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Phylogenetics and Biogeography of the Guiana Shield Pencil Catfishes, Genus *Trichomycterus*

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The relationships of Guiana Shield members of *Trichomycterus* are examined using three mitochondrial genes (Cytochrome Oxidase 1, Cytochrome b, and 16s) and one nuclear gene (RAG 2). A time-calibrated phylogeny is implemented to examine how diversification is related to the timing of major geographical events in the Guiana Shield. There was topological discordance among the gene trees; however, they overall suggest the presence of two subclades. The *T. guianensis* subclade consists of the strongly supported relationships of *T.* sp. 'Potaro, elongate' (Potaro R.) sister to *T. guianensis* (Potaro R.) + *T.* sp. 'Ireng, spotted' (Ireng R.). The *T. cf. guianensis* subclade consists of the strongly supported sister group relationship of *T. cf. guianensis* (Mazaruni, Potaro, and potentially Caroni Rivers) + *T.* sp. 'Mazaruni, plain' (Mazaruni R.). Weakly supported as sister to this is *T.* sp. 'Kusad Mountain' (Takutu R.), and sister to all other members of the subclade is *T. conradi* (Ireng and Potaro Rivers). The dated phylogeny suggests that the Guiana Shield clade is derived from a lowland ancestor that entered the Proto-Berbice and/or Proto-Essequibo Rivers ~17.4 Ma. The Proto-Berbice contained the lowland portions of the Ireng and Takutu Rivers, and the Proto-Essequibo contained the lowland portions of the Potaro and Mazaruni Rivers, but the histories of the upland portions of the Ireng, Potaro, and Mazaruni Rivers are less clear and have been thought to have drained northward in what we are referring to as the Grand Pakaraima River in which all members of the *T. guianensis* clade (except for *T.* sp. 'Kusad Mountain' and possibly *T. conradi*) were found. We interpret two geodispersal events into the Grand Pakaraima River and a potential vicariance event ~9.4 Ma between uplands and lowlands. The likely formation of the modern Guiana Shield rivers occurred 3.8–1.9 Ma, with the modern Ireng River being captured first by the Proto-Essequibo ~3.8 Ma and finally consolidated ~1.9 Ma when captured by the Rio Branco.

THE Trichomycteridae is a diverse family of Neotropical catfishes (Siluriformes) distributed throughout most of South America and southern Central America (Fernández, 2017). The family is distinguishable from other catfishes chiefly by the presence of opercular and interopercular odontodes as well as a pair of rictal barbels (Baskin, 1973; de Pinna, 1989; Fernández, 2017). Some species utilize their odontodes to aid in climbing waterfalls (Armbruster, 2011), allowing them to inhabit extreme reaches of headwaters and otherwise depauperate Andean mountain streams (Baskin, 1973).

Currently, the Trichomycteridae contains 421 species, with 72% (305) found within the subfamily Trichomycterinae. Of these, 116 species have been described within the last ten years (Fricke et al., 2023). There are currently nine recognized genera in the Trichomycterinae, *Eremophilus* von Humboldt 1805 (1 sp.), *Trichomycterus* Valenciennes 1832 (199 spp.), *Hatcheria* Eigenmann 1909 (1 sp.), *Sclero-nema* Eigenmann 1917 (10 spp.), *Rhizosomichthys* Miles 1943 (1 sp.), *Bullockia* Arratia, Chang, Menu-Marque, and Rojas 1978 (1 sp.), *Ituglanis* Costa and Bockmann 1993 (31 spp.), *Silvinichthys* Arratia 1998 (7 spp.), and *Cambeva* Katz, Barbosa, Mattos, and Costa 2018 (54 spp.). Within the Trichomycterinae, several recent taxonomic and phylogenetic studies support the monophyly of many species, although deeper relationships remain largely unresolved (Ochoa et al., 2017; Katz et al., 2018; Hayes et al., 2020; Fernández et al., 2021). To date, the most comprehensive phylogenetic assessment of the Trichomycteridae sequenced ultra-conserved elements (UCEs) for 139 species (Ochoa et al., 2020). Results from this study found the genus *Trichomycterus* to be non-monophyletic and inferred two major lineages within the monophyletic subfamily Trichomycterinae: 1) the *Trichomycterus* Lineage (*Cambeva*, *Scleronema*, and *Trichomycterus sensu stricto*) that is distributed throughout the Brazilian Shield and southeastern South America and 2) the *Eremophilus* Lineage (*Bullockia*, *Eremophilus*, *Ituglanis*, and the remainder of *Trichomycterus*) that is distributed throughout the Andes and Guiana Shield as well as tropical, cis-Andean lowlands (see Ochoa et al., 2020: figs. 3, 4, or a simplified cladogram in Supplemental File S1; see Data Accessibility). Although this was the most lineage-inclusive study of the entire family, about two-thirds of known species were not included (mostly trichomycterines), and many additional species are yet to be described. Given the large gaps in geographic sampling and recent rapid diversification of lineages, the deeper relationships within the subfamily Trichomycterinae are dubious at best, and *Trichomycterus* remains non-monophyletic. However, we follow the suggestions of Ochoa et al. (2020), which supplies a clade-based framework for testing evolutionary and biogeographic hypotheses within smaller groups of the Trichomycteridae.

One group of particular interest is the *Trichomycterus guianensis* clade, within the *Eremophilus* lineage (Ochoa et al., 2020). The *T. guianensis* clade conservatively contains at least 11 species (several undescribed) that are endemic to

