Diversification of the sleepers (Gobiiformes: Gobioidei: Eleotridae) and evolution of the root gobioid families

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ARTICLE INFO

ABSTRACT

Eleotridae (sleepers) and five smaller families are the earliest diverging lineages within Gobioidei. Most inhabit freshwaters in and around the Indo-Pacific, but Eleotridae also includes species that have invaded the Neotropics as well as several inland radiations in the freshwaters of Australia, New Zealand, and New Guinea. Previous efforts to infer phylogeny of these families have been based on sets of mitochondrial or nuclear loci and have yielded uncertain resolution of clades within Eleotridae. We expand the taxon sampling of previous studies and use genomic data from nuclear ultraconserved elements (UCEs) to infer phylogeny, then calibrate the hypothesis with recently discovered fossils. Our hypothesis clarifies ambiguously resolved relationships, provides a time-scale for divergences, and indicates the core crown Eleotridae diverged over a short period 24.3–26.3 Ma in the late Oligocene. Within Eleotridae, we evaluate diversification dynamics with BAMM and find evidence for an overall slowdown in diversification over the past 35 Ma, but with a sharp increase 3.5 Ma in the genus *Mogurnda*, a clade of brightly colored species found in the freshwaters of Australia and New Guinea.

1. Introduction

The gobiiform suborder Gobioidei is dominated by the high-diversity families Gobiidae and Oxudercidae, with species distributed globally in nearly all tropical and temperate aquatic habitats and including a significant presence on coral reefs. Gobiidae, Oxudercidae, and Thalasseleotrididae together comprise the gobioid crown clade, but outside that radiation are six smaller families that represent the earlier-branching gobioid lineages and are mostly distributed in the freshwaters of the Indo-Pacific. Within the family Eleotridae (sleepers) there are independent radiations of species in freshwater habitats of Australia, New Guinea, and New Zealand that are notably more diverse than their

relatives and may represent localized instances of accelerated diversification. To explore the timing and rates of these radiations, we infer a new calibrated phylogeny of the earlier branching gobioid families and use BAMM to estimate the diversification dynamics of eleotrid clades.

Eleotridae includes 132 species classified in 20 genera and is globally distributed in freshwater and brackish (rarely marine) habitats, with species diversity highest in Australia, New Guinea, and New Zealand (Fricke et al., 2022). The closely related Butidae includes 46 species classified in 10 genera and inhabits fresh to marine waters in Asia, Africa, Australia, and Oceania, with the highest species diversity in the Indo-Australian Archipelago (Fricke et al., 2022). Within Gobioidei, Eleotridae is the third most species-rich lineage (behind Gobiidae and

Oxudercidae) and those three are the only gobioid lineages with a circumglobal distribution (Thacker, 2015). Related smaller families include Xenisthmidae (wrigglers, 16 species in marine habitats of the Indo-Pacific), Milyeringidae (blind cave gudgeons, 5 species with a remarkable disjunct distribution in subterranean caves in Madagascar and Western Australia), Odontobutidae (freshwater sleepers, 25 species in the freshwaters of eastern Asia), and Rhyacichthyidae (loach gobies, 3 species in fast-flowing streams of coastal China, southern Japan, Indonesia, Philippines, New Caledonia, Vanuatu, and the Solomon Islands). These disparate lineages are not a monophyletic group (Butidae is sister to the remaining gobioid families: Thalasseleotrididae, Gobiidae, and Oxudercidae), instead they represent a rootward grade of early-

Table 1

Genus, species, locality, voucher code, SRA Project number, and SRA BioSample code for species used in UCE analyses. Institutional abbreviations in voucher codes are as follows: ABTC: Australian Biological Tissue Collection, South Australian Museum, Adelaide, Australia; ANSP: Academy of Natural Sciences, Philadelphia, Pennsylvania, USA; FMNH: Field Museum of Natural History, Chicago, Illinois, USA; LACM: Natural History Museum of Los Angeles County Fish Tissue Collection, Los Angeles, California, USA; NTM: Museum and Art Gallery of the Northern Territory, Australia; NCSM: North Carolina State Museum of Natural Sciences, Raleigh, North Carolina, USA; NMNZ: Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand; YFTC: Yamanashi Prefectural Fisheries Technology Center, Yamanashi, Japan. Species with codes beginning with PU or IOD are pending accession at the ABTC, the specimen with code beginning with MUE is pending accession at the Western Australian Museum, Perth, Western Australia, and the specimen with code beginning with TM is uncatalogued at the University of California, Los Angeles, California, USA.

