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Explosion of goby fish diversity at the Eocene-Oligocene transition

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ABSTRACT

A rapid drop of sea level at the Eocene-Oligocene transition (EOT; 34–33 Ma) triggered a marine mass extinction event and the turnover of terrestrial fauna, but its influence on the diversification of nearshore marine fish communities is unclear. Goby fishes (*Acanthomorpha*: *Percomorpha*: *Gobiiformes*) provide an ideal system to investigate the hypothesis that ecological opportunity at the EOT triggered the proliferation of coastal marine fishes. However, despite more than 30 years of molecular evolutionary research, divergence time estimates for gobies are widely variable, incomplete with respect to sampling of taxonomic families and sub-familial lineages, and far older than evident by the modest fossil record. Here we use 1,314 ultraconserved element (UCE) sequences sampled from 121 species, including all gobiiform families and sub-familial goby lineages, to infer phylogeny and node ages under species tree and relaxed molecular clock models. Our time-calibrated phylogenomic hypothesis reconciles molecular clock- and fossil-based estimates for gobiiform diversification, dating the origin of *Apogonidae* and *Gobioidei* to the uppermost Late Cretaceous, with lower to middle Paleogene divergence of the gobioid backbone and an explosion of goby lineages at the EOT. Our results support a remarkably recent evolutionary origin of goby families and stimulate new questions on the seemingly exceptional diversity of the group.

1. Introduction

The Eocene-Oligocene transition (EOT; 34–33 Ma) marks a major shift from the warm conditions of the Eocene to the cool climate of the Oligocene, which led to the first full Antarctic glaciation, changes in ocean circulation and productivity, and a marine mass extinction event (Coxall and Pearson, 2007; Hutchinson et al., 2021). Ecological opportunities likely arose as the sea levels quickly fell, triggering coastal erosion and the formation of dynamic marginal marine habitats (Edinger and Risk, 1994; Harzhauser et al., 2007; Zachos et al., 2001), but linking the EOT to the evolutionary success of diverse nearshore fish communities (McCord et al., 2021) has not been thoroughly explored with phylogenomic data.

Inhabiting reef and marginal marine habitats of all oceans and continents except Antarctica, the approximately 2,740 species of *Gobiiformes* represent a taxonomically rich, ecologically diverse and globally extensive radiation of small fishes. Composed of iridescent nurseryfishes (*Kurtidae*), bioluminescent cardinalfishes (*Apogonidae*), colorful sanddivers (*Trichonotidae*), hardy loach gobies (*Rhyacichthyidae*), sleepers (*Odontobutidae*, *Eleotridae*, *Thalasseleotrididae*) and gudgeons (*Milyeringidae*, *Butidae*), sublime wrigglers (*Xenisthmidae*) and charismatic gobies (*Oxudercidae* and *Gobiidae*; Fig. 1a), *Gobiiformes* is among the earliest-diverging of the major radiations of percomorph fishes, with stem age estimates reaching the lower Late Cretaceous (Alfaro et al., 2018; Betancur-R et al., 2017; Ghezelayagh et al., 2022; Hughes et al., 2018; Near and Thacker, 2024). *Gobioidei* contains 2,331 species and has been repeatedly identified as having accelerated diversification rates relative to other lineages of ray-finned fishes (Near et al., 2013; Rabosky et al., 2018, 2013). *Gobiidae* (1,408 species) and *Oxudercidae* (680 species) are remarkably diverse, and the division of each into sub-familial lineages facilitates investigation by making the groups more tractable. In terms of average numbers of living species, the oxudercid and gobiid

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goby lineages are nearly twice the size of other acanthopterygian fish families, with about 110 species per sub-familial goby lineage compared to 57 species per acanthopterygian fish family (Agorreta et al., 2013; Near and Thacker, 2024; Thacker, 2015, 2013; Thacker and Roje, 2011).

In addition to their high species diversity, gobiiform fishes exhibit a remarkable variety of ecological specializations, including semi-terrestriality and air breathing, a variety of mutualisms, and a host of unusual reproductive innovations such as forehead brooding and bi-directional sex change (Cole, 2010; Patzner et al., 2011). Investigating the evolutionary origins and significance of these ecological specializations with respect to diversity and distribution requires a robust time-calibrated phylogenetic hypothesis, yet despite more than three decades of concerted molecular phylogenetics and evolutionary research, gobiiform divergence time estimates remain widely variable among relaxed molecular clock-based studies and between molecular- and paleontological-based research programs (Alfaro et al., 2018; Ghezelayagh et al., 2022; Reichenbacher et al., 2018; Thacker, 2015, 2014).

The phylogenetic classification of *Gobiiformes* includes two orders, 12 families and 19 sub-familial goby lineages (Fig. 1a; Table S1) (Betancur-R et al., 2017; Near and Thacker, 2024; Thacker, 2009; Thacker et al., 2015). We follow the taxonomy of Near and Thacker (2024), including the convention of italicizing all taxon names. Consistent phylogenetic relationships are resolved using both multilocus Sanger-sequence and phylogenomic datasets (Alfaro et al., 2018; Betancur-R et al., 2017; Ghezelayagh et al., 2022; Hughes et al., 2018; Thacker et al., 2015), although the relationships among the deeply-branching nurseryfishes (*Kurtidae*) and cardinalfishes (*Apogonidae*), the placement of paedomorphic infantfishes (*Schindleria* spp.), and the resolution of the Small-Eyed Goby (*Austrolethops wardi*) are uncertain (Agorreta et al., 2013; Alfaro et al., 2018; Eschmeyer et al., 2016; Ghezelayagh et al., 2022; Johnson and Brothers, 1993; Kuang et al., 2018; Thacker and Roje, 2011; Tornabene et al., 2017). Phylogenomic studies resolve nurseryfishes and cardinalfishes either as reciprocal (Ghezelayagh et al., 2022) or sequential (Alfaro et al., 2018; Kuang et al., 2018) sister clades to *Gobioidei*. Infantfishes are placed in their own family in the taxonomy presented in (Eschmeyer et al., 2016) despite consensus among morphological and molecular phylogenetic studies that resolve *Schindleria* within *Gobiidae* (Johnson and Brothers, 1993). Within *Gobiidae*, molecular phylogenies place *Schindleria* in either the *Gobiodon* or the *Gunnellichthys* goby lineage, and the taxon is considered "rogue" because it reduces branch support and alters topology in molecular phylogenetic analyses (Agorreta et al., 2013; McCraney et al., 2020; Tornabene et al., 2017). The Small-Eyed Goby has not been sampled in any molecular studies to date, and uncommon traits such as herbivory, lack of scales, and commensalism with burrowing shrimp have precluded classification of this species to any goby lineage using morphological characters (Thacker and Roje, 2011).