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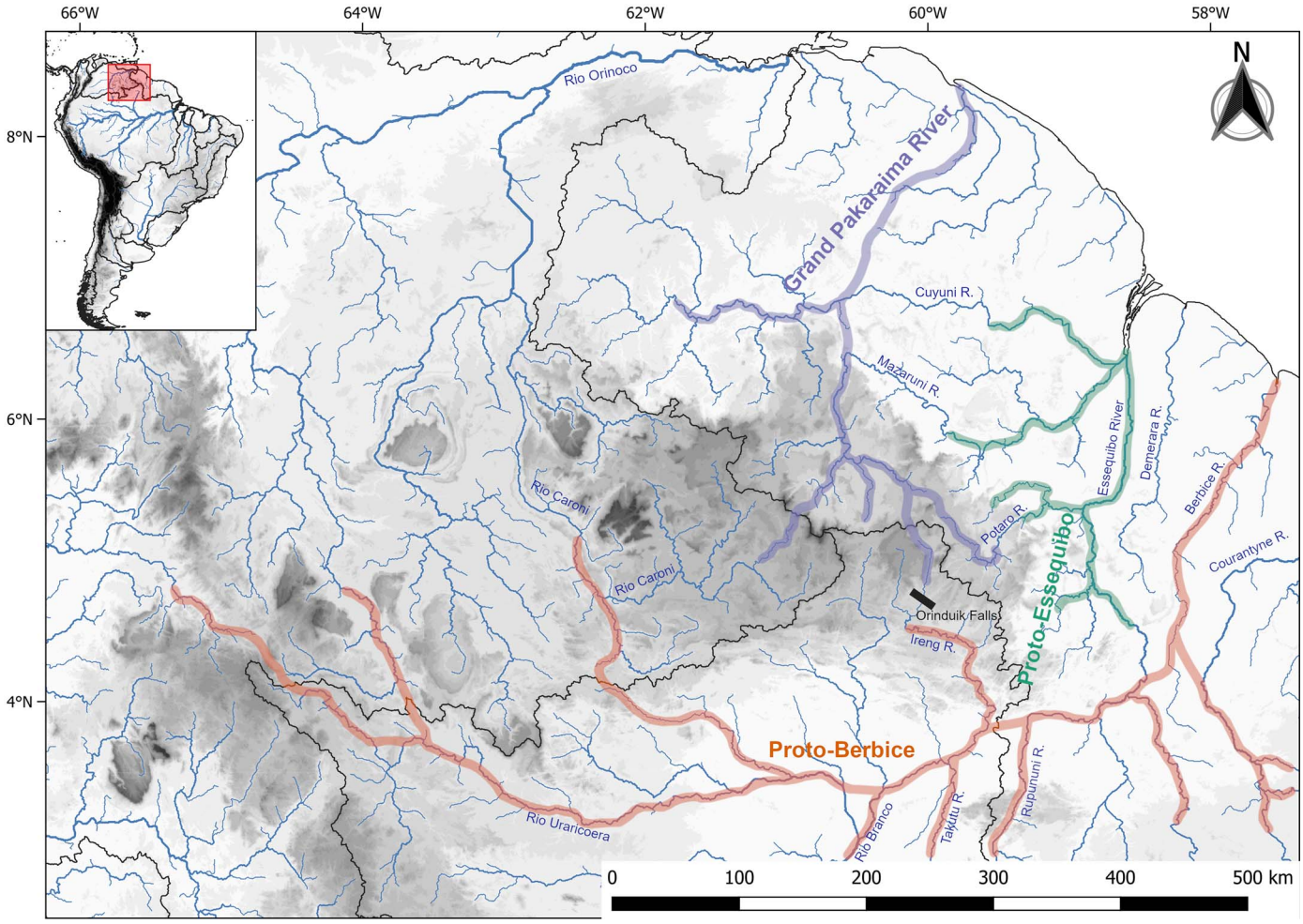

Fig. 1. Map of the rivers of the Guiana Shield with hypothesized routes of the Proto-Berbice (red), Proto-Essequibo (green), and Grand Pakaraima River (purple).

the Guiana Shield region of South America, which represents a biogeographic area of interest given its complex geologic history and high levels of biodiversity and endemicity. Furthermore, headwaters from this region divide and flow into three major river basins of South America—the Amazon, Orinoco, and Essequibo River basins.

The Guiana Shield is the northern portion of the Amazonian Craton, separated from the rest of the craton (Brazilian Shield) by the Amazon River. The basement of the Guiana Shield rock is highly durable, metamorphic rock with the central part of the shield covered in sandstones of the Roraima Supergroup that were laid down in the Proterozoic (~2 billion years ago). These sandstones remained buried until the late Jurassic (163.5–145 mya) when they were block uplifted during the Takutu Event. Today, the Guiana Shield exists to the west as the low Pakaraima Mountains (Hartmann, 2002; Santos et al., 2003). The old age of the Pakaraimas is in stark contrast to the young, lowland Amazon basin that neighbors it, which is roughly 10–15 million years old (Méndez-Camacho et al., 2021). This juxtaposition of old and young geologic features lends itself to studying events that may have shaped the current diversity and biogeography of freshwater fishes. These events include marine incursions, in which the highlands of the Guiana Shield may have provided high-elevation refugia for freshwater fishes (Hubert and Renno, 2006). Additionally, river capture events are hypothesized to play an important role in shaping patterns of fish biodiversity by allowing for dispersal and subsequent isolation of riverine species (Lundberg et al., 1998; Winemiller et al., 2008; Lujan and Armbruster, 2011). Temporary connections of rivers in Guyana, such as those that occur between the Takutu and the Essequibo Rivers over the Rupununi Wetlands, indicate the early stages of river capture and are seasonal passageways for dispersal and ensuing isolation (Lujan and Armbruster, 2011; de Souza et al., 2012, 2020). Despite a lengthy and intricate geologic history, much consideration for the diversification of Neotropical freshwater fishes has been given to the recent Andean Orogeny and formation of the modern Amazon, even though the major lineages of freshwater fishes are much older than this event (Lundberg et al., 1998).

Before the formation of the modern Amazon River, the paleo-rivers of the Guiana Shield such as the Proto-Berbice River would have been the dominant river systems of the area (Fig. 1). The formation of the Proto-Berbice River began with the Takutu Event, which involved the formation of a graben separating the Pakaraima and Kanuku Mountains in the late Jurassic. This graben is extended as a failed rift that is currently demarcated by the Berbice River and Berbice

Trough, with the rift eventually terminating at the mid-Atlantic rift. The Takutu graben formed Paleolake Maracanta, which began transition to a fluvial system in the late Cretaceous (Berrangé, 1975; Crawford et al., 1985). Major tributaries of the Proto-Berbice included the Uraricoera, Cotinga, Takutu, Ireng, Rupununi, upper Essequibo, Demerara, Berbice, New, and upper Courantyne Rivers. It has been further hypothesized based on the distribution of some species that the upper courses of Orinoco tributaries like the Ventuari, Caura, and Caroni possibly also flowed into the Proto-Berbice (Lujan and Armbruster, 2011), and this has largely proven predictive of species distributions (Taphorn et al., 2010; Lujan et al., 2017; Armbruster et al., 2021).