Genus	Species	Locality	Voucher	SRA Project	SRA BioSample
Allomogurnda	*nesolepis*	Kali Tiri, near Dabra, Papua, Indonesia	PU P147	PRJNA774634	SAMN22568898
Bostrychus	*sinensis*	Rapid Creek levee, Darwin Harbour, NT	NTM A02999	PRJNA774634	SAMN22568905
Bostrychus	*zonatus*	Freshwater drain, Ludmilla Ck, NT	NTM A03000	PRJNA774634	SAMN22568906
Bunaka	*gyrinoides*	Mulgrave River, QLD	NTM A04309	PRJNA774634	SAMN22568908
Butis	*butis*	Bargara, Innes Park Ck, QLD	LACM T-000045	PRJNA774634	SAMN22568909
Butis	*koilomatodon*	Coastal waters, NT	NTM A06131	PRJNA774634	SAMN22568910
Calumia	*papuensis*	Milne Bay, PNG	MUE-18–064	PRJNA774634	SAMN22568913
Dormitator	*maculatus*	Palm Beach, Florida	LACM T-000017	PRJNA774634	SAMN22568919
Eleotris	*perniger*	Quebrada Juan Diego, Puerto Rico	NCSM54870	PRJNA774634	SAMN22568920
Eleotris	*picta*	Punta de Mita, Mexico	LACM T-000019	PRJNA774634	SAMN22568921
Eleotris	*smaragdus*	Twin Cays, Belize	LACM T-000042	PRJNA348720	SAMN05915063
Giuris	*margaritacea*	Ross River, Townsville, QLD	LACM-T000073	PRJNA659476	SAMN15908314
Gobiomorphus	*australis*	Williams Creek, Georges River, NSW	PU14-4GA-1	PRJNA659476	SAMN15908308
Gobiomorphus	*basalis*	Karapiro Stream, Waikato River, NZ	NMNZ P.061390	PRJNA659476	SAMN15908300
Gobiomorphus	*breviceps*	Ashley River, NZ	LACM T-000064	PRJNA774634	SAMN22568928
Gobiomorphus	*coxii*	Nepean River, NSW	PU14-85GC-1	PRJNA659476	SAMN15908310
Gobiomorphus	*hubbsi*	Waitao Stream (Tauranga Harbor), NZ	NMNZ P.061391	PRJNA659476	SAMN15908306
Gobiomorus	*dormitor*	Rio de la Plata, Puerto Rico	NCSM68278	PRJNA774634	SAMN22568929
Guavina	*micropus*	La Palma San Miguel Estuary, Panama	LACM T-uncat	PRJNA774634	SAMN22568932
Hemieleotris	*latifasciata*	Rio Cardenas, Corozal, Panama	LACM-T000018	PRJNA774634	SAMN22568933
Hypseleotris	*aurea*	Gascoyne River, East Carnarvon, WA	IOD4-68432	PRJNA774634	SAMN22568934
Hypseleotris	*barrawayi*	Katherine River, NT	NTM A05849	PRJNA774634	SAMN22568935
Hypseleotris	*bucephala*	Blacks Creek, Pioneer River, QLD	PU02-44MCG-X	PRJNA774634	SAMN22568959
Hypseleotris	*compressa*	Trunding Creek, Embley River, Weipa, QLD	LACM-T000104	PRJNA659476	SAMN22889036
Hypseleotris	*cyprinoides*	Ularimbin Creek, East Sepik, PNG	LACM-T000139	PRJNA774634	SAMN22568941
Hypseleotris	*galii*	Macleay River, Bellbrook, NSW	PU99-41HG-1	PRJNA774634	SAMN22568939
Hypseleotris	*klunzingeri*	Back Creek, Cooyar, QLD	PU99-51WCG-X	PRJNA774634	SAMN22568956
Incara	*multisquamata*	East Arm Darwin Harbour, NT	NTM A09702	PRJNA774634	SAMN22568967
Kribia	*nana*	Niger River, Guinea, West Africa	LACM T-000077	PRJNA774634	SAMN22568971
Leptophilypnus	*panamensis*	Rio Caimito, Panama	LACM T-000979	PRJNA774634	SAMN22568972
Leptophilypnus	*panamensis*	Rio Caimito, Panama	LACM T-000980	PRJNA774634	SAMN22568973
Microphilypnus	cf. *amazonicus*	Rio Yutaje Manapiare-Ventuari, Venezuela	ANSP180531	PRJNA774634	SAMN22568976
Microphilypnus	*ternetzi*	Manari River, Guyana	ANSP180643	PRJNA774634	SAMN22568977
Milyeringa	*veritas*	Northwest Cape, WA	ABTC22891-1	PRJNA774634	SAMN22568978
Mogurnda	*adspersa*	Ross River, Townsville, QLD	LACM T-000069	PRJNA348720	SAMN05915085
Mogurnda	*cingulata*	Unasim Wok, Fly River, PNG	NTM A01896	PRJNA774634	SAMN22568980
Mogurnda	*clivicola*	Belyando River, Bakoolama Waterhole, QLD	PU14-35MO-1	PRJNA774634	SAMN22568981
Mogurnda	*oligolepis*	Royston Ck, WA	NTM A03190	PRJNA774634	SAMN22568982
Odonteleotris	*macrodon*	Bindoola Ck estuary, WA	NTM A03213	PRJNA774634	SAMN22568986
Odontobutis	*obscura*	Souro River, Japan	LACM-T000002	PRJNA348720	SAMN05915089
Ophiocara	*porocephala*	Palmer Creek, Innes Park, QLD	LACM-T000001	PRJNA348720	SAMN05915091
Oxyeleotris	*altipinna*	Lake Aiwaso, Indonesia	NTM A06732	PRJNA774634	SAMN22568988
Oxyeleotris	*aruensis*	Myall Creek, Weipa, QLD	NTM A04676	PRJNA774634	SAMN22568989
Oxyeleotris	*fimbriata*	Uk Fon, Fly River, PNG	NTM A01887	PRJNA774634	SAMN22568990
Oxyeleotris	*lineolata*	Adelaide River, NT	LACM-T000022	PRJNA659476	SAMN15908316
Oxyeleotris	*nullipora*	Howard River, NT	LACM-T000596	PRJNA774634	SAMN22568992
Oxyeleotris	*selhemi*	Adelaide River, NT	LACM-T000006	PRJNA774634	SAMN22568993
Perccottus	*glenii*	Dniestr River, Russia	LACM-T000032	PRJNA659476	SAMN15908317
Philypnodon	*grandiceps*	Wollondilly River, NSW	PU14-167PG-1	PRJNA659476	SAMN15908311
Philypnodon	*macrostomus*	Orara River, NSW	PU14-53PM.1	PRJNA659476	SAMN15908309
Prionobutis	*microps*	Rapid Creek, Darwin Harbour, NT	NTM A01427	PRJNA774634	SAMN22569000
Ratsirakia	*legendrei*	Sakalava River, Madagascar	LACM T-000007	PRJNA774634	SAMN22569003
Rhyacichthys	*aspro*	Yutsun River, Iriomote Island, Japan	YFTC 25,765	PRJNA758064	SAMN22936341
Sineleotris	*saccharae*	Hainan, China	LACM T-uncat	PRJNA774634	SAMN22569008
Tateurndina	*ocellicauda*	Aquarium specimen (PNG)	LACM-T000066	PRJNA774634	SAMN22569010
Trichonotus	*filamentosus*	South China Sea	YFTC 24,183	PRJNA758064	SAMN22936425
Typhleotris	*madagascariensis*	Toliara, Madagascar	FMNH116498	PRJNA774634	SAMN22569011
Xenisthmus	*polyzonatus*	Dongsha Atoll, Taiwan	TM-2015–021	PRJNA774634	SAMN22569013
Xenisthmus	sp.	Santa Cruz Island, Solomon Islands	LACM-T000009	PRJNA774634	SAMN22569014

diverging lineages within Gobioidei (Thacker et al., 2015), and we refer to them collectively as the root gobioids (equivalent to the "basal gobioids" of Thacker and Hardman, 2005).

