

# Time calibrated phylogenomic analysis of cyclostome divergence, diversity and biogeography

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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<sup>1,2</sup>
- Origin of Galápagos hagfish biodiversity an open question<sup>3</sup>

## 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 GenBank<sup>4</sup>
- Missing mtDNA markers retrieved from RNA-seq data using Nucleotide BLAST<sup>5</sup>

### Ortholog filtering (Orthofinder<sup>6</sup>) and sequence alignment (MAFFT<sup>7</sup>, MACSE<sup>8</sup>)

- Identified orthologs across genome/RNA-seq taxa using Orthofinder<sup>6</sup>
- Filtered to retain orthologs present in ≥75% of taxa (392 orthologs retained)
- Aligned orthologs and non-coding mtDNA (12S, 16S) sequences using MAFFT<sup>7</sup>
- Aligned coding mtDNA (COI, CYTB) sequences using MACSE<sup>8</sup>

Figure 1. Workflow for phylogenetic data collection and analysis

### Phylogenomic tree (IQ-TREE<sup>9</sup>)

- Tree estimated from data matrix of 392 filtered ortholog alignments used as separate partitions under MFP+MERGE using IQ-TREE<sup>9</sup>
- Resulting topology received high support for all nodes; used as a constraint for further analysis.

### Mitochondrial tree (IQ-TREE<sup>9</sup>)

- mtDNA partition data (12S, 16S, COI, CYTB) and preceding constraint tree analyzed under MFP+MERGE using IQ-TREE<sup>9</sup>

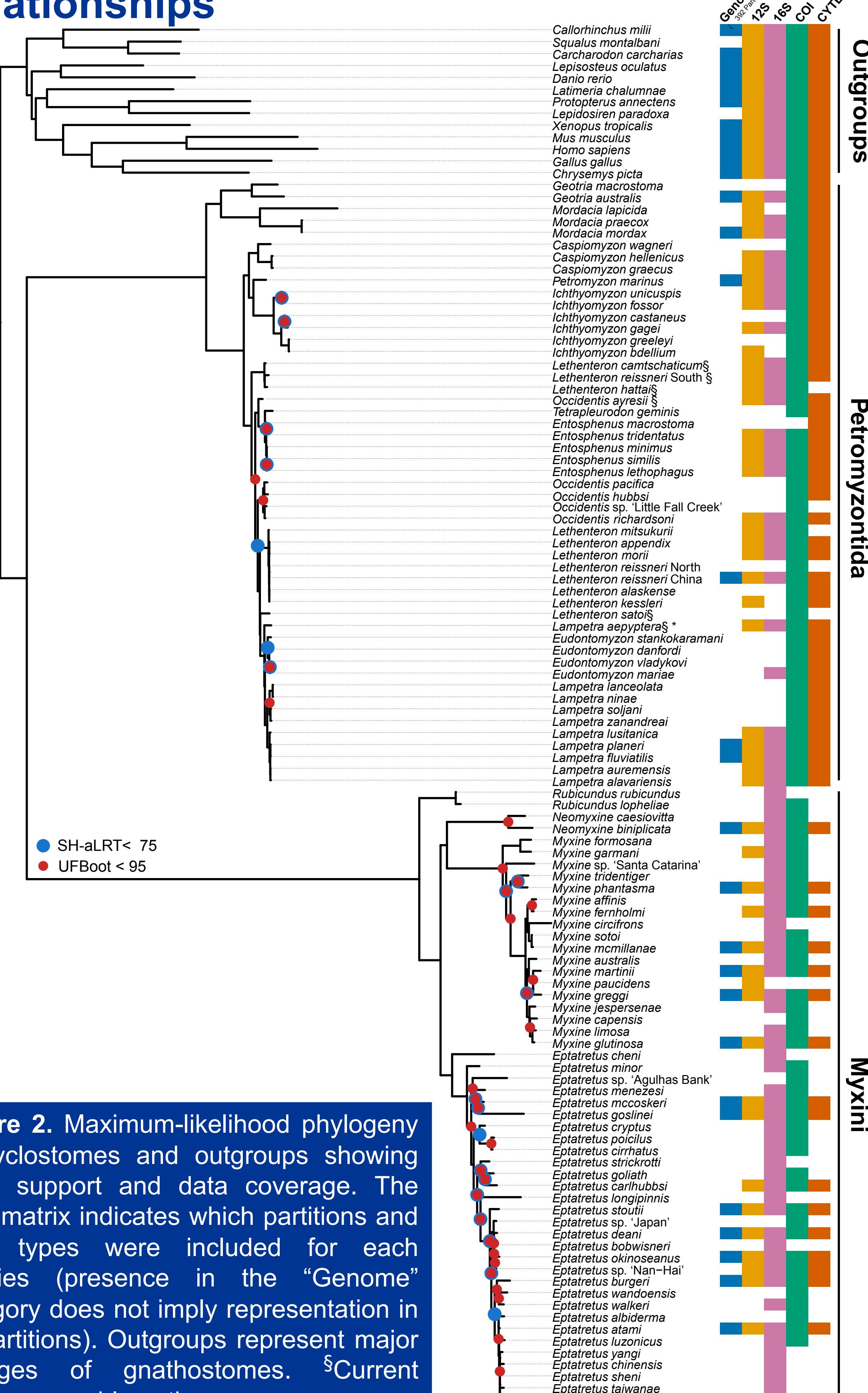
### Time calibration (MCMCTree<sup>10</sup>)