Less understood is what was happening in the upper courses of the rivers through the Cenozoic. McConnell (1968) suggests that the presence of large, braided sections of rivers in the Guiana Shield represent areas where stream capture has taken place. The highly durable rock of the Guiana Shield prevents captured rivers from consolidating into a single channel. These stream capture events also result in sudden changes in the direction of flow. Based on these observations, he suggested that the upper course of the Mazaruni once continued northward to the Atlantic Ocean taking with it part of the Potaro and Cuyuni Rivers. Uplift around the Pliocene (McConnell, 1968: 517, states only "end-Tertiary") diverted the Cuyuni, Mazaruni, and Potaro to the east where the rivers flow in unconsolidated channels before falling off the escarpment in a series of rapids or large falls like Kaieteur Falls. Here, we term the paleoriver that contained portions of the upper Cuyuni, Mazaruni, and Potaro as well as possibly the upper Caroni and upper Ireng the Grand Pakaraima River (Fig. 1).

The present distributions of species on the Guiana Shield can be used to hypothesize the ancient relationships among the major rivers of South America by implementing phylogenetic studies of small-bodied, positively rheophilic, headwater specialist species that have wide geographic ranges (Lujan and Armbruster, 2011). The Trichomycteridae matches each of these criteria (Fernández, 2017), and future studies of this group may very well provide insight for many paleogeographic puzzles concerning the rivers of South America. Understanding the historic biogeography of this region is paramount to managing the futures of these rivers, which are of ecological importance to thousands of species as well as to the livelihoods and cultures of many Indigenous communities.

In this study, we assess the phylogenetic relationships of the *T. guianensis* clade of the *Eremophilus* lineage of the Trichomycterinae. The *T. guianensis* clade includes four described species (*T. celsae*, *T. conradi*, *T. guianensis*, and *T. lewi*) and at least six undescribed species: *T.* sp. 'Kusad Mountain,' *T.* sp. 'Ireng, spotted,' *T.* sp. 'Gran Sabana,' *T.* sp. 'Mazaruni, plain,' *T.* sp. 'Potaro, elongate,' and *T. cf. guianensis* (Akin et al., unpubl.). These species are all found in highland regions of the Guiana Shield around the tricorner of Brazil, Guyana, and Venezuela, except for *T.* sp. 'Kusad Mountain,' a disjunct species known only from Kusad Mountain (located along the Takutu River south of the Kanuku Mountains of Guyana; Fig. 2). In this study, we provide a time-calibrated phylogeny for the *Eremophilus* lineage, including novel sequences for *T.* sp. 'Kusad Moutain.' We combine the phylogenetic hypotheses formed by this paper as well as the broader phylogenetic hypothesis proposed by Ochoa et al. (2020) alongside our time calibration and the current interpretation of South American geology to infer phylogeographic patterns for Guiana Shield rivers.

MATERIALS AND METHODS

Data sampling.—No field work was done specific to this study. We sequenced tissues that were loaned to us by the Royal Ontario Museum (ROM) from a natural history collection made in the upper Takutu River (Amazon River drainage) in Guyana in 2013 by D. C. Taphorn, M. Kolmann, M. Ignace, and L. Kalicharan. For *Trichomycterus conradi*, all sequences from the Potaro River are represented by a lone individual, and sequences from the Ireng River are represented by one individual in the *rag2* analysis, three individuals in the *coI* and *16s* analyses, and no sequences of *cytb* were available from individuals of *T. conradi* from the Ireng River. For *T. cf. guianensis*, there are no tissues available for collections of this species from the upper Caroni River in Venezuela. All other species in this study are currently known as endemics to single drainages. Not included in this study due to lack of tissues are *T. celsae* and *T. lewi*.

Molecular data.—We obtained tissue samples of five specimens of *Trichomycterus* sp. 'Kusad Mountain' from ROM (see Table 1). We extracted whole genomic DNA using Qiagen DNeasy Blood and Tissue Extraction Kit following manufacturer protocol (Qiagen Sciences Inc., Germantown, MD). We amplified four genes, three mitochondrial (*16s*, *coI*, and *cytb*) and one nuclear (*rag2*). Target loci were selected to maximize overlap with previous studies (Ochoa et al., 2017; Hayes et al., 2020). We amplified our target loci with polymerase chain reactions (PCR) using primer and protocols implemented by previous studies (see Table 2; Ochoa et al., 2017; Hayes et al., 2020). Our detailed PCR protocol is found in Supplemental File S2 (see Data Accessibility). We sent unpurified PCR products to an external sequencing facility (GENEWIZ, Cambridge, MA) for DNA sequencing. We edited and aligned raw sequence data using the MAFFT algorithm (Katoh et al., 2002) as implemented in the program Geneious Prime v.11.0.14 (Kearse et al., 2012).

We downloaded additional sequence data for ingroup taxa from 24 individuals of the *Trichomycterus guianensis* clade from previously published studies from the NCBI GenBank (see Table 1; e.g., Ochoa et al., 2017; Hayes et al., 2020) and verified the identities of all ingroup taxa with museum voucher specimens. We trimmed alignments for all 29 individuals within the *T. guianensis* clade to the following lengths for consistency with previously published sequences (Ochoa et al., 2017; Hayes et al., 2020): *16s*, 466 bp; *coI*, 521 bp; *cytb*, 858 bp; and *rag2*, 885 bp.