Eleotridae includes mostly low diversity genera, each with one or a few species. In freshwaters, the exceptions are three radiations in Australia, New Guinea, and New Zealand: *Mogurnda* in New Guinea and Australia (27 described species); *Hypseleotris* in Australia (18 species); and *Gobiomorphus* in New Zealand (9 species; Thacker et al. (2022b) resolved the two Australian *Gobiomorphus* species as sister to *Philypnodon*, indicating that they should be moved to *Philypnodon* or assigned to a new genus). Compared to the diversity of some reef-dwelling genera in Gobiidae (121 species in *Eviota*; 108 species in *Trimma*), these radiations are not large. However, they are notable compared to their sister genera and for the fact that they occur in freshwater and in restricted geographic areas.

The evolutionary relationships of genera within Eleotridae have been difficult to infer consistently, due in part to the shallow divergences separating most clades within the family. Previous studies have yielded conflicting topologies, with many clades in common but little confidence as to the relationships among them. Phylogenies of Gobioidei (Agorreta et al., 2013; Thacker, 2009) and those focusing on the root gobioids (Thacker & Hardman, 2005; Thacker, 2017) used data from nuclear and mitochondrial gene sequences. Our study expands both the taxonomic and character coverage of previous studies, with near-complete sampling of genera, and uses an abundant source of nuclear phylogenomic data: ultraconserved elements (UCEs). We calibrate the topology with fossils and assess diversification patterns in Eleotridae using BAMM, to investigate whether the radiations of *Hypseleotris*, *Mogurnda*, and *Gobiomorphus* represent instances of elevated diversification in Australia, New Guinea, or New Zealand.

2. Material and methods

2.1. Sampling and UCE analysis

To infer phylogeny for the root gobioids, we assembled a dataset of 59 individuals representing 58 species (two individuals of *Leptophilypnus panamensis* were included) of Eleotridae, Butidae, Xenisthmidae, Milyeringidae, Odontobutidae, Rhyacichthyidae and the outgroup family Trichonotidae. Samples not obtained from museum tissue collections were collected in various localities throughout Australia, New Guinea, New Zealand, and Taiwan between 2014 and 2019 using electrofishers or seine nets and euthanized with an overdose of clove oil (species, voucher, and locality information are provided in Table 1).

We extracted genomic DNA from tissues using the either the QIAamp Fast DNA tissue kit or the DNeasy blood and tissue kit (Qiagen Inc., Germantown, MD, USA), quantified DNA concentration with a Qubit 2.0 Fluorometer (Invitrogen Co., Carlsbad, CA, USA), and submitted the extracted DNA to Arbor Biosciences (Ann Arbor, MI, USA) for library preparation, enrichment, and UCE sequencing. The UCE enrichment and sequencing were performed using targeted probe sets Acanthomorpha 0.5Kv1 (2001 baits for 500 UCE loci; Faircloth et al., 2013) or 1Kv1 (2628 baits for 1341 UCE loci; Alfaro et al., 2018). Following library preparation, enrichment, and sequencing, we cleaned reads of adapter contamination and low-quality bases using the parallel *illumiprocessor.py* wrapper for *Trimmomatic* (Bolger et al., 2014). We used *phyluce* to assemble the quality- trimmed reads with *SPAdes* (Bankevich et al., 2012), the *phyluce* "mapping" workflow to map reads to contigs, mark duplicates, and compute assembly coverage, and implemented the "correction" workflow to trim low-coverage, low-quality base calls from assemblies (Faircloth, 2015).

We matched the probes to assembled contigs for all samples with the 1Kv1 probeset, with minimum coverage and identity threshold parameters set to 80% (Faircloth et al., 2013), then tabulated an incomplete matrix of match counts and extracted fasta-formatted UCE sequences for each locus and each taxon. We then aligned UCE sequences with *MAFFT*

7.4, using the L-INS-i option (local pairwise alignment) (Katoh et al., 2002), cleaned locus names from sequence alignments, and trimmed alignments with *trimAl* "automated1" option (Castresana, 2000). As a final step, we screened alignments for minimum taxonomic coverage requirements of 75% and 95%, and concatenated alignments into sequential format.

2.2. Phylogenetic reconstruction and calibration

The 75% taxon complete matrix included 868 loci and 546,840 base pairs; the 95% taxon complete matrix yielded 353 loci and 235,982 base pairs. We partitioned the matrices using the sliding -window site characteristics based on site entropies (SWSC-EN) method (Tagliacollo and Lanfear, 2018) which groups UCE sites based on their estimated entropy, a proxy for the rate of evolution. We then generated a partitioning scheme for the resultant data blocks with Partition Finder 2.1.1, using the rcluster search algorithm and GTRGAMMA model with fit evaluated using the corrected Aikake information criterion (Lanfear et al., 2014; Lanfear et al., 2017), and obtained 625 subsets for the 75% taxon complete matrix and 318 subsets for the 95% taxon complete matrix. We performed partitioned maximum likelihood analyses using RAxML 8.2.12 (Stamatakis, 2014) at the CIPRES science gateway (phylo.org; Miller et al., 2010), applying the GTRGAMMA substitution model to each partition and assessing support for nodes using non-parametric bootstrapping set to finish based on the autoMRE majority rule criterion. We also ran three replicates of a Bayesian phylogenetic analysis for both the 75% and 95% taxon complete matrices, each with 1 million generations and different starting seeds but with the same SWSC-EN partitioning, using ExaBayes on CIPRES (Aberer et al., 2014). To confirm that the effective sample sizes for each run exceeded 200, we examined log files with Tracer 1.7 (Rambaut et al., 2018), and computed 50% majority rule consensus trees from the posterior tree distribution using TreeAnnotator 2.6.7 (Bouckaert et al., 2019). To examine potential gene-tree/species-tree discordance across our UCE data, we computed individual gene trees for each UCE locus in the 95% taxon complete dataset with RAxML and summarized them into a species tree under the multispecies coalescent model using ASTRAL 5.6.3, with node support measured as local posterior probability (Mirarab and Warnow, 2015; Sayyari and Mirarab, 2016).

