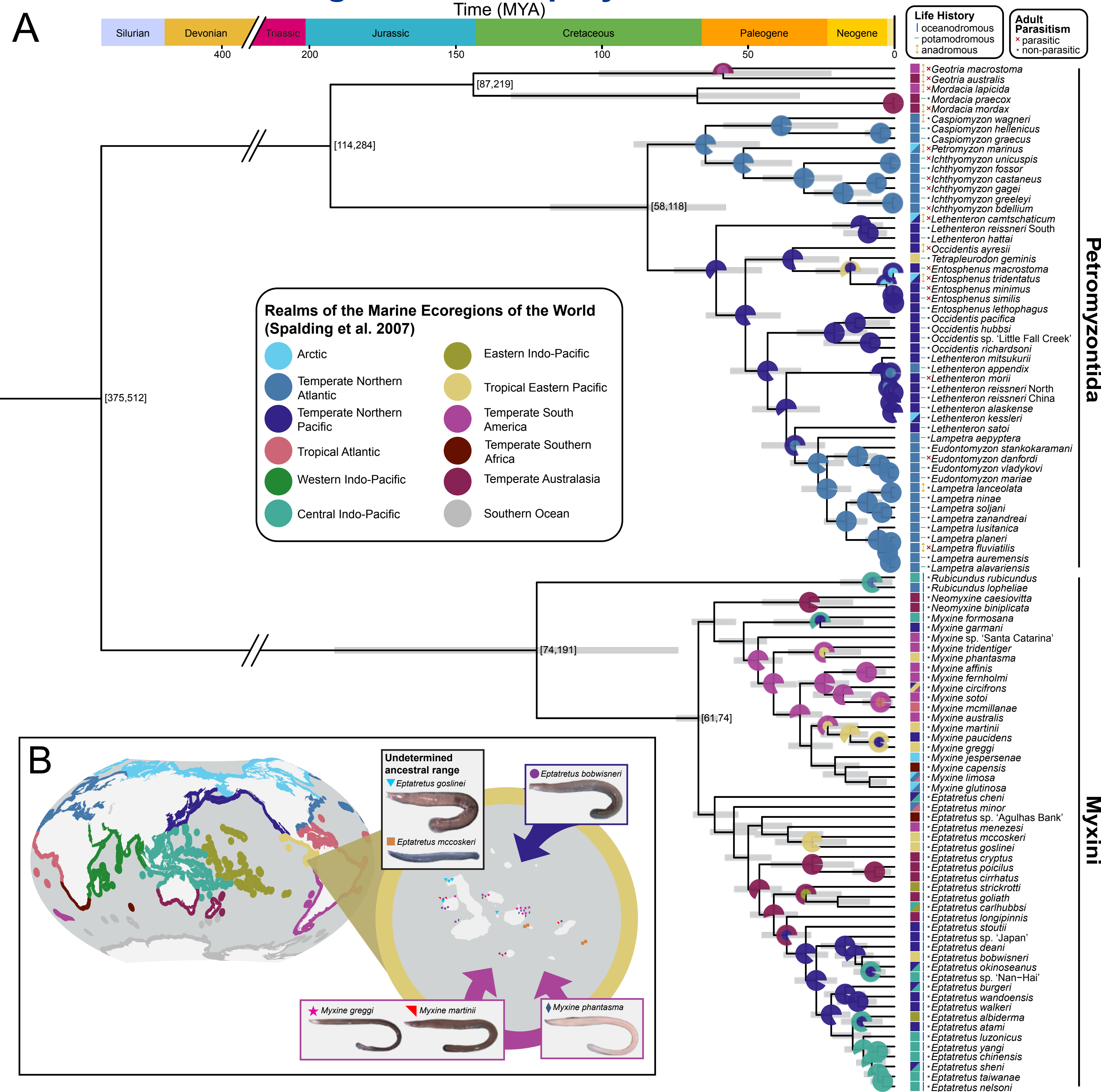


Figure 3. (A) Time-calibrated phylogeny of cyclostomes with pie charts showing inferred ancestral geographic ranges. Multiple colors within a pie represent the full inferred range, and pie completeness reflects confidence in the reconstruction. Node bars indicate 95% confidence intervals and ranges that fall within the axis break are shown in brackets. Colored boxes preceding taxon names indicate present-day ranges; for freshwater and anadromous species, ranges are assigned to the nearest marine ecoregion at the river outlet. Symbols following range boxes and preceding taxon names denote life-history and parasitism. (B) Marine ecoregions¹⁴ with inset showing the field collection sites of sequenced hagfish species in the Galápagos Islands, photos from Mincarone et al. (2021). Arrows indicate hypotheses for the colonization Galápagos Islands during the past 40 MY, based on reconstructed ancestral ranges as shown in A.

Results/Discussion

Molecular monophyletic validation (Fig. 2):

- Cyclostomes are a monophyletic clade
- Support for monophyly of hagfish genera *Eptatretus*, *Myxine*, *Neomyxine*, and *Rubicundus*
- Lamprey relationships well supported and consistent with recent analyses¹

Timetree analysis (Fig. 3A):

- Hagfish and Lamprey lineages split ~430 MYA
- Like lampreys (Brownstein 2023), most hagfish diversity originated in the past 100 million years
- *Rubicundis* is earliest branching (~120) extant hagfish clade, close to *Tethymyxine* fossil¹⁵
- *Myxine/Eptatretus* split, and LCA of Petromyzontidae ~66 MYA (end Cretaceous mass extinction)

Biogeographic analysis (Fig. 3B)

- Both lampreys and hagfish show biogeographical correlates with diversity
- Galápagos-endemic hagfishes related to both Pacific and South America species, but do not follow typical patterns of migration explained by surface currents.

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