Estimates of divergence times of *Gobiiformes* vary widely among molecular studies and between molecular and paleontological inferences. Phylogenomic studies have estimated ages of *Gobiidae* under the independent log-normal (ILN) rates model that range from the early Oligocene (33.6 Ma) (Alfaro et al., 2018) to the late Paleocene (56.7 Ma)

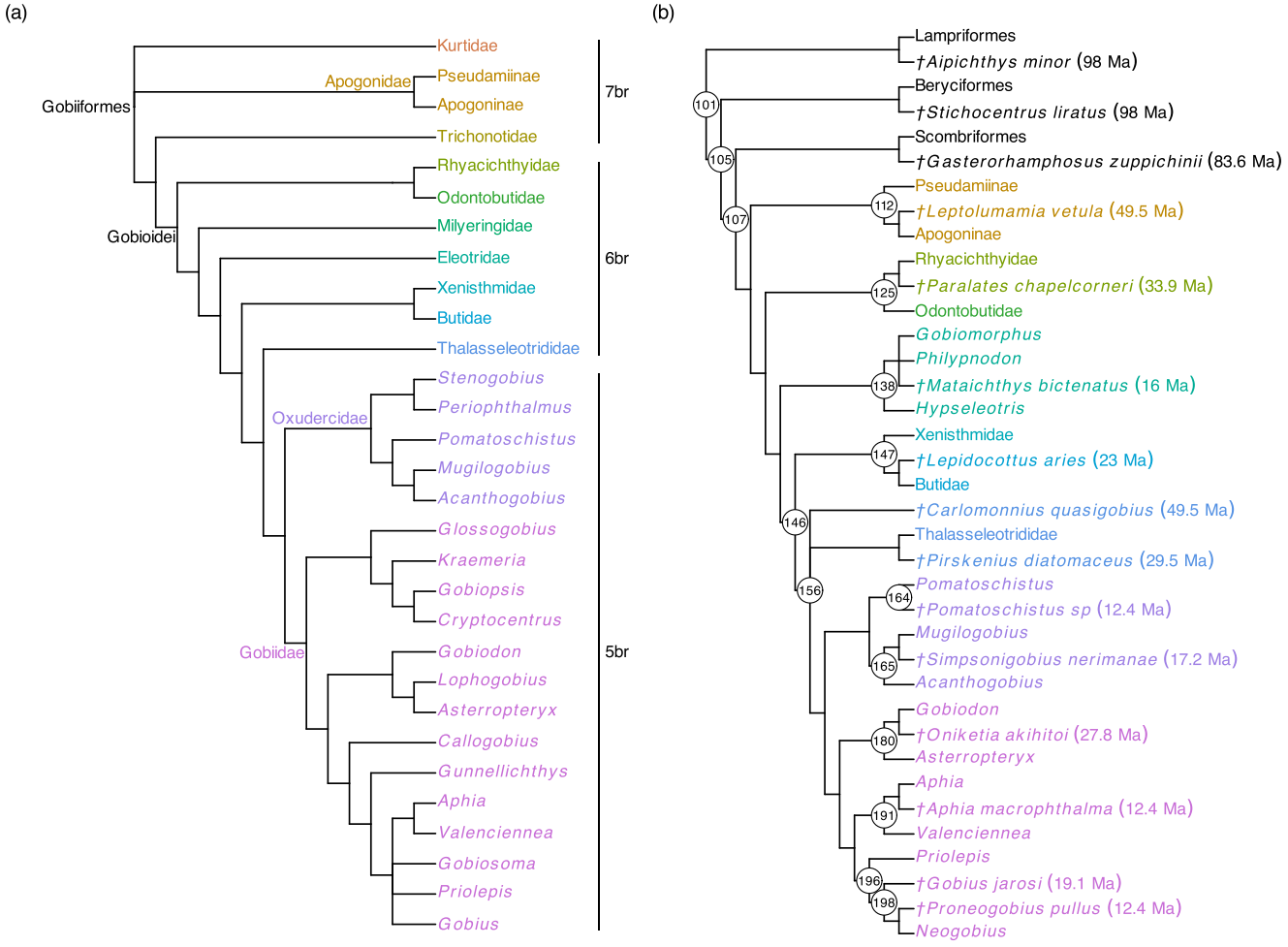


Fig. 1. (a) Gobiiformes phylogeny according to (Betancur-R et al., 2017; Near and Thacker, 2024; Thacker, 2009; Thacker et al., 2015). br = branchiostegal-rayed. (b) Skeleton tree showing fossil placements according to (Bannikov and Fraser, 2016; Brownstein, 2023; Davesne et al., 2016, 2014; Dirnberger et al., 2024; Gierl et al., 2022; Marramà et al., 2022; Schwarzhans et al., 2017, 2012). Node numbers correspond to nodes in Tabs. S2–S3, Fig. 4a, and Fig. S5.

(Ghezelayagh et al., 2022). These and other examples of relaxed molecular clock node dates (Chakrabarty et al., 2012; Thacker, 2015, 2014) precede macroevolutionary time scales supported by gobioid fossils by 20 Myr or more (Reichenbacher et al., 2018).

The fossil record of *Gobiiformes* is modest but well studied. Articulated skeletal remains appear in the lower Eocene (Ypresian; c. 50 Ma), with five *Apogonidae* and one gobioid species (family *incertae sedis*) described from the fossil-*Lagerstätten* of Bolca (Bannikov, 2014; Bannikov and Carnevale, 2016; Bannikov and Fraser, 2016). A second early gobioid species not placed into any extant family appears in the upper Eocene of England (Priabonian; c. 34 Ma) and the lower Oligocene of France (Rupelian; c. 32 Ma) (Gierl and Reichenbacher, 2017). Gobioid fossils placed as sister groups to *Thalasseleotrididae* and *Butidae* (Reichenbacher et al., 2020; Reichenbacher and Přikryl, 2024), and to *Gobiidae* (Marramà et al., 2022) begin to appear during the Oligocene, followed by the lower to middle Miocene appearance of *Gobiomorphus* (*Eleotridae*) (McDowall et al., 2006), *Pomatoschistus* (*Oxudercidae*) (Carnevale et al., 2006), *Mugilogobius* (*Oxudercidae*), *Aphia* (*Gobiidae*), and *Gobius* (*Gobiidae*) goby lineages (Dirnberger et al., 2024). Collectively, the articulated skeletal fossil record suggests divergence of *Apogonidae* and *Gobioidei* before the early to middle Eocene, appearance of extinct gobioid stem lineages until the late Eocene, and the evolutionary origin of extant sub-familial goby lineages beginning in the middle Oligocene and lasting through the middle Miocene.

Here we seek to infer phylogenetic relationships of *Kurtidae* and *Apogonidae* within *Gobiiformes*, resolve placements of *Schindleria* and *Austrolethops* among *Gobiidae*, estimate comprehensive divergence times of the gobiiform radiation, and compare molecular- and fossil-based estimates of the evolutionary origins of gobiiform clades. Our analyses utilize ultraconserved element (UCE) sequences sampled from all gobiiform families and sub-familial goby lineages. We infer a time-calibrated phylogenomic hypothesis with Bayesian relaxed molecular clock models, and compare our molecular clock divergence time estimates to fossil-based divergence times independently estimated from stratigraphic distributions of fossil outgroup taxa. Our analysis yields a comprehensive time-calibrated phylogenomic tree for *Gobiiformes*. This phylogenomic framework will facilitate the investigation into the mechanisms responsible for the ecological and evolutionary success of *Gobiiformes*.