Topologies obtained in the RAxML and ExaBayes analyses for both the 75% and 95% taxon complete matrices were all identical, so we used the 95% taxon complete matrix and hypothesis for time-calibration with MCMCTree, using the two-step approximate likelihood calibration procedure as implemented in PAML 4.8 and with a conservative age constraint for the root of 120 Ma (Yang and Rannala, 2006; Yang, 2007; dos Reis and Yang, 2019). We repeated the analysis three times, each with 1 million generations and discarding the first 10,000 generations as burnin and report the intermediate from among the three very similar results. We applied five fossil calibrations, all based on fossil finds from the last decade:

Carlomonnius quasigobius (Bannikov and Carnevale, 2016). This specimen is a skeleton with the imprint of an otolith from the Eocene reef deposits of Monte Bolca, with an inferred age of 50.0 Ma (age range estimate 47.8–53.0 Ma). It displays a mix of morphological characters and is ambiguously placed in the total evidence analyses of Gierl et al. (2022), either at the root of all gobioid families except Odontobutidae and Rhyacichthyidae, or higher in the tree (within Butidae) in an analysis with restricted taxon sampling. Due to the age of this fossil and its mosaic assortment of morphological characters, we place this calibration at the root of the Eleotridae + Butidae + Xenisthmidae clade.

Lepidocottus aries (Gierl et al., 2013). This remarkably preserved specimen consists of a full skeleton and otoliths, from the latest Oligocene (23.5 Ma, age range estimate 23.0–24.0 Ma) of southern France. Its morphology is consistent with placement as a stem lineage of Butidae, a position confirmed by the analyses of Gierl et al. (2022), and we assign the calibration to the root of the Butidae clade.

Mataichthys bictenatus (Schwarzhans et al., 2012). A skeleton with otoliths from the early Miocene (17.5 Ma, age range estimate 16.0–19.0 Ma) of New Zealand, this fossil most closely resembles *Gobiomorphus* but with slight differences in epural fusion and anal fin ray count and is hypothesized to be an ancestor to *Gobiomorphus* and *Philypnodon*. We place the calibration at the root of the *Gobiomorphus* + *Philypnodon* clade.

Micropercops pomahaka (Schwarzhans et al., 2017). Otoliths only, from the late Oligocene (25.1 Ma, age range estimate 23.0–27.3 Ma) of southern New Zealand. *Micropercops* is part of Odontobutidae, and we place this calibration at the root of our sampled species (genera *Perccottus*, *Odontobutis*, and *Sineleotris*) based on the phylogeny of Li et al. (2018) that resolved *Micropercops* outside and sister to our sampled genera.

Paralates chapelcorneri (Gierl and Reichenbacher, 2017). Skeletons without otoliths from the late Eocene of southern England, 36.0 Ma (age range estimate 33.9–37.8 Ma). This fossil exhibits a mix of characters, and its placement is uncertain in the analyses of Gierl et al. (2022), although it has six branchiostegal rays and therefore is among the root gobioids (five branchiostegal rays is a consistent characteristic of Gobiidae, Oxudercidae, and Thalasseleotrididae). Gierl and Reichenbacher (2017) described some similarities between *Paralates chapelcorneri* and the eleotrid genus *Gobiomorphus*, although other characters differ, and it is recovered in a more rootward position in their analyses. We conservatively place this calibration at the root of Eleotridae.

2.3. Analyses of diversification within Eleotridae

To explore the tempo of species diversification within the largest family of root Gobioidei, Eleotridae, we constructed a lineage through time (LTT) plot and computed the gamma statistic (Pybus and Harvey, 2000) with the R package phytools 1.0–3 (Revell, 2012), and used BAMM 2.5.0 (Rabosky, 2014) to evaluate lineage diversification rates and test for accelerated diversification among eleotrid clades. For these analyses, we removed one individual of *Leptophilypnus panamensis,* the ambiguously identified *Microphilypnus* cf. *amazonicus* (potentially a synonym of *M. ternetzi*), and all representatives of Butidae, Xenisthmidae, Milyeringidae, Odontobutidae, Rhyacichthyidae, and Trichonotidae from the calibrated UCE phylogeny, such that only Eleotridae were included and each species was represented by one terminal in the tree. To account for incomplete taxon sampling, we calculated sampling fractions for each genus and included the unsampled genera in the counts for their likely closest relatives: *Belobranchus* (two species) was included with *Bunaka* (Keith et al., 2012), and *Caecieleotris* (one species) was counted with *Eleotris* (Walsh and Chakrabarty, 2016). Once the sampling probabilities were tabulated, we set the priors given the phylogeny using the R package BAMMtools 2.1.10 (Rabosky et al., 2014) and ran the analysis for 10 million generations. We then again used BAMMtools to examine the output, ensure chains had converged, check the effective sample sizes (ESS) for numbers of shifts and log likelihood, and extract diversification rates across the tree and in specific clades.

3. Results

3.1. Phylogeny of root Gobioidei

The phylogenies obtained from both the RAxML and ExaBayes analyses on both the 75% and 95% taxon complete datasets were identical, and the calibrated topology is shown in Fig. 1. The ASTRAL species tree resolved a similar topology, with most nodes well-supported except for those along the backbone of the crown Eleotridae (Fig. 2). The primary difference between the RAxML/ExaBayes tree and the ASTRAL tree is that the pairing of *Hypseleotris* and *Leptophilypnus* is not resolved in the ASTRAL tree, consistent with the poor support that pairing obtains in the concatenated species tree and the shallow resolution among major lineages in the Eleotridae crown clade.