For the time-calibrated phylogeny, we downloaded additional sequence data of one or two representatives per species from previously published studies for members of the *Eremophilus* lineage from the NCBI GenBank that contained at least three loci, prioritizing alignments that contained all four loci (see Table 1). These criteria yielded 27 additional individuals of the *Eremophilus* lineage to include in a time-calibrated analysis with our 29 individuals from the *T. guianensis* clade. We did not verify species identities with

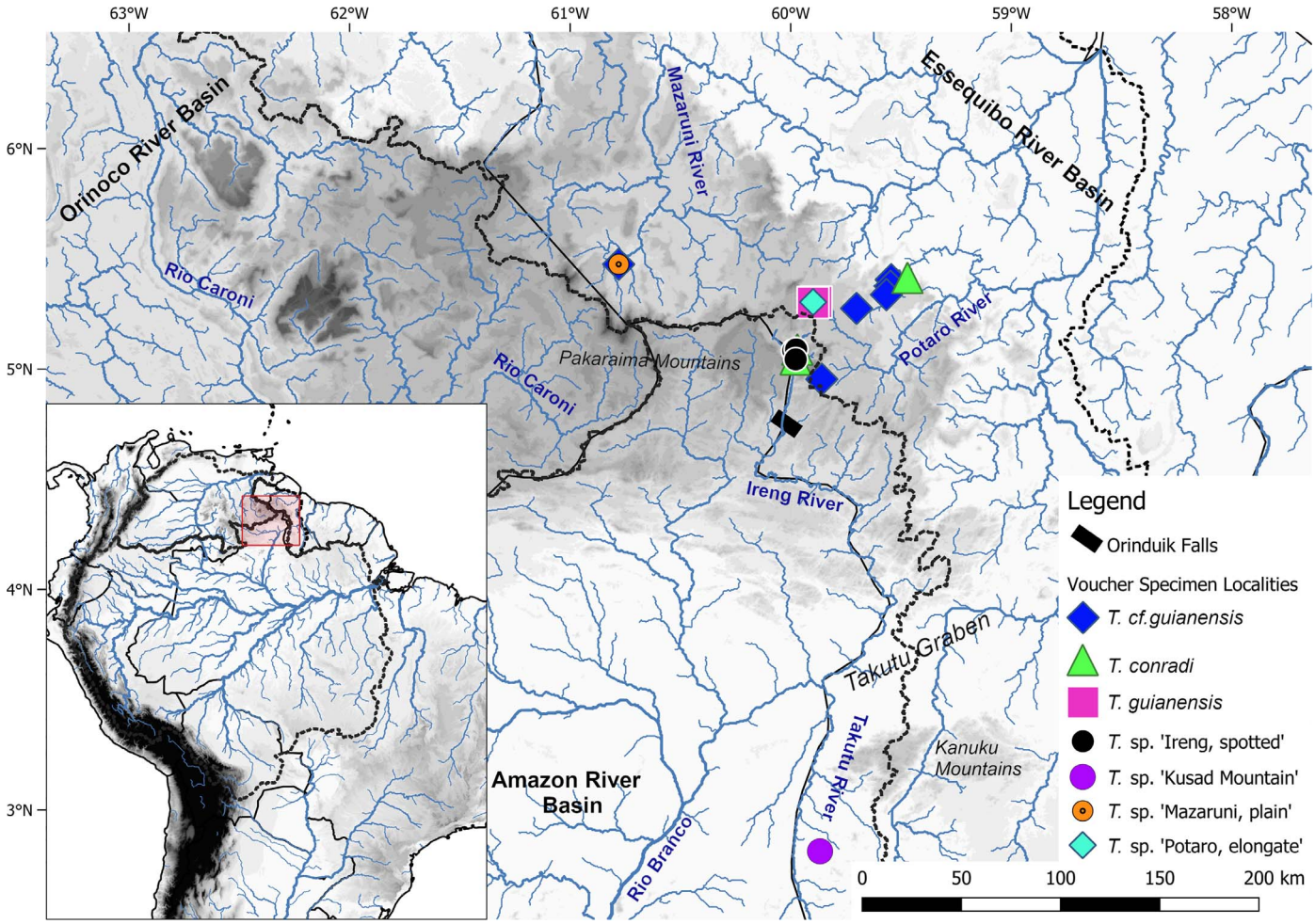

Fig. 2. Localities of Guiana Shield voucher specimens of *Trichomycterus* sequenced.

museum vouchers for these outgroup taxa. We trimmed these alignments as above.

Phylogenetic analysis.—We analyzed individual gene trees of the *T. guianensis* clade with one individual of *Scleronema minutum* as an outgroup to assess whether individual genes may be used for barcoding (e.g., Reis and de Pinna, 2023) and to inform phylogeographic discussion of topologies estimated with different rates of diversification. We then analyzed a concatenated dataset (2,730 bp) including all 29 individuals of the *T. guianensis* clade with *S. minutum* as an outgroup. We used only one outgroup taxon because the *T. guianensis* clade was found to be monophyletic by studies using more extensive taxonomic sampling with the same loci, but without resolution for interpreting deeper relationships within the subfamily (Supplemental File S3; see Data Accessibility; Ochoa et al., 2017; Hayes et al., 2020; Akin, 2022).

We inferred phylogenetic relationships using a Bayesian inference (BI) approach in the program MrBayes v3.2.6 via the CIPRES web portal (Huelsenbeck and Ronquist, 2005; Miller et al., 2010). We partitioned protein-coding genes by codon position and estimated substitution models simultaneously by using the lset function (Huelsenbeck and Ronquist, 2005). We similarly partitioned the concatenated analysis by both gene and codon position. For all gene tree analyses, we used a Markov chain Monte Carlo (MCMC) to sample the posterior for $2*10^7$ generations. To ensure the chains converged and to reduce autocorrelation among samples, we performed two independent runs with four chains (three heated, one cold). We sampled tree topologies from the posterior every 1,000 generations. For the concatenated tree analysis, we used MCMC to sample the posterior for $1.5*10^7$ generations. We summarized the posterior and trees in MrBayes v3.2.6 using the default 25% burn-in. To ensure that each of the MCMC runs had sufficiently mixed for all estimated parameters, we used the program Tracer v1.7 to visualize posterior sampling and calculate estimated sample sizes (ESS) for each parameter. We consider parameters with ESS $>$ 200 to have converged (Rambaut et al., 2018). We report the resulting 50% majority rule consensus phylogeny and report support values in posterior probabilities (PP); values $\geq$0.95 are considered as strong support and any value $<$0.95 to be weakly supported.

Time calibration.—We estimated the time-calibrated phylogeny under a fossilized birth death (FBD) model in BEAST v2.6 (Stadler, 2009; Bouckaert et al., 2014; Heath et al., 2014; Barido-Sottani et al., 2018). The FBD model approach is optimal for the *Eremophilus* lineage because it allows for a primary calibration and fossils do not need to be assigned to a specific node, rather the fossil can be treated as a tip in the entire tree or constrained to an *a priori* clade within the data, and it integrates the fossil occurrence times into the

Table 1. Voucher specimens used in phylogenetic analyses and associated information.