2. Material and methods

2.1. Sequence processing

We collected ultraconserved element (UCE) sequence data for 121 species, including 113 gobiiform species encompassing all families and sub-familial lineages, and eight outgroup species representing *Percomorpha*, *Acanthopterygii*, and *Acanthomorpha*. We used standard protocols for DNA extraction and quantification, Illumina library preparation, and targeted enrichment of 1,314 UCE loci ascertained for acanthomorph phylogenetic inference (Alfaro et al., 2018; Faircloth et al., 2015; Glenn et al., 2019). We utilized the PHYLUCE pipeline to assemble, align and trim sequences, and filter incomplete matrices for recovery of 75 % and 95 % of taxon samples (Faircloth, 2016, 2013). We provide additional details of our laboratory methods in the supplementary material.

2.2. Phylogenetic analysis

We used two strategies to estimate species trees from 75 % and 95 % matrices. First, we inferred phylogenies from the concatenated-partitioned dataset with maximum likelihood (ML) and Bayesian analysis with IQ-TREE and ExaBayes, respectively (Aberer et al., 2014; Chernomor et al., 2016; Izquierdo-Carrasco et al., 2011; Kalyaanamoorthy et al., 2017; Minh et al., 2020). Second, we used the multi-species coalescent (MSC) model-based, hybrid branch length- and branch support-weighted gene tree summary method of wASTRAL-Hybrid to infer species trees from ML gene trees (Zhang et al., 2018; Zhang and Mirarab, 2022). We provide additional details of our phylogenetic analyses in the supplementary material.

2.3. Node dating

We adopted two approaches for node dating. First, we estimated node ages of 15 clades where articulated skeletal fossils are either confidently placed with phylogenetic methods or tentatively placed with comparative morphology (Table S2; Fig. 1b; Fig. S5). We used a method developed by (Hedman, 2010) that was first applied to bony fish phylogeny by (Friedman et al., 2013) to estimate probable time intervals for the evolutionary origin of clades from the cumulative geological ages of successive fossil outgroups to the clades. This method integrates stratigraphic and phylogenetic data to estimate the age of the most recent common ancestor (MRCA) of the group in which a fossil can be placed, given the minimum geological age of the fossil, the minimum geological ages of a sequence of stratigraphically-consistent (i.e., sequentially younger or contemporaneous) fossil outgroup taxa, and an arbitrary maximum geological age for all of the fossils analyzed (Hedman, 2010).

Second, we estimated node dates under relaxed molecular clock models using the approximate likelihood method in MCMCTree (dos Reis and Yang, 2011; Yang and Rannala, 2006). To hasten runtimes, we reduced the total number of taxa in the 75 % ML/Bayesian topology by pruning lineages down to four or fewer tips (Fig. S5). We partitioned loci into nine quantiles of substitution rates estimated under the strict molecular clock model (Fig. S6) and computed gradient and Hessian matrices of the likelihood function at ML estimates of branch lengths inferred under GTR + Γ_5 models (dos Reis and Yang, 2019).

The phylogenetic placements of several fossil gobioids are uncertain because they represent extinct lineages and few synapomorphies are available for placements of extant lineages (Gierl et al., 2022). Posterior node ages can be inaccurate if estimated using mistaken or conflicting fossil calibrations on internal nodes, and the assignment of one correct fossil calibration to the root node can be just as effective as two consistent calibrations if evolutionary rates are saturated in phylogenomic data (Dos Reis and Yang, 2013). Therefore, we opted to calibrate prior node age densities with a single gamma-distributed root age constraint by assigning the fossil-based node age estimate of †*Aipichthys minor* to the acanthomorph root (i.e., the MRCA of *Lampris incognitus* and *Holocentrus rufus*; Table S2; Fig. 1b; Fig. S5). We sampled chains generated under two Bayesian relaxed molecular clock models, including the independent log-normal rates (ILN) model, and the geometric Brownian motion (autocorrelated) rates (GBM) model (dos Reis et al., 2016).

We used three strategies to assess posterior relaxed molecular clock node ages with respect to evolutionary rate saturation in the phylogenomic data, model selection, and fossil-based node ages. First, we prepared infinite sites plots to evaluate evolutionary rate saturation and uncertainty of divergence times arising from our phylogenomic sequencing and alignment partitioning strategy (Dos Reis and Yang, 2013). Second, we conducted Bayesian model selection experiments on a random sample of 1 % of loci (Reis et al., 2018). Third, we examined the correlation of relaxed molecular clock node dates with fossil-based node dates independently estimated from the geological age distributions of fossil outgroups. We provide additional details of our node dating analyses in the supplementary material.

3. Results

3.1. Sequence matrices

We recovered 1,289 loci from the 1,314 UCE probes, with an average of 913.5 loci per species (Table S1). The 75 % complete matrix included 908 loci and 471,195 characters with 28.6 % missing data, and the 95 %

complete matrix included 215 loci and 128,431 characters with 15.6 % missing data. The reduced 75 % matrix prepared for node dating included 100 species and 909 UCE loci, comprising 466,737 characters with 320,390 alignment patterns.

3.2. Phylogenetic relationships

Unabbreviated phylograms estimated with ML/Bayesian and MSC strategies are shown in Figs. S1–S2 and Figs. S3–S4, respectively. ML/Bayesian phylogenies inferred using concatenated-partitioned datasets are robust to inference methods and matrix designs, except for one branch within *Apogoninae* with different resolutions from analyses using the 75 % and 95 % matrices (Fig. 2; Figs. S1–S2). MSC species trees are congruent with those inferred in the analyses using ML and Bayesian approaches, with the exception of a few branches within *Apogonoidei*, *Apogoninae*, *Eleotridae*, and *Gobiidae* (Figs. S3–S4).

Kurtus and *Apogonidae* are resolved as sister lineages with strong branch support; however, in the 75 % MSC tree *Kurtus* is resolved with limited branch support as a sequential sister group to a clade containing *Apogonidae*, *Trichonotidae*, and *Gobioidei* (local posterior probability = 0.98; Fig. S3). Infantfishes (*Schindleria*) and the Small-Eyed Goby (*Austrolethops wardi*) are resolved in the *Gunnellichthys* lineage with uncertain placement, as either *Austrolethops* or *Schindleria* are resolved as the earliest-diverging branch in ML, Bayesian, and MSC trees, respectively (Figs. S1–S4). We provide additional details of phylogenetic results and discussion in the supplementary material.

3.3. Node ages

Chronograms estimated under the ILN and GBM models are shown in Figs. 3 and S7 respectively. Node age estimates are given in Table 1. Regression coefficients of infinite sites plots are nearly identical among ILN and GBM node ages (Fig. S9). Similarly, correlations and associated *p*-values of relaxed molecular clock ages versus fossil outgroup ages estimated under ILN and GBM models are in close agreement (Fig. 4). Bayesian model selection experiments indicated the ILN model was better for explaining evolutionary rate variation in a small, random sample of 1 % of UCE loci, with posterior model probabilities and Bayes factors favoring the ILN model in seven out of the nine UCE loci tested (Table S5). Since the GBM model was selected in two out of nine UCE loci analyzed, we present the node age estimates under both relaxed molecular clock models.