Our hypothesis supports the family-level relationships among root Gobioidei resolved in many other studies: first diverging in the suborder is a clade of Rhyacichthyidae + Odontobutidae, followed by Milyeringidae, then Butidae + Xenisthmidae sister to Eleotridae (Thacker et al., 2015). Within the sleeper clade Eleotridae, we resolve several clades: first diverging is an Indo-Pacific/Malagasy grouping of *Ratsirakia, Giurus, Tateurndina, Allomogurnda,* and *Mogurnda*, consistent with Thacker & Hardman (2005), Agorreta et al. (2013) and Thacker (2009, 2017). The remainder of Eleotridae is arrayed into four clades along a shallow backbone. The first to diverge is a clade including *Calumia* (widespread on Indo-Pacific reefs) and *Bunaka* (inhabits Indo-Pacific swamps, estuaries, and mangroves), and the circumtropical euryhaline *Eleotris* (including *Erotelis*). Next is the pairing of *Philypnodon* and *Gobiomorphus*, species of which inhabit fresh and brackish waters in Australia and New Zealand. *Philypnodon* is the sister taxon to the two Australian species of *Gobiomorphus*, separated from the New Zealand *Gobiomorphus* species and likely warranting assignment to *Philypnodon* or a distinct genus (e.g. *Krefftius*) pending morphological revision, as previously resolved in a more detailed phylogeny of the genera given in Thacker et al. (2022b).
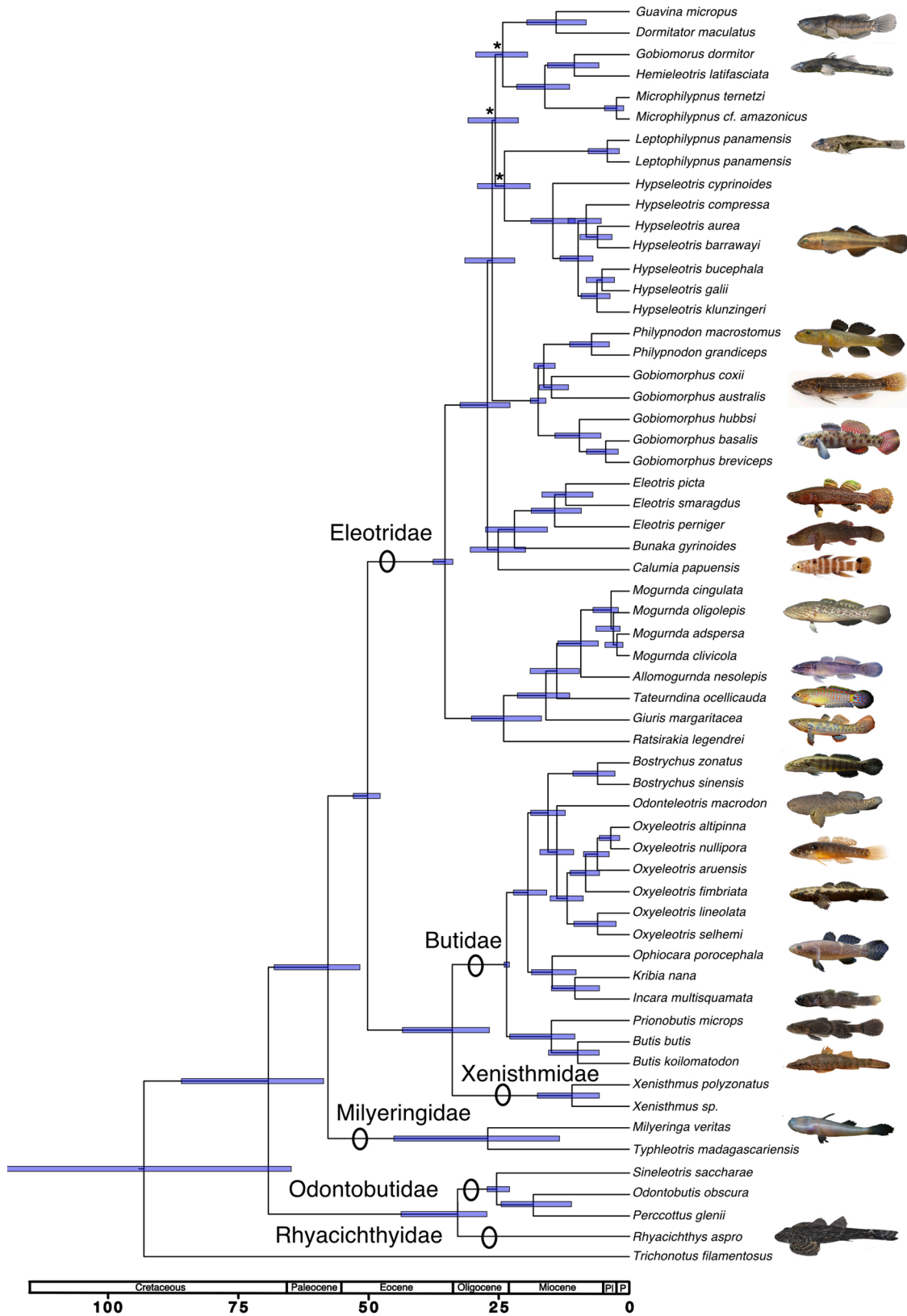
The two crown clades of Eleotridae are one containing the Neotropical euryhaline and freshwater genera *Gobiomorus*, *Hemieleotris*, *Microphilypnus*, *Dormitator* and *Guavina*, sister to a grouping between the Neotropical freshwater genus *Leptophilypnus* and the Indo-Pacific (mostly Australian) genus *Hypseleotris* (euryhaline to freshwater; that genus includes *Kimberleyeleotris*, (Thacker et al., 2022a)). Relationships within and among *Hypseleotris* and the other eleotrid genera have placed *Hypseleotris* variably among eleotrid clades (Thacker and Hardman, 2005; Thacker and Unmack, 2005; Thacker, 2017). Notably, in none of the earlier hypotheses is *Hypseleotris* grouped closely with *Philypnodon*, *Mogurnda*, *Gobiomorphus*, or other eleotrid genera common in Australia, New Zealand, or New Guinea. Instead, we place *Hypseleotris* as sister to the Neotropical miniature genus *Leptophilypnus*, although that placement was unsupported in the ASTRAL tree, and the relationships of both *Hypseleotris* and *Leptophilypnus* should be conservatively interpreted as unresolved within the crown Eleotridae clade.