Subfamily	Clade	Species	Cat #	Tissue	Country	Drainage	*16s*	*co1*	*cytb*	*rag2*
Trichomycteridae — *Trichomycterus* lineage	Minutum	*Scleronema minutum*	LBP 3310	LBPV19841	Brazil	Laguna dos Patos (Atlantic)	KY807234	KY857957	KY858031	KY858184
Trichomycterinae— *Eremophilus* lineage	Areolatus	*Bullockia maldonadoi*	LBP 3112	LBPV 19795	Chile	Biobio (Pacific)	KY807202	KY857926	—	KY858166
Trichomycterinae— *Eremophilus* lineage	Areolatus	*Trichomycterus areolatus*	LBP 3118	LBPV 19819	Chile	Toltén (Pacific)	KY807241	KY857964	KY858036	KY858188
Trichomycterinae— *Eremophilus* lineage	Areolatus	*Trichomycterus areolatus*	LBP 997	LBPV 10320	Chile	Paicavi (Pacific)	KY807240	KY857963	—	KY858187
Trichomycterinae— *Eremophilus* lineage	Chapmani	*Trichomycterus cf. transandianus*	LBP 19844	LBPV 77965	Colombia	Magdalena	KY807277	KY857999	KY858067	KY858216
Trichomycterinae— *Eremophilus* lineage	Chapmani	*Trichomycterus transandianus*	LBP 19845	LBPV 77967	Colombia	Magdalena	KY807285	KY858007	KY858073	KY858222
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67129	AUFT 10166	Guyana	Ireng River drainage	MT025525	MT017634	—	MT017607
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67129	AUFT 10168	Guyana	Ireng River drainage	MT025526	MT017635	—	MT017608
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67129	AUFT 10169	Guyana	Ireng River drainage	MT025527	MT017636	—	MT017609
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67129	AUFT 10170	Guyana	Ireng River drainage	MT025528	MT017637	—	MT017610
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67154	AUFT 10234	Guyana	Ireng River drainage	MT025531	MT017640	—	MT017611
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67172	AUFT 10310	Guyana	Ireng River drainage	MT025534	MT017643	—	MT017613
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Ireng, spotted'	AUM 67179	AUFT 10276	Guyana	Ireng River drainage	MT025532	MT017641	—	MT017612
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Mazaruni, plain'	AUM 83791	ROMT 06183	Guyana	Mazaruni River drainage	MT025535	MT017644	MT017626	MT017614
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Mazaruni, plain'	AUM 83791	ROMT 06184	Guyana	Mazaruni River drainage	MT025536	MT017645	MT017627	—
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Potaro, elongate'	AUM 62949	AUFT 6596	Guyana	Potaro River drainage	MT025523	—	MT017628	MT017615
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Potaro, elongate'	AUM 62949	AUFT 6597	Guyana	Potaro River drainage	MT025524	—	MT017629	MT017616
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Kusad Mountain'	ROM 95880	ROMT 14915	Guyana	Takutu River drainage	PP886079	PP891395	PP944606	PP944607
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Kusad Mountain'	ROM 95880	ROMT 14922	Guyana	Takutu River drainage	PP886082	PP891397	PP944605	PP944608
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Kusad Mountain'	ROM 95880	ROMT 14925	Guyana	Takutu River drainage	PP886081	PP891396	PP944603	PP944609
Trichomycterinae— *Eremophilus* lineage	Guianensis	*T.* sp. 'Kusad Mountain'	ROM 95880	ROMT 14926	Guyana	Takutu River drainage	PP886080	—	PP944602	PP944610

Table 1. Continued.

Subfamily	Clade	Species	Cat #	Tissue	Country	Drainage	*16s*	*coI*	*cytb*	*rag2*
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T.* sp. 'Kusad Mountain'	ROM 95880	ROMT 14927	Guyana	Takutu River drainage	—	PP891394	PP944604	PP944611
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	AUM 62902	AUFT 2186	Guyana	Potaro River drainage	MT025521	MT017631	MT017617	MT017603
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	AUM 83790	ROMT 06185	Guyana	Mazaruni River drainage	MT025537	MT017646	MT017618	—
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	AUM 83790	ROMT 06186	Guyana	Mazaruni River drainage	MT025538	MT017647	MT017619	—
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	AUM 89932	ROMT 12696	Guyana	Potaro River drainage	MT025539	MT017648	MT017620	MT017600
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	AUM 91392	ROMT 15527	Guyana	Potaro River drainage	MT025540	MT017649	MT017621	MT017601
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	ROM 91500	ROMT 15575	Guyana	Potaro River drainage	MT025541	MT017650	MT017622	MT017602
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. cf. guianensis*	LBP 17444	LBPV 69015	Guyana	Potaro (Essequibo)	KY807251	KY857974	KY858043	—
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. conradi*	AUM 67137	AUFT 10212	Guyana	Ireng River drainage	MT025529	MT017638	—	MT017604
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. conradi*	AUM 67137	AUFT 10213	Guyana	Ireng River drainage	MT025530	MT017639	—	—
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. conradi*	AUM 67194	AUFT 10294	Guyana	Ireng River drainage	MT025533	MT017642	—	—
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. conradi*	AUM 91436	ROMT 15595	Guyana	Potaro River drainage	MT025542	MT017651	MT017623	MT017605
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. guianensis*	AUM 62932	AUFT 6563	Guyana	Potaro River drainage	—	MT017633	MT017625	MT017606
Trichomycterinae—*Eremophilus* lineage	Guianensis	*T. guianensis*	AUM 63677	AUFT 2110	Guyana	Potaro River drainage	MT025520	MT017630	MT017624	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis amazonicus*	LBP 11003	LBPV50532	Brazil	Mamoré (Madeira)	KY807212	KY857936	KY858017	KY858175
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis amazonicus*	LBP 16129	LBPV 66849	Brazil	Tapajós (Amazonas)	KY807225	KY857948	—	KY858181
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis boitata*	LBP 14546	LBPV 60865	Brazil	Jacuí (Laguna dos Patos)	KY807272	KY857994	KY858063	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis cf. amazonicus*	UFRJ 9943	UFRJ 9943	Brazil	Rio Morcego	MF434082	MK123683	MK123705	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis cf. eichhorniarum*	LBP 1916	LBPV 14038	Brazil	Paraguay (Paraná)	KY807220	KY857943	—	KY858179
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis cf. eichhorniarum*	LBP 7667	LBPV 36473	Brazil	Aquidauana (Paraguay)	KY807216	KY857940	KY858021	KY858177
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis cf. parkoi*	LBP 5407	LBPV 27103	Brazil	Jari (Amazonas)	KY807221	KY857944	KY858024	—

Table 1. Continued.