Overlapping 95 % CI's indicated age estimates for the deepest nodes in the tree, including *Gobiiformes*, *Apogonoidei*, *Apogonidae*, and *Gobioidei* are not different among Bayesian relaxed clock models (Table 1; Fig. S8). In contrast, posterior mean age estimates for many shallow nodes varied with respect to the clock models among gobiiform clades. The node ages estimated for *Kurtidae* and *Apogoninae*, and for sampled tribes of *Apogoninae* are far younger under the ILN model. The reverse pattern appeared in *Gobioidei*, with moderately older ages estimated under the ILN model for all nodes except the stem of *Rhyacichthyidae*, and *Odontobutidae*, *Milyeringidae*, and *Butidae* (Table 1; Fig. S8). We provide additional details of node dating results and discussion in the supplementary material.

4. Discussion

Our phylogeny is the first to include all *Gobiiformes* families and goby lineages and estimate divergence times across the entire group. Relationships among families are resolved in accordance with previous phylogenetic studies (Agorreta et al., 2013; Ghezelayagh et al., 2022; McCraney et al., 2020; Thacker et al., 2023, 2015; Tornabene et al., 2013), and we provide a comprehensive resolution of relationships of goby lineages within *Oxudercidae* and *Gobiidae*. We show that the largest gobioid families *Eleotridae*, *Oxudercidae*, and *Gobiidae* arose at or near the EOT, and underwent rapid lineage-level diversification through the Oligocene and Miocene.

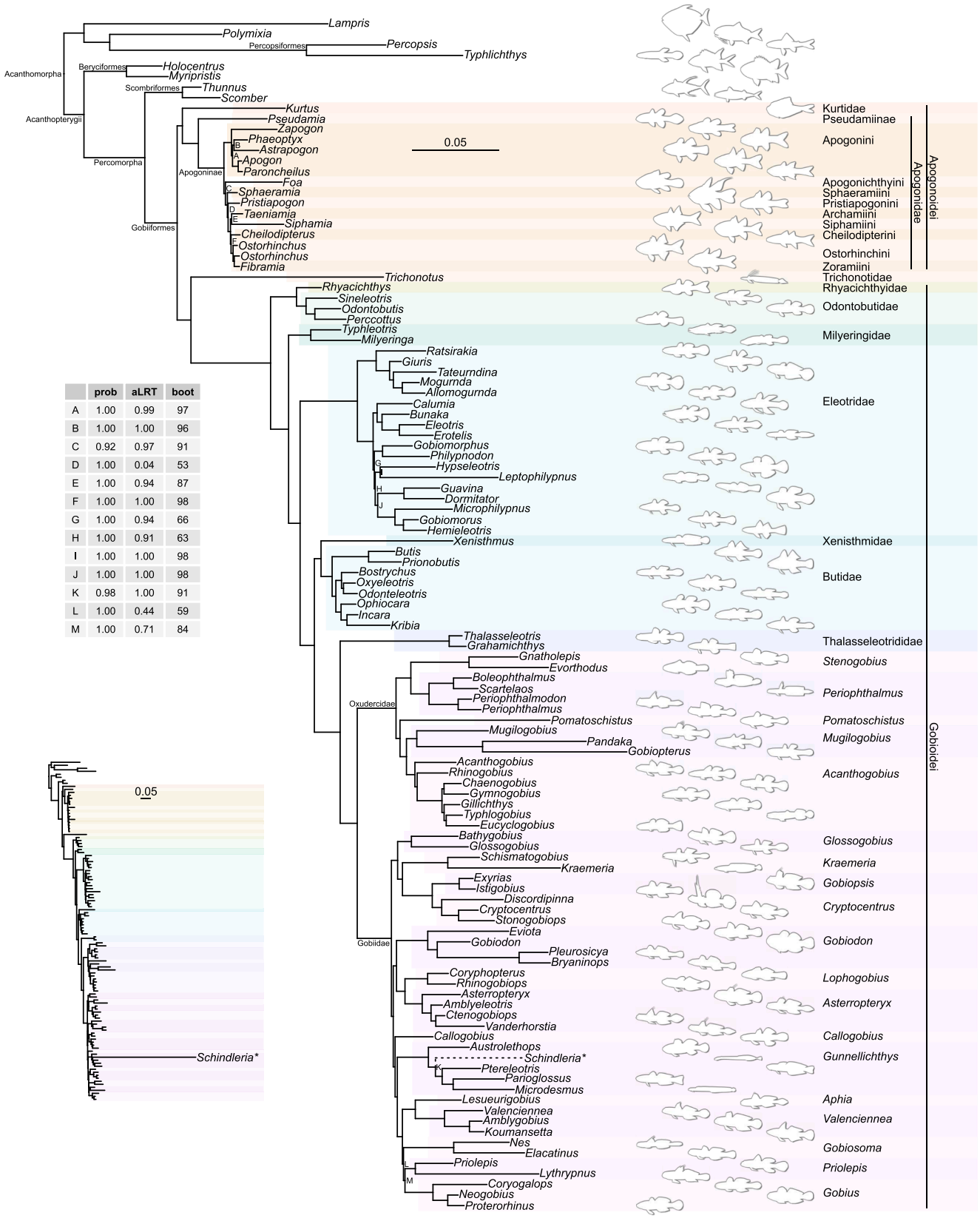
4.1. Phylogenetic uncertainty of Apogonoidei

Variable placements of *Kurtus* and *Apogonidae*, either as a monophyletic group (Fig. 2; Figs. S1–S3) or as sequential (Fig. S4) branching groups relative to *Gobioidei* have been inferred among different phylogenomic studies (Alfaro et al., 2018; Ghezelayagh et al., 2022; Kuang et al., 2018), and multilocus concatenated analyses and MSC species trees (McCraney et al., 2020). Given the consistent differing relationships of nurseryfishes and cardinalfishes resolved in various datasets and phylogenetic studies, and the lack of any well-characterized morphological synapomorphy supporting the grouping (Johnson, 1993), the monophyly of *Apogonoidei* remains controversial. The deepest branches in the species-rich *Apogoninae* are short and poorly supported, and a slightly different topology is resolved in each phylogenetic analysis of our UCE dataset. The phylogenetic uncertainty of *Apogonoidei* may have resulted from a history of rapid diversification and incomplete lineage sorting. Alternatively, chromosomal rearrangement and diploid number variation, which have been shown to cause phylogenetic uncertainty in bony fish phylogeny (Faircloth et al., 2013; Hughes et al., 2018; Parey et al., 2023) may underlie the incomplete resolution of *Apogonoidei*. Chromosomal rearrangement and diploid number variation within *Apogonidae* was recently discovered and attributed to repeated pericentric (i.e., centromere-containing) inversions and chromosomal fusions (dos Santos et al., 2023). The diploid value of percomorph fishes is typically 2n = 48, whereas the diploid value is slightly reduced (2n = 44) in the Nurseryfish *Kurtus gulliveri* (Ezaz et al., 2006), and remarkably variable in cardinalfishes (2n = 34–46). For instance, diploid values of six species in Atlantic *Apogon* and *Phaeoptyx* are 2n = 34 (Rivlin et al., 1988), 2n = 35 (Rivlin et al., 1987), 2n = 36 (de Araújo et al., 2010; dos Santos et al., 2023) and 2n = 38 (Rivlin et al., 1986), whereas the diploid value 2n = 46 is broadly conserved in the Indo-West Pacific *Nectamia* (Rivlin et al., 1986), *Fibramia* (Kasiroek et al., 2017), *Sphaeramia* (dos Santos et al., 2023; Kasiroek et al., 2022, 2017; Ojima and Kojima, 1985), and *Pterapogon* (dos Santos et al., 2023; Kasiroek et al., 2017). Furthermore, 18S rDNA and 5S rDNA are syntenic in Atlantic cardinalfishes but located on different chromosomes in the Indo-West Pacific species (dos Santos et al., 2023). Whole genome duplication events followed by chromosomal inversions and translocations present insidious challenges to phylogenetic inference when taxon sampling is broad (Faircloth et al., 2013; Hughes et al., 2018; Parey et al., 2023). A phylogenomic approach with expanded taxon sampling of *Apogonidae*, combined with micro- and macro-synteny analyses of high quality, long-read whole genome reference assemblies for nurseryfishes and cardinalfishes may be necessary to resolve the phylogenetic uncertainty of *Apogonoidei*, as this approach has settled other controversies deep in the animal phylogeny (Steenwyk and King, 2024).