Our hypothesis concurs with earlier inferences of phylogeny for root Gobioidei (Thacker and Hardman, 2005; Thacker, 2017) in recovering *Butis* as the earliest diverging lineage in Butidae, placed as sister to *Prionobutis*. Two clades diverge within the crown Butidae, one including *Ophiocara*, *Incara*, and *Kribia*, sister to a clade containing *Bostrychus*, *Odonteleotris*, and a monophyletic *Oxyeleotris*. The most prominent disagreements between this and the earlier hypotheses are the dwarf species *Oxyeleotris nullipora* is recovered with the other *Oxyeleotris* species, rather than as a separate lineage, and that we recover *Xenisthmus* (Xenisthmidae) as the sister to Butidae, rather than as nested within Eleotridae as inferred by Thacker (2003) and Thacker & Hardman (2005).

3.2. Temporal patterns of divergence and diversification

The inferred stem age for Gobioidei is 93.12 Ma (95% highest posterior density interval [HPD] 64.87–121.58 Ma), in the late Cretaceous. This estimate is comparable to those inferred by Alfaro et al. (2018), Hughes et al. (2018), and Ghezelayagh et al. (2022), who recovered stem ages for Gobioidei ranging from 95 to 110 Ma. We resolve a crown age for Gobioidei of 69.25 Ma (95% HPD 58.64–85.92); within Gobioidei we estimate a crown age for Eleotridae of 35.36 Ma (95% HPD 33.84–37.64) and for Butidae of 23.53 Ma (95% HPD 23.0–24.0). The earliest diverging eleotrid clade, containing *Mogurnda* and its relatives, has a crown age of 24.10 Ma (95% HPD 16.85–30.28 Ma) in the late Oligocene. Divergence between the four remaining eleotrid lineages was rapid and also took place in the late Oligocene, an estimated 24.3–26.3 Ma.

Australian *Hypseleotris* and New Zealand *Gobiomorphus* are resolved with crown ages in the Miocene at 9.82 Ma (95% HPD 6.98–13.30 Ma)

(caption on next page)

and 9.58 Ma (95% HPD 5.45–14.26 Ma), respectively, as is the clade of *Allomogurnda* plus *Mogurnda* at 9.3 Ma (95% HPD 5.95–13.70 Ma). *Mogurnda* alone is much younger, with a crown age of 3.50 Ma (95% HPD 2.10–9.62 Ma) in the Pliocene. The BAMM analysis of Eleotridae obtained ESS values over 1,000 and comparisons of Bayes factors for different shift regimes weakly supported a diversification model with an acceleration of diversification only in the genus *Mogurnda*; no shifts or rate accelerations were inferred for *Hypseleotris* or *Gobiomorphus*. The Bayes factor for a shift in *Mogurnda* compared to a zero-shift model was 1.15, a negligible result given that Bayes factors of > 20 are considered to provide strong evidence for a rate shift (Rabosky, 2014; Rabosky et al., 2017). However, the diversification rate in *Mogurnda* was 0.262 lineages/my, more than twice the background diversification rate in Eleotridae of 0.115 lineages/my. No other lineages show rate increases. Fig. 3 shows the BAMM reconstruction of the diversification acceleration in *Mogurnda*, along with the lineages through time (LTT) plot and rates through time (RTT) plots. The LTT plot shows a slight uptick in rate of new lineages around the late Oligocene when the crown clades of Eleotridae were arising, and the inferred value of the gamma statistic was −2.70 (p = 0.007), indicating a significant slowing of diversification over time (Pybus and Harvey, 2000). The RTT plot also shows an overall slowing of speciation, except for a slight increase over the last 3.5 Ma, corresponding with the origin and diversification of *Mogurnda* species.

4. Discussion

4.1. Relationships among root Gobioidei

Our phylogeny agrees in many respects with those presented in the earlier studies of Thacker & Hardman (2005), Agorreta et al. (2013), Thacker (2009, 2017), Thacker et al. (2015), McCraney et al. (2020), and Ghezelayagh et al. (2022), with the strongest concordant results being the placement of a *Ratsirakia, Tateurndina, Giurus, Allomogurnda* and *Mogurnda* clade as the earliest diverging lineage within the family, the sister relationship of *Philypnodon* and *Gobiomorphus*, and a clade containing most of the Neotropical genera (*Guavina, Dormitator, Gobiomorus,* and *Hemieleotris*, plus the miniature *Microphilypnus*). Although the divergences among the four core Eleotridae clades are shallow, we resolve *Bunaka, Calumia,* and the circumglobal *Eleotris* as diverging first, followed by the Australian/New Zealand clade containing *Gobiomorphus* and *Philypnodon*. The most crownward clade contains *Hypseleotris, Leptophilypnus,* and the Neotropical genera *Microphilypnus, Hemieleotris, Gobiomorus, Guavina,* and *Dormitator*. Interrelationships of these clades have traditionally been difficult to resolve, and we demonstrate that they diverged rapidly over a 2-million-year period in the late Oligocene, 24.3–26.3 Ma.

Our phylogenetic hypothesis, like those of Thacker and Hardman (2005) and Thacker (2017), indicates that Eleotridae invaded the Neotropics at least twice, once among the worldwide *Eleotris* species and again in the lineage leading to *Guavina, Dormitator, Gobiomorus, Hemieleotris,* and *Microphilypnus*. It is possible that *Leptophilypnus* constitutes a third neotropical invasion, given that its placement as sister to *Hypseleotris* rather than to the other neotropical genera is ambiguously resolved. *Leptophilypnus* and *Microphilypnus,* both miniaturized but inhabiting different areas in the neotropics (Thacker et al., 2006), were recovered as sister taxa in Thacker (2017) but not in the UCE hypothesis. Relationships among genera of Butidae also agree in most respects with Thacker & Hardman (2005), Agorreta et al. (2013) and Thacker (2009, 2017), with much of the disagreement likely due to the inclusion here of several more taxa.