Subfamily	Clade	Species	Cat #	Tissue	Country	Drainage	*16s*	*coI*	*cytb*	*rag2*
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis cf. ramiroi*	LBP 15293	LBPV 63262	Brazil	Paranã (Tocantins)	KY807276	KY857998	—	KY858215
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis eichhorniarum*	LBP 4686	LBPV 24825	Brazil	Paraguay (Paraná)	KY807215	KY857939	KY858020	KY858176
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis goya*	LBP 17137	LBPV 68599	Brazil	Dos Couros (Tocantins)	KY807223	KY857946	—	KY858180
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis goya*	LBP 19465	LBPV 77969	Brazil	das Brancas (Tocantins)	KY807278	KY858000	—	KY858217
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis herberti*	LBP 2442	LBPV 16211	Brazil	Araguaia (Tocantins)	KY807211	KY857935	KY858016	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis herberti*	LBP 676	LBPV 8028	Brazil	Pirai (Paraná)	KY807224	KY857947	KY858025	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis parahybae*	LBP 10703	LBPV 49719	Brazil	Macabú (Atlantic)	KY807219	—	KY858023	KY858178
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis parkoi*	LBP 14153	LBPV 59188	Brazil	Tapajós (Amazonas)	KY807213	KY857937	KY858018	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*Ituglanis parkoi*	LBP 7995	LBPV 37376	Brazil	Arinos (Tapajós)	KY807226	KY857949	KY858026	—
Trichomycterinae—*Eremophilus* lineage	*Ituglanis*	*T. cf. conradi*	AUM 51758	AUFT 4743	Suriname	Maroni River drainage	MT025522	MT017632	—	—
Trichomycterinae—*Eremophilus* lineage	Mutisii	*Eremophilus mutisii*	no voucher	ANSP11306	Colombia	Magdalena	KY807207	KY857931	—	KY858171
Trichomycterinae—*Eremophilus* lineage	Mutisii	*Trichomycterus cachiraensis*	LBP 19832	LBPV 77943	Colombia	Magdalena	KY807248	KY857971	—	KY858195
Trichomycterinae—*Eremophilus* lineage	Mutisii	*Trichomycterus sandovali*	LBP 19833	LBPV 77947	Colombia	Magdalena	KY807261	KY857985	KY858052	KY858205

Table 2. Primers used for polymerase chain reactions (PCR).

Locus	Primer	Primer sequence 5′–3′	Source
16s	16Sa-L	ACGCCTGTTTATCAAAAACAT	Palumbi (1996)
	16Sb-H	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
coI	FishF1	TCAACCAACCACAAAGACATTGGCAC	Ward et al. (2005)
	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	Ward et al. (2005)
cytb	Cytb Siluri F	CCA CCG TTG TAA TTC AAC TA	Villa-Verde et al. (2012)
	Cytb Siluri R	GAT TAC AAG ACC GGC GCT TT	Villa-Verde et al. (2012)
rag2—step one	164F	AGCTCAAGCTGCGYGCCAT	Oliveira et al. (2011)
	RAG2-R6	TGRTCCARGCAGAAGTACTTG	Lovejoy and Collette (2001)
rag2—step two	176R	GYGCCATCTCATTCTCCAACA	Oliveira et al. (2011)
	Rag2Ri	AGAACAAAAGATCATTGCTGGTCGGG	Oliveira et al. (2011)

tree prior (Heath et al., 2014). We used the program BEAUTi to assign model parameters and generate the XML input file for BEAST (Bouckaert et al., 2014). We partitioned our data by gene, and protein-coding genes were partitioned by codon according to the following scheme: (1,2) + 3. To identify the best fitting substitution model under a Bayesian framework, we used the program bModelTest (Bouckaert and Drummond, 2017). We included the only fossil that has been confirmed as a member of the Trichomycteridae as a primary calibration (Bogan and Agnolin, 2009), and although it cannot be accurately diagnosed using morphology to a generic level, we make an assumption that it belongs to the *Eremophilus* lineage (most likely the *T. areolatus* clade) based on biogeographic hypotheses described by Bogan and Agnolin (2009) as well as the recovery of a southern Andes clade of trichomycterines (consistent with the *T. areolatus* clade) by Fernández et al. (2021). This fossil was discovered in the Monte Hermoso formation (aged 5.3–4.5 Ma) near Bahía Blanca, Argentina (Tomassini et al., 2013). To treat our fossil as a branch tip, we toggled the tip dates parameter and assigned the fossil taxon as a tip dated 5.2 Ma. We used an uncorrelated lognormal relaxed molecular clock with default values. We set the following required parameters to estimate divergence times under the FBD model. To create a proper distribution for the clock prior, we set the ucldMean to an exponential prior density with a mean of ten. We used a normal prior restricted on the interval [0.01, 0.19] for diversification rate based on the estimated diversification rate of Neotropical otophysan fishes (Miller and Román-Palacios, 2021). We used an uninformative prior (uniform [0, 1]) for turnover, as it is difficult to calculate, but is related to derivations of the other parameters (Barido-Sottani et al., 2018). We used an exponential prior with a mean of 0.2 on the interval [0, 1] for sampling proportion (i.e., the likelihood of sampling a fossil of an extant taxon within the *Eremophilus* lineage is probably low when considering only one fossil has been recovered for what is over 120 presently described extant species). Finally, for ρ we used a beta prior (alpha = 10, beta = 20, [0.01, 0.99]) based on an assumption that roughly 33% ([17.9%, 47.9%; 95%CI]) of all extant species (including those undescribed) within the *Eremophilus* lineage are included in this tree. Additionally, we constrained the monophyly of all *Eremophilus* lineage members excluding the *E. mutisii* clade, making that clade the outgroup for this analysis. We ran two independent (MCMC) chains for $5*10^7$ generations. We sampled parameters and trees every 2,500 generations. We checked sufficient mixing of parameters (ESS > 200) for both independent runs as well as the combined run using Tracer v1.7 (Rambaut et al., 2018). We combined trees from both runs using LogCombiner v1.10.4 with a 10% burn-in resampled at an interval of 5,000 (Suchard et al., 2018). Because the fossil was only included to inform the FBD model and could attach to any lineage, we then pruned it using the FullToExtantTreeConverter plug-in in BEAUTi (Bouckaert et al., 2014; Barido-Sottani et al., 2018). We input combined trees into TreeAnnotater v1.10.4 to obtain the maximum credibility tree and posterior probabilities (Suchard et al., 2018). We then visualized the tree with a geological timescale in R (R Core Team, 2022) using the *strap* package (Bell and Lloyd, 2015). We assessed support for relationships with posterior probability values ≥ 0.95 considered strong support and values < 0.95 considered not supported.