4.2. Phylogenomic systematics of Gobioidei

Wrigglers (*Xenisthmus*) are resolved as a sister group to *Butidae* with complete branch support in all analyses. Previous studies using mitochondrial DNA sequences placed *Xenisthmus* within *Eleotridae* (Thacker, 2009, 2003), however the placement was unexpected since wrigglers are strictly marine and reef-associated, which contrasts with the generally freshwater or brackish habitat preferences of eleotrid sleepers. Agorreta and Rüber (2012) resolved *Xenisthmus* as the sister lineage of *Butidae* in their reanalysis of (Akihito et al., 2000) mitochondrial data, and (Hoese and Gill, 1993) identified a morphological character (extrascapulae; dermal bones posterior of the neurocranium) in *Xenisthmus* and most of *Butidae* that is not present in *Eleotridae*, both of which support the distinctiveness of *Xenisthmus* resolved here. The resolution of *Xenisthmus* in this study unequivocally supports resurrection of *Xenisthmidae* in the phylogenetic classification of *Gobiiformes*.

The rogue taxon *Schindleria* is resolved within the *Gunnellichthys*



	prob	aLRT	boot
A	1.00	0.99	97
B	1.00	1.00	96
C	0.92	0.97	91
D	1.00	0.04	53
E	1.00	0.94	87
F	1.00	1.00	98
G	1.00	0.94	66
H	1.00	0.91	63
I	1.00	1.00	98
J	1.00	1.00	98
K	0.98	1.00	91
L	1.00	0.44	59
M	1.00	0.71	84

Fig. 2. *Gobiiformes* phylogeny inferred from concatenated-partitioned Bayesian analysis of UCE loci. Tips are collapsed to the genus rank. Branches with uncertain Bayesian posterior probability (prob), SH-like approximate likelihood ratio test (aLRT), or non-parametric bootstrap support (boot) are labelled. *The long branch to *Schindleria* in the inset tree is reduced by one order-of-magnitude in the main tree.

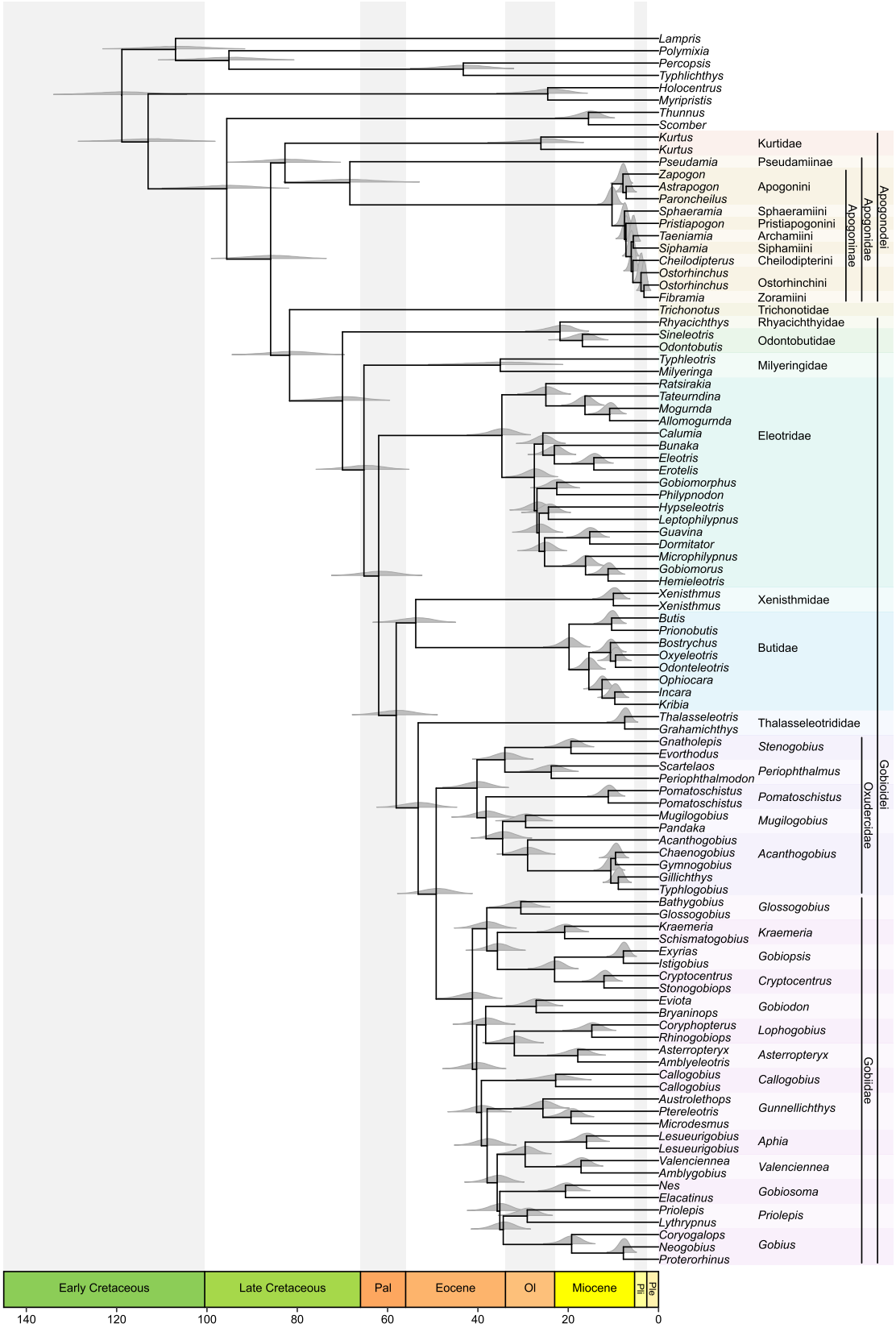


Fig. 3. Bayesian relaxed molecular clock divergence times of *Gobiiformes* estimated under the independent log-normal (ILN) rates model. Posterior density tails extend to 0.5% of the maximum height.