- Calibrated select nodes using literature-based priors (gathered using TimeTree<sup>11</sup>)
- 20,000 samples from the posterior (freq=10) summarized in time-calibrated phylogeny

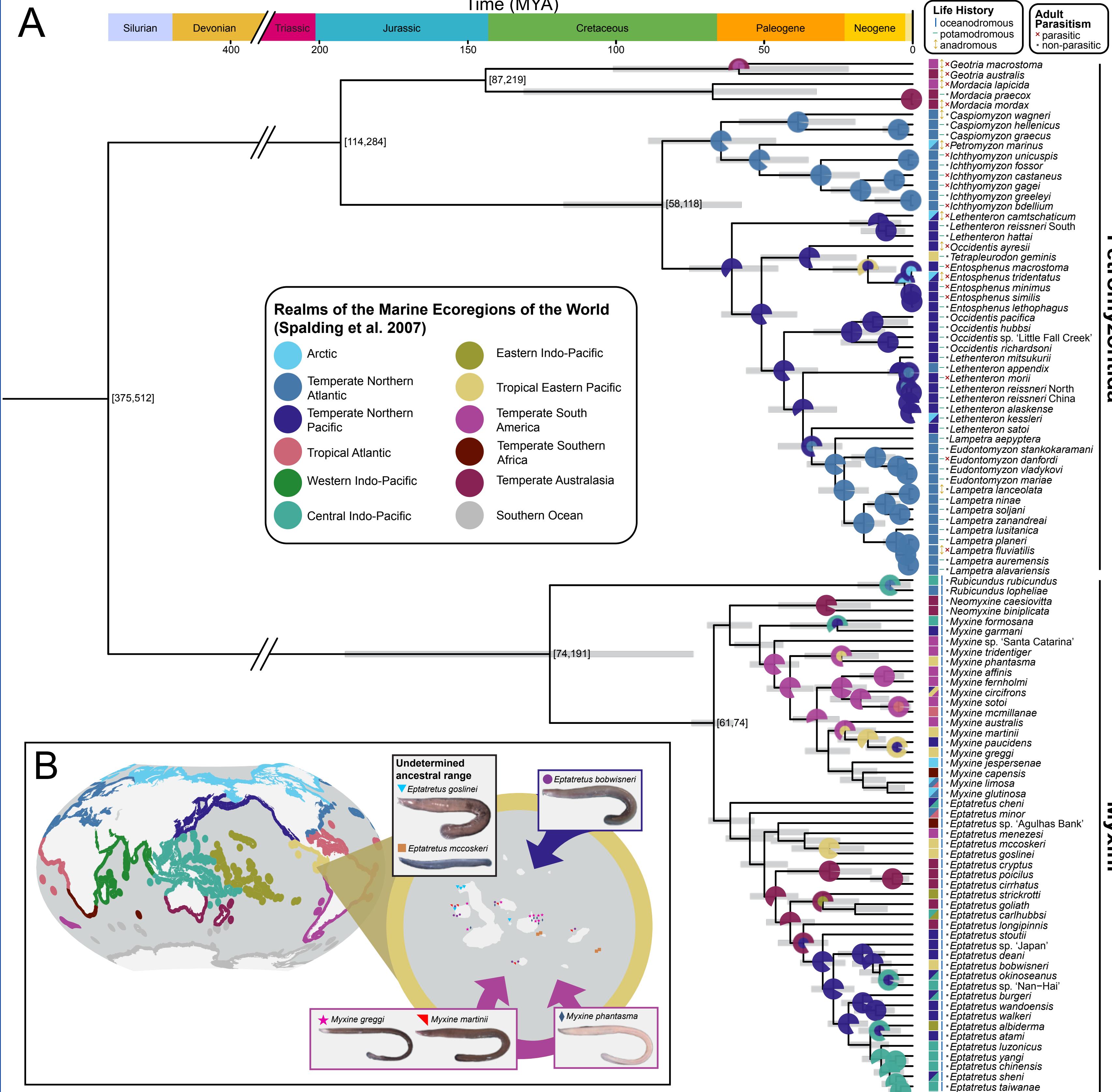
### Biogeographic reconstruction (BioGeoBEARS<sup>12</sup>)

- Modern ranges of taxa determined using distribution information gathered on FishBase<sup>13</sup>
- Reconstructed ancestral ranges of taxa using realms of the Marine Ecoregions of the World<sup>14</sup>
- Filtered out low-confidence reconstructions (<50%)

## Phylogenomic analysis clarifies cyclostome relationships



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



## 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 analyses<sup>1</sup>

### 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* fossil<sup>15</sup>
- 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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