RESULTS

The gene trees consistently recovered the reciprocal monophyly of all species (except for *Trichomycterus conradi* in *rag2*); however, there is topological discordance among the gene trees, and interspecific relationships were generally unable to be resolved (Fig. 3). The *coI* analysis recovers *T.* sp. 'Kusad Mountain' in a polytomy with *T. conradi* and an unsupported grouping of *T. guianensis* and *T.* sp. 'Ireng, spotted' (PP = 0.74), and this polytomy is recovered as a branch in a polytomy with *T. cf. guianensis* and *T.* sp. 'Mazaruni, plain.' For the *cytb* analysis, *T.* sp. 'Kusad Mountain' is recovered as sister to all other lineages (PP = 1.00), which are recovered as a polytomy consisting of *T. conradi*, a strongly supported clade (PP = 1.00) containing *T. guianensis* and *T.* sp. 'Potaro, elongate,' and another strongly supported clade (PP = 1.00) containing *T.* sp. 'Mazaruni, plain' and *T. cf. guianensis*. For the *16s* analysis, *T.* sp. 'Potaro, elongate' was recovered as sister to the rest of the clade (PP = 1.00), and a clade containing *T. guianensis* and *T.* sp. 'Ireng, spotted' was recovered as sister to a clade containing *T. conradi*, *T.* sp. 'Mazaruni, plain,' *T.* sp. 'Kusad Mountain,' and *T. cf. guianensis* (PP = 0.95) in which a grouping of two populations of *T. conradi* that had weak support (PP = 0.71) was recovered with weak support as sister to the remainder of the clade (PP = 0.92), and *T.* sp. 'Mazaruni, plain' was recovered as sister to *T.* sp. 'Kusad Mountain' + *T. cf. guianensis*, but the relationship between *T.* sp. 'Kusad Mountain' and *T. cf. guianensis* is not well supported (PP = 0.73). The *rag2* gene tree consists of a polytomy of *T. conradi* (Potaro), a supported sister relationship of *T.* sp. 'Mazaruni, plain'

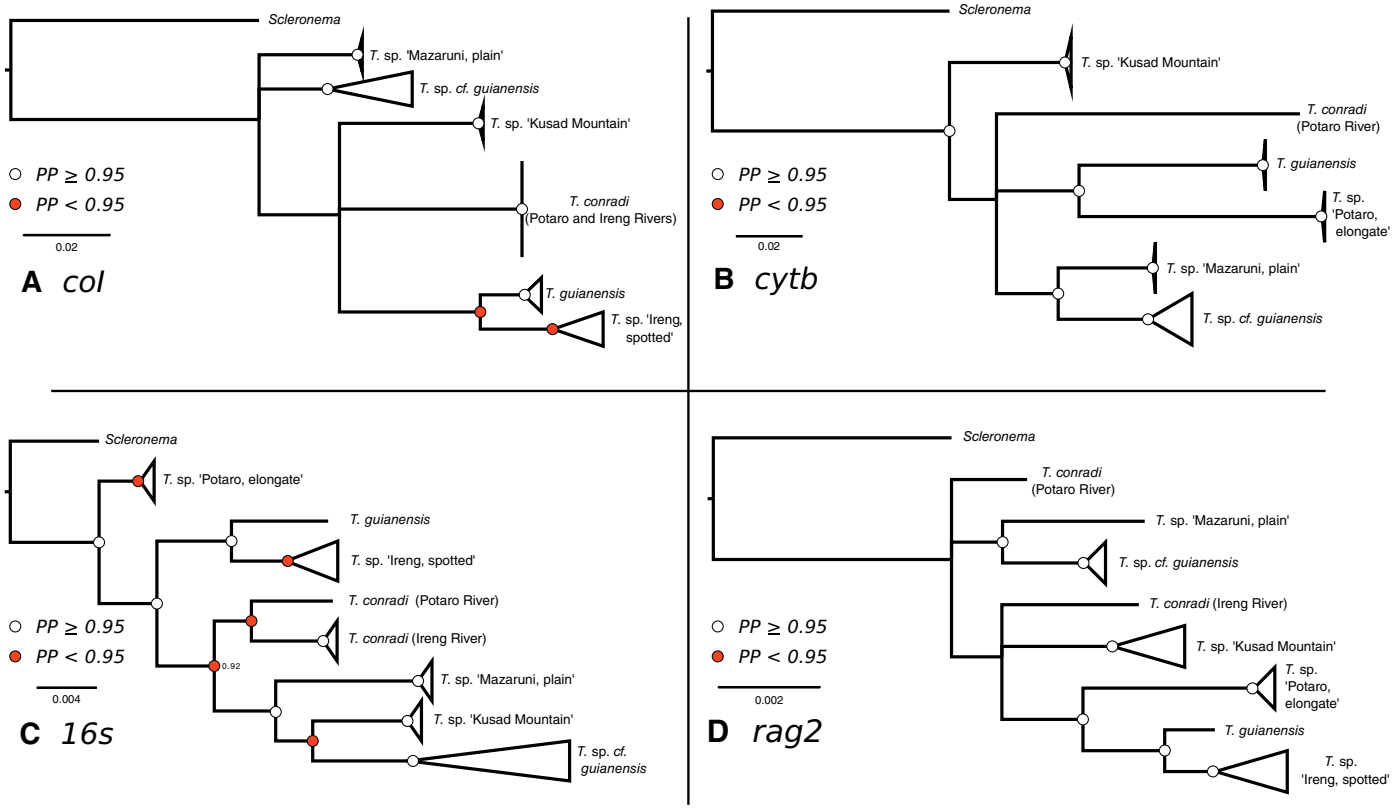

Fig. 3. Gene trees for (A) *coI*, (B) *cytb*, (C) *16s*, and (D) *rag2*. Red circles represent posterior probabilities less than 0.95, and white circles represent posterior probabilities greater than or equal to 0.95.

and *T. cf. guianensis*, and a polytomy of *T.* sp. 'Kusad Mountain,' *T. conradi* (Ireng), and a well-supported (PP = 1.00) clade of *T.* sp. 'Potaro, elongate' sister to a well-supported (PP = 1.00) sister group of *T. guianensis* and *T.* sp. 'Ireng, spotted.'