Table 1
Node ages estimated under Independent log-normal (ILN) and Geometric Brownian motion (GBM) Bayesian relaxed molecular clock models. Ages are given in Ma with 95 % credible intervals. Divergence times overlapping the Eocene-Oligocene transition (EOT; 34–33 Ma) are boldfaced.

Lineage	Clade age		Stem age	
	ILN	GBM	ILN	GBM
Gobiiformes	85.9 (75.4, 96.5)	89.0 (79.2, 99.5)	95.6 (84.1, 107.3)	100.7 (89.9, 111.9)
Apogonoidei	82.7 (72.6, 93.2)	86.8 (77.4, 97.2)	85.9 (75.4, 96.5)	89.0 (79.2, 99.5)
Kurtidae	**26.0 (18.2, 35.0)**	61.0 (50.7, 71.6)	82.7 (72.6, 93.2)	86.8 (77.4, 97.2)
Apogonidae	68.4 (55.4, 80.8)	77.9 (68.8, 87.4)	82.7 (72.6, 93.2)	86.8 (77.4, 97.2)
Pseudamiinae	–	–	68.4 (55.4, 80.8)	77.9 (68.8, 87.4)
Apogoninae	10.3 (8.7, 12.0)	52.6 (44.7, 60.5)	68.4 (55.4, 80.8)	77.9 (68.8, 87.4)
Trichonotidae	–	–	81.7 (71.4, 91.6)	84.1 (74.2, 93.6)
Gobioidei	70.0 (61.3, 78.7)	61.5 (53.9, 69.4)	81.7 (71.4, 91.6)	84.1 (74.2, 93.6)
Rhyacichthyidae	–	–	21.8 (16.7, 27.1)	44.6 (37.2, 51.7)
Odontobutidae	16.9 (12.5, 21.7)	**36.1 (29.6, 43.4)**	21.8 (16.7, 27.1)	44.6 (37.2, 51.7)
Milyeringidae	**35.0 (23.6, 48.0)**	43.0 (36.7, 49.6)	65.2 (57.1, 73.5)	54.9 (48.0, 62.3)
Eleotridae	**34.7 (29.8, 39.9)**	**28.8 (23.8, 33.9)**	62.0 (54.4, 70.0)	50.9 (44.3, 57.6)
Xenisthmidae	10.0 (7.3, 12.7)	7.9 (5.3, 10.4)	53.8 (46.8, 60.9)	43.7 (38.1, 49.8)
Butidae	19.8 (16.5, 23.5)	**37.9 (32.8, 43.2)**	53.8 (46.8, 60.9)	43.7 (38.1, 49.8)
Thalasseleotrididae	7.5 (5.4, 9.5)	5.6 (3.8, 7.5)	53.2 (46.3, 59.7)	**39.1 (33.7, 44.4)**
Oxudercidae	40.2 (35.0, 45.7)	26.3 (22.5, 30.1)	49.2 (43.2, 55.6)	**35.2 (30.4, 40.1)**
Stenogobius	19.4 (15.5, 23.1)	11.2 (9.1, 13.5)	**34.0 (29.1, 38.8)**	22.7 (19.2, 26.0)
Periophthalmus	23.8 (19.2, 28.4)	17.4 (14.7, 20.3)	**34.0 (29.1, 38.8)**	22.7 (19.2, 26.0)
Pomatoschistus	11.1 (8.5, 14.1)	5.0 (3.7, 6.2)	**38.2 (33.2, 43.5)**	25.3 (21.6, 29.0)
Mugilogobius	**29.4 (25.0, 33.9)**	20.7 (17.6, 23.9)	**34.5 (29.9, 39.5)**	23.5 (20.1, 27.1)
Acanthogobius	**29.0 (24.3, 33.6)**	21.0 (17.9, 24.2)	**34.5 (29.9, 39.5)**	23.5 (20.1, 27.1)
Gobiidae	41.2 (36.1, 46.6)	26.9 (23.2, 31.0)	49.2 (43.2, 55.6)	**35.2 (30.4, 40.1)**
Glossogobius	**30.5 (25.7, 35.4)**	21.2 (18.2, 24.5)	**38.0 (33.2, 43.1)**	25.1 (21.7, 28.9)
Kraemeria	20.8 (16.7, 25.0)	13.6 (11.4, 16.0)	**35.7 (31.0, 40.5)**	23.8 (20.4, 27.3)
Gobiopsis	7.8 (5.8, 10.1)	5.9 (4.7, 7.2)	23.0 (19.2, 27.1)	15.7 (13.2, 18.2)
Cryptocentrus	12.1 (9.2, 15.1)	8.3 (6.7, 9.9)	23.0 (19.2, 27.1)	15.7 (13.2, 18.2)
Gobiodon	27.1 (22.7, 31.7)	17.4 (14.8, 20.3)	**38.3 (33.3, 43.2)**	25.4 (21.8, 29.1)
Lophogobius	14.8 (10.8, 19.0)	13.0 (10.8, 15.4)	**31.9 (27.1, 36.9)**	22.6 (19.4, 26.0)
Asterropteryx	17.9 (13.4, 22.6)	15.0 (12.6, 17.6)	**31.9 (27.1, 36.9)**	22.6 (19.4, 26.0)
Callogobius	22.8 (16.6, 29.2)	19.3 (16.3, 22.4)	39.2 (34.3, 44.4)	25.8 (22.2, 29.6)
Gunnellichthys	25.6 (21.4, 30.1)	16.6 (13.9, 19.2)	**38.0 (33.1, 42.9)**	25.1 (21.6, 28.8)
Aphia	16.0 (12.3, 19.8)	12.9 (10.8, 15.1)	**29.5 (25.4, 34.0)**	20.2 (17.2, 23.2)
Valenciennea	17.2 (13.4, 20.7)	12.1 (10.1, 14.2)	**29.5 (25.4, 34.0)**	20.2 (17.2, 23.2)
Gobiosoma	20.6 (16.7, 24.8)	12.3 (10.1, 14.5)	**35.1 (30.6, 39.8)**	23.4 (20.1, 26.9)
Priolepis	**29.1 (24.9, 33.5)**	20.2 (17.4, 23.4)	**34.4 (29.9, 38.9)**	23.0 (19.8, 26.5)
Gobius	19.2 (15.3, 23.2)	14.6 (12.2, 17.0)	**34.4 (29.9, 38.9)**	23.0 (19.8, 26.5)

lineage with strong support. Similar to previous analyses using multilocus nuclear and mitochondrial datasets (Agorreta et al., 2013; Tornabene and Deis, 2017), terminal and subtending branches to *Schindleria* are remarkably long (Figs. S1–S4), which may be the result of high substitution rates or multiple sequence alignment errors. Resolution of *Austrolethops* to the *Gunnellichthys* lineage was unexpected, but not unreasonable when considering the remarkable eco-morphological diversity of the wormfishes and dartfishes, which includes body elongation associated with infaunal habitats, as well as morphological adaptations associated with pelagic schooling behavior (Allen and Erdmann, 2012).