4.2. Diversification patterns among root Gobioidei

Radiations of Eleotridae in Australia and New Guinea (*Mogurnda* and *Allomogurnda*), New Zealand (*Gobiomorphus*), and Australia (*Hypseleotris*) all arose in the late Miocene (9.30–9.82 Ma) but we identify a diversification rate acceleration only in the genus *Mogurnda*. We confirm the results of Thacker et al. (2022a) in showing that the *Hypseleotris* radiations in both northwestern and eastern Australia have diversified steadily, without significant rate shifts, but depart from the results of Thacker et al. (2022b) in failing to recover a diversification rate shift within New Zealand *Gobiomorphus*. This discrepancy is likely due to the broader sampling outside of *Gobiomorphus* and reduced sampling within *Gobiomorphus* in this hypothesis, given that Thacker et al. (2022b) inferred an increase in diversification only in the exclusively freshwater crown clade, containing six species of which only two were sampled here. We infer crown ages for Australian *Hypseleotris* at 9.82 Ma (95% HPD 6.98–13.30 Ma) and for New Zealand *Gobiomorphus* at 9.58 Ma (95% HPD 5.45–14.26 Ma), in the Miocene. The radiation of *Mogurnda* and *Allomogurnda* in New Guinea and Australia is slightly younger at 9.30 Ma (95% HPD 5.95–13.70 Ma), with *Mogurnda* alone much younger at 3.50 Ma (95% HPD 2.10–9.62 Ma) in the Pliocene.

Hypseleotris species in Australia have evolved and diversified in a stable continental landscape that has shown little significant tectonic activity since approximately 80 Ma in the late Cretaceous (Unmack, 2013), and we infer no accelerations in diversification for the group since its origin in the Miocene. We also recover a Miocene origin for *Gobiomorphus* in New Zealand, in keeping with the emergence of the islands approximately 17 Ma following a marine transgression in the late Oligocene to early Miocene 22–17 Ma (Mildenhall et al., 2014; Craw et al., 2016). Unlike the acceleration inferred for exclusively freshwater *Gobiomorphus* by Thacker et al. (2022b) we do not infer accelerated diversification in *Gobiomorphus* as a whole, likely due to differences in sampling of the *Gobiomorphus* crown clade. We infer an acceleration of diversification in the genus *Mogurnda* in Australia and New Guinea beginning in the Pliocene. New Guinea originated with the accretion of an archipelago of much smaller island terranes that formed in the Eocene to early Oligocene and consolidated sometime in the middle to late Miocene (Baldwin et al., 2012; Unmack et al., 2013; Toussaint et al., 2014). Concordant patterns of high Pliocene diversification in New Guinea have been recovered among beetles (Toussaint et al. 2014) and geckos (Tallowin et al., 2018), supporting the hypothesis that invasion of New Guinea was rapid and occurred as soon as the island had grown and uplifted to the point of supporting freshwater habitats.
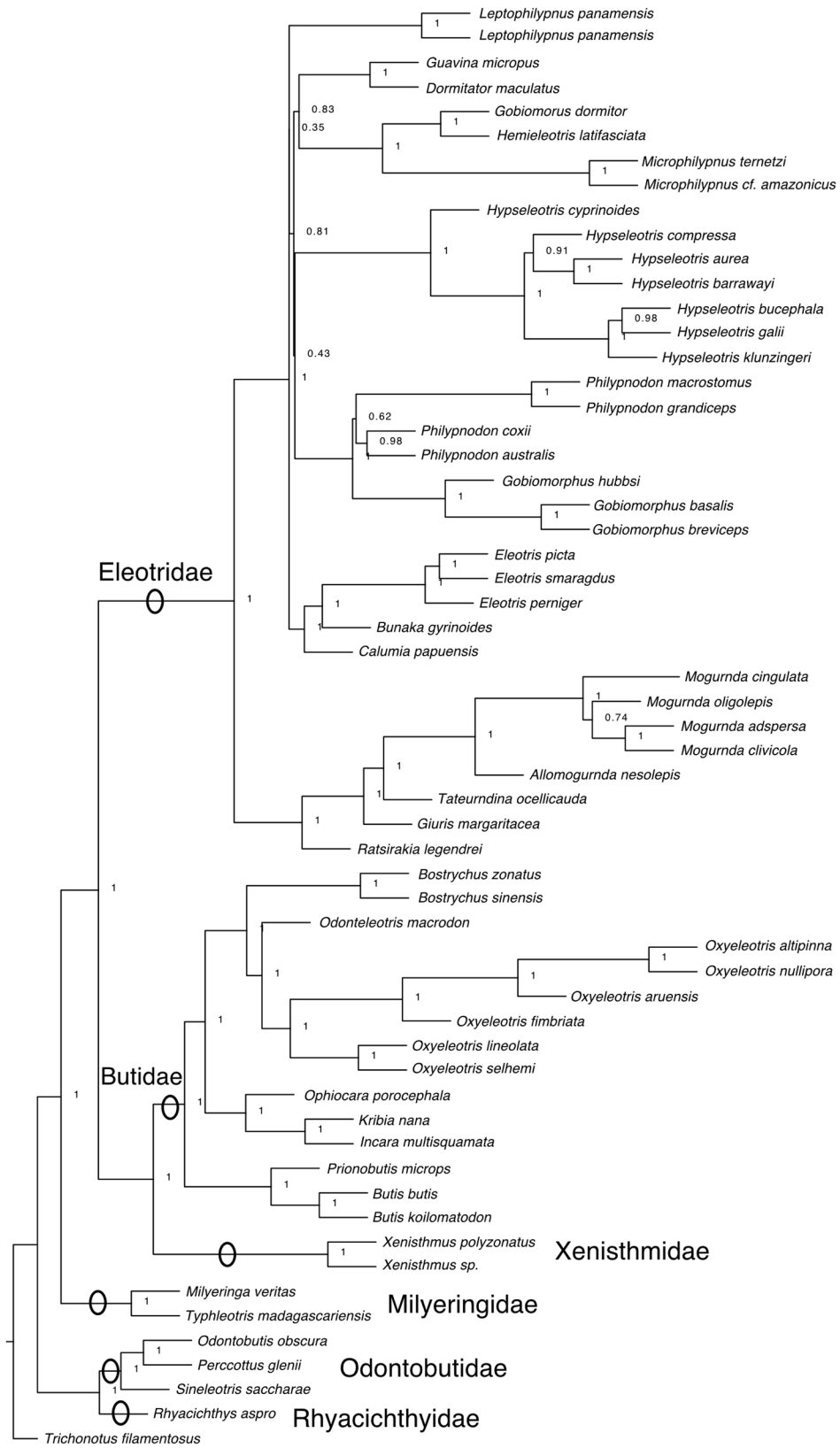
Fig. 2. ASTRAL tree of root Gobioidei based on 95% taxon complete matrix of UCE data.
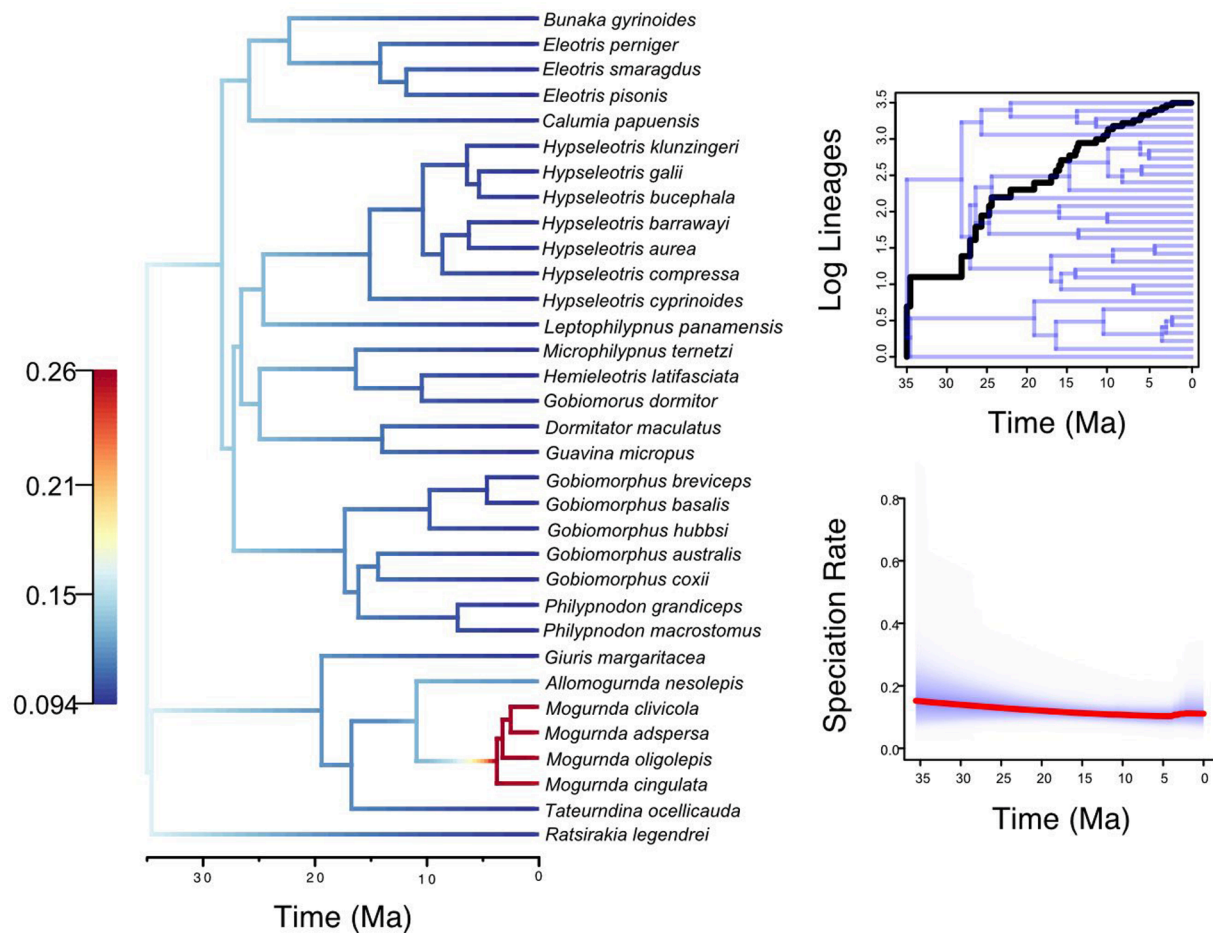
Fig. 3. UCE phylogeny for Eleotridae, with diversification assessed with BAMM on the UCE tree corrected for sampling fraction by genus (left), and LTT and RTT (right). The recent uptick in speciation rate shown in the RTT graph corresponds with the origin and diversification of *Mogurnda*. Colored scale indicates diversification rate in lineages/million years.