4.3. Fossil calibration, evolutionary rate saturation, and relaxed molecular clock model selection

Our fossil-based node dating strategy was enabled by recent advances in gobioid paleoichthyology, which include the redescription of several articulated skeletal fossils and resolution of some fossil taxa in total evidence phylogenetic frameworks integrating molecular and morphological data (Dirnberger et al., 2024; Gierl et al., 2022; Reichenbacher and Přikryl, 2024). Despite these advances, the placement of several fossil gobioids remains uncertain, and we did not assign fossil constraints to calibrate internal node age priors. We assigned a single fossil age constraint to the root node, estimated posterior node ages with uniform prior densities generated under the birth–death-sampling model, and assessed evolutionary rate saturation in the phylogenomic data with infinite sites plots (Dos Reis and Yang, 2013).

We found strong goodness-of-fit in our infinite sites plots, with 93 % of the variation CI widths explained by posterior mean divergence times under both ILN and GBM models (Fig. S9). This result suggests that evolutionary rates are saturated in our phylogenomic data matrix and that adding additional sequence data may not improve the precision of our divergence time estimates, given the single fossil age constraint that we assigned to the root node. The slope of our infinite sites plots suggests that for every 1 Ma of divergence time, 0.28 Ma and 0.27 Ma of uncertainty are expected under ILN and GBM models, respectively (Fig. S9), which is comparable to levels obtained from phylogenomic node dating of primates (dos Reis et al., 2012). The similar goodness-of-fit and slope of infinite sites plots drawn under ILN and GBM models indicate that the uncertainty of our node age estimates is robust to Bayesian relaxed molecular clock model selection. While additional sequence data may not improve the precision of divergence times estimated using the single fossil root constraint, the uncertainty we observed might be reduced with the addition of fossil constraints assigned to internal nodes.

To reduce the computational burden, we performed Bayesian model selection experiments on a random sample of 1 % of gene trees, and found support for the ILN model in seven out of nine UCE loci analyzed (Table S5). We recognize two potential limitations of our approach. First, we did not account for variable taxon sampling in the 75 % matrix, which arises from differences in library quality and sequence capture efficiency, and could possibly be a confounding factor in computing posterior model probabilities and Bayes factors. Second, we cannot exclude the possibility that support for the ILN model in seven out of the nine experiments was due to chance alone, and repeating the Bayesian model selection experiments with different or larger samples of UCE loci

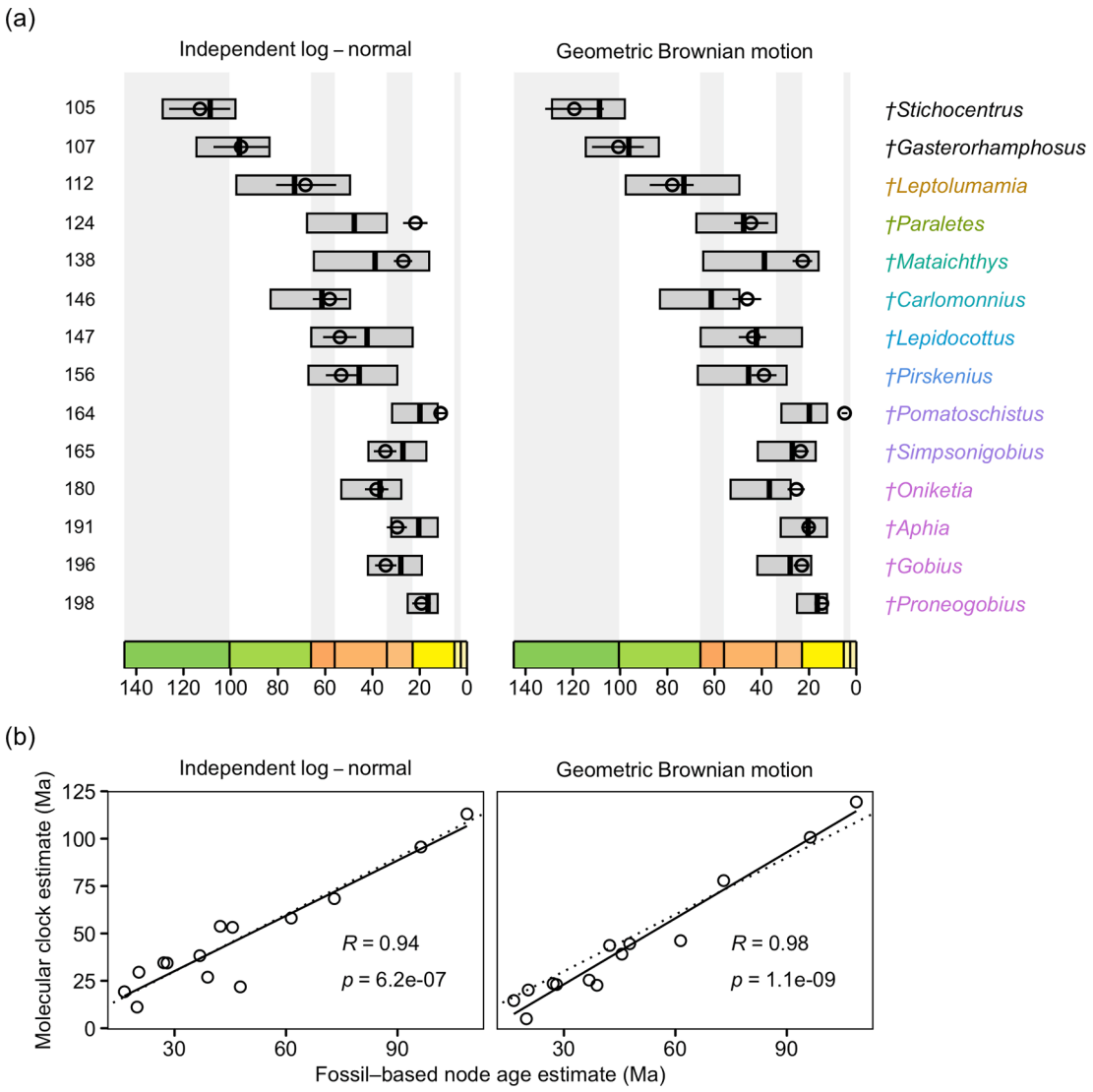


Fig. 4. Node ages estimated under relaxed molecular clock models are congruent with node ages independently estimated from the ages of fossil outgroups to the node. (a) Relaxed molecular clock node age estimates and 95% credible intervals are pointranges. Fossil outgroup-based node age estimates and 95% credible intervals are grey crossbars. Node numbers in the left margin correspond to nodes in Tabs. S2–S3, Fig. 1b, and Fig. S5. (b) Pearson correlation (*R*). Regression and rule lines are solid and dotted, respectively.

might reveal greater support for the GBM model. Given the wide disparity among the ILN and GBM node age estimates for the depauperate families *Kurtidae*, *Rhyacichthyidae*, and *Odontobutidae*, future research should consider incomplete and variable taxon sampling in the design of Bayesian relaxed molecular clock model selection experiments.