5. Conclusions

This phylogeny includes the densest sampling of root gobioid families to date and is the first to use UCE data, complementing the phylogenomic hypothesis of Kuang et al. (2018) that used a dataset of nuclear exon markers to infer phylogeny of the gobioid families and outgroups. Our calibrations are based on fossils described in the last decade, yielding a timescale for divergences among the early branches of the gobioid tree that accords well with larger-scale hypotheses of acanthopterygian evolution. Although the majority of the relationships are well-supported, some uncertainty still exists as to the resolution of the four crown clades within Eleotridae, which we infer to have diverged rapidly during a 2-million-year interval in the late Oligocene. Diversification within eleotrid lineages has been steady overall, with the exception of a jump in diversification rate in the genus *Mogurnda* over the last 3.5 million years, a radiation with diversification centered in the freshwater habitats of New Guinea.

CRediT authorship contribution statement

Christine E. Thacker: Conceptualization, Data curation, Resources, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **W. Tyler McCraney:** Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Richard C. Harrington:** Data curation, Resources, Writing – review & editing. **Thomas J. Near:** Data curation, Resources, Writing – review & editing. **James J. Shelley:** Resources, Funding acquisition, Writing – original draft, Writing – review & editing. **Mark Adams:** Resources, Funding acquisition, Writing – review & editing. **Michael P. Hammer:** Resources, Funding acquisition, Writing – review & editing. **Peter J. Unmack:** Conceptualization, Resources, Funding acquisition, Writing – review & editing.

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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