4.4. Comparison of molecular and paleontological time scales of Gobiiformes

Several ancient cardinalfish species have been described from the Eocene fossil-*Lagerstätten* of Bolca (Bannikov and Fraser, 2016). The early divergence of *Pseudamia* estimated in our study is congruent with the placement of Oligocene †*Oligopseudamia* (33.9–27.8 Ma) as a stem group of *Pseudamiinae* (Marrama' et al., 2022), and Eocene (c. 49.5 Ma) †*Leptolumamia* (Fig. 4) and †*Eoapogonini* as stem groups of *Apogoninae* (Bannikov and Fraser, 2016). Previous phylogenomic studies have estimated ages of *Apogoninae* across the Miocene (10.6 Ma, Alfaro et al., 2018; 17.4 Ma, Ghezelayagh et al., 2022) and Oligocene (28.3 Ma, Hughes et al., 2018) (Table S4). The precise Miocene emergence of *Apogoninae* that we estimate under the ILN model corroborates the phylogenomic timescale of (Alfaro et al., 2018), and suggests a remarkably rapid rate of speciation in cardinalfishes (Table S4). However, given the 5-fold difference of estimates under the ILN and GBM models, and the rich Eocene fossil record of *Apogoninae*, additional research with expanded taxon sampling and fossil integration is warranted.

The stem age estimate of *Rhyacichthyidae* under the ILN model is inconsistent with placement of late Eocene (33.9 Ma) †*Paralates* (Gierl and Reichenbacher, 2017) as a sister group to *Rhyacichthyidae* (Dirnberger et al., 2024), however the molecular and paleontological age estimates are congruent under the GBM model. The Miocene age of the *Gobiomorphus* plus *Philypnodon* clade estimated under both ILN and GBM models aligns with the tentative placement of †*Mataichthys* (19–16 Ma) as a stem group of this Australian/New Zealand lineage (Schwarzhans et al., 2012). Divergence times of *Butidae* (23 Ma) estimated here agree with the placement of †*Lepidocottus aries* (Gierl et al., 2013), either as a stem group under the ILN model (Fig. 3), or in the

crown group under the GBM model (Fig. S7). Divergence times of *Thalasseleotrididae* are congruent with the placement of the Oligocene †*Laubeichthys gracilis* (31.8–28.6 Ma) (Reichenbacher and Přikryl, 2024) plus †*Pirskenius* (33.9–27.8 Ma) (Přikryl, 2014) clade as a sister group to *Thalasseleotrididae* (Dirnberger et al., 2024; Gierl et al., 2022).

The Miocene divergence of sand gobies (*Pomatoschistus* lineage) estimated under the ILN model is consistent with the paleontological evidence (Schwarzhans et al., 2017), however the Pliocene age estimated under the GBM model is younger (Fig. 4). EOT and lower Miocene divergence times of the *Mugilogobius* lineage estimated under the ILN and GBM models, respectively, are consistent with the placement of †*Simpsonigobius nerimanae* (17.2 Ma) in the *Mugilogobius* lineage (Dirnberger et al., 2024).

Oligocene divergence of the *Gobiodon* lineage estimated under the ILN model is in synchrony with the tentative placement of †*Oniketia akihitoi* (33.9–27.8 Ma) as a stem group of *Eviota* (Marramà et al., 2022), whereas the middle Miocene age estimated under the GBM model is later than the paleontological time scale. The Miocene divergence date of the *Aphia* lineage is in synch with fossil evidence (e.g., †*Pseudolesueurigobius manfredi*, 12 Ma, (Reichenbacher and Bannikov, 2022); †*Aphia macrophthalma*, 12.4 Ma, (Schwarzhans et al., 2017), and similar to a Bayesian relaxed molecular clock-based estimate (Dirnberger et al., 2024) (Table S4). Miocene divergence times of the *Gobius* lineage estimated under both ILN and GBM models are synchronous with the placements of †*Gobius jarosi* (19.1 Ma) (Gierl et al., 2022; Reichenbacher et al., 2018) and †*Proneogobius pullus* (12.4 Ma) (Schwarzhans et al., 2017) (Fig. 4a).

4.5. Explosive diversification of goby lineages at the Eocene-Oligocene transition

Our node dating analyses reveal two novel patterns concerning the evolution of goby fishes. First, the diverse families *Gobiidae* (1,408 species) and *Oxudercidae* (680 species) are remarkably young in relation to other percomorph groups of comparable species diversity, with divergence time estimates dating the evolutionary origins of goby clades to the upper Eocene (41.2–40.2 Ma) and the middle Oligocene (26.9–26.3 Ma) under ILN and GBM relaxed molecular clock models, respectively. Indeed, *Gobiidae* and *Oxudercidae* are exceptionally diverse relative to Late Cretaceous clade age estimates of the only other percomorph families with comparably-high levels of species diversity: the freshwater *Cichlidae* (1,786 species) and the marine *Labridae* (564 species), which are both approximately twice as old as either goby family (Hughes et al., 2023; Matschiner et al., 2020). Second, diversification of most of the 14 gobiid goby lineages and all 5 oxudercid goby lineages occurred rapidly, within timeframes spanning ~ 6 Myr and ~ 4 Myr under the ILN and GBM models, respectively (Fig. 3; Fig. S7). Rapid diversification is common in the evolutionary history of many percomorph lineages, but the timing of the goby explosion is unusual. In contrast to other percomorph radiations that were associated with the K-Pg boundary (Alfaro et al., 2018), goby diversification occurred at or near the EOT (Table 1), a period of accelerated global cooling which saw the formation of permanent polar ice sheets, a rapid drop of sea levels, major changes in ocean circulation, and a marine mass extinction event (Coxall and Pearson, 2007; Hutchinson et al., 2021).

5. Conclusions

With over 2,000 species in *Gobiidae* and *Oxudercidae*, gobies comprise the most diverse clade of marine vertebrates, yet despite more than 30 years of concerted molecular phylogenetics research, a comprehensive timescale dating the evolutionary origins and divergence of goby lineages has been unattainable. This study provides the first analysis of relaxed molecular clock divergence times for all 19 goby lineages. Our results reconcile paleontological and molecular timescales for evolutionary origins, and reveal remarkably young clades composed of exceptional species diversity. Future research combining a megaphylogeny of *Gobiiformes* with the robust phylogenomic framework of this study will enable the macroevolutionary analysis of goby fishes with unprecedented power.

CRediT authorship contribution statement

W. Tyler McCraney: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Christine E. Thacker:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization, Investigation, Project administration, Resources, Validation. **Brant C. Faircloth:** Writing – review & editing, Resources, Data curation, Validation. **Richard C. Harrington:** Writing – review & editing, Resources, Data curation, Validation. **Thomas J. Near:** Writing – review & editing, Resources, Data curation, Validation. **Michael E. Alfaro:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization, Methodology, Validation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108342.

Data availability

Sequence alignments, partition models, and phylogenetic trees are available on the corresponding Dryad Digital Repository: https://doi.org/10.5061/dryad.mw6m9067p.

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