The concatenated analysis recovered all species as monophyletic and a topology consisting of two subclades within the *Trichomycterus guianensis* clade (Fig. 4). The *T. guianensis* subclade strongly supports a sister relationship of *T. guianensis* and *T.* sp. 'Ireng, spotted' (PP = 1.00) with *T.* sp. 'Potaro, elongate' sister to that grouping (PP = 1.00). The *T. cf. guianensis* subclade consists of *T. cf. guianensis* sister to *T.* sp. 'Mazaruni, plain' (PP = 1.00), with a weakly supported relationship to *T. conradi* (PP = 0.52), and weak support for *T.* sp. 'Kusad Mountain' sister to all members of the subclade (PP = 0.78).

The time calibration recovered the same two subclades within the *T. guianensis* clade as the concatenated analysis (PP = 0.99), but a slightly different topology for the *T. cf. guianensis* subclade (Fig. 5), with the only change in this analysis being that *T. conradi* is recovered as sister to the remainder of the *T. cf. guianensis* subclade (PP = 0.97). The estimates of the time calibration place the oldest divergence within the *Eremophilus* lineage at 18.48 Ma ([5.52, 37.57] 95% HPD). The focal group of this study, the *T. guianensis* clade, is estimated to have diverged at 17.37 Ma ([5.02, 35.04] 95% HPD), with the two subclades diverging at 14.69 Ma ([3.93, 30.2] 95% HPD). Within the *T. guianensis* subclade, *T.* sp. 'Potaro, elongate' is estimated to have diverged at 9.43 Ma ([1.72, 20.02] 95% HPD) and *T. guianensis* and *T.* sp. 'Ireng, spotted' separated at 3.77 Ma ([0.64, 8.63] 95% HPD). Within the *T. cf. guianensis* subclade, *T. conradi* is estimated to have diverged at 11.75 Ma ([2.8, 24.64] 95% HPD) with the Ireng River and Potaro River populations separating at 1.97 Ma ([0.2, 4.94] 95% HPD). *Trichomycterus* sp. 'Kusad Mountain' is estimated to have diverged at 9.4 Ma ([2.14, 20.25] 95% HPD), and *T.* sp. 'Mazaruni, plain' and *T. cf. guianensis* separated at 5.23 Ma ([0.85, 11.62] 95% HPD) with the Potaro River and Mazaruni River populations of *T. cf. guianensis* separating at 1.88 Ma ([0.36, 4.27] 95% HPD).

DISCUSSION

All four gene trees recovered the reciprocal monophyly of each species except for *Trichomycterus conradi* in the *rag2* analysis, but because reciprocal monophyly of this species was recovered in the *coI* analysis (PP = 1.00), and weakly supported in the *16s* analysis (PP = 0.71), we believe the non-monophyletic relationship in *rag2* to be due to the overall low level of disparity of sequences within this locus. This is expected given nuclear genes are known to have lower substitution rates compared to mitochondrial loci (see Supplemental File S4; see Data Accessibility). Akin (2022) could not find morphological differences between the two populations that could be used describe the Ireng population as a new species; however, museum collections of this species are incredibly rare ($n = 14$ specimens known to the authors) and more sampling in these headwaters is needed. The generally well-supported monophyly of all species recovered by the *coI* tree provides support to the conclusion of Reis and de Pinna (2023) that *coI* may be helpful to identify different species of *Trichomycterus* through DNA

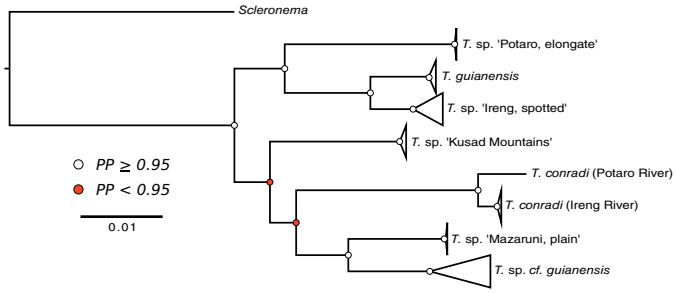

Fig. 4. Concatenated BI tree estimated using MrBayes. Red circles represent posterior probabilities less than 0.95, and white circles represent posterior probabilities greater than or equal to 0.95.

barcoding; however, this should only be used as a supplementary tool alongside morphological evidence whenever specimens are available.

Our multi-locus phylogenetic analyses recovered two subclades within the *Trichomycterus guianensis* clade. When comparing the results from MrBayes and estimated by BEAST, the topologies differ in which lineage is recovered as sister to all other members of the *T. cf. guianensis* subclade. In the MrBayes phylogeny, *T. conradi* is recovered as sister to the rest of the subclade, whereas in the BEAST tree, *T.* sp. 'Kusad Mountain' is sister to the rest of the subclade. This inconsistency may be the result of either limited geographic and species sampling or insufficient phylogenetic informativeness in the four loci used. Genetic samples are lacking from at least three known and two suspected species of *Trichomycterus* from the Pakaraimas, as well as any number of potentially unknown species. Based on the specimens examined by Akin (2022), there do not appear to be any clear synapomorphies that would separate these subclades; however, as more species are sequenced, discovered, and included within these subclades, a more thorough revision of their osteology should be considered.

Our divergence time estimates are roughly 5–10 million years younger than the estimates made by Ochoa et al. (2017) and Costa et al. (2022), but the estimates made in those papers are well within the highest probability distributions (HPDs) reported here. Ochoa et al. (2017) did not report HPDs, and Costa et al. (2022) used a much stronger prior for their secondary time calibration than should be used, potentially resulting in narrower HPDs. The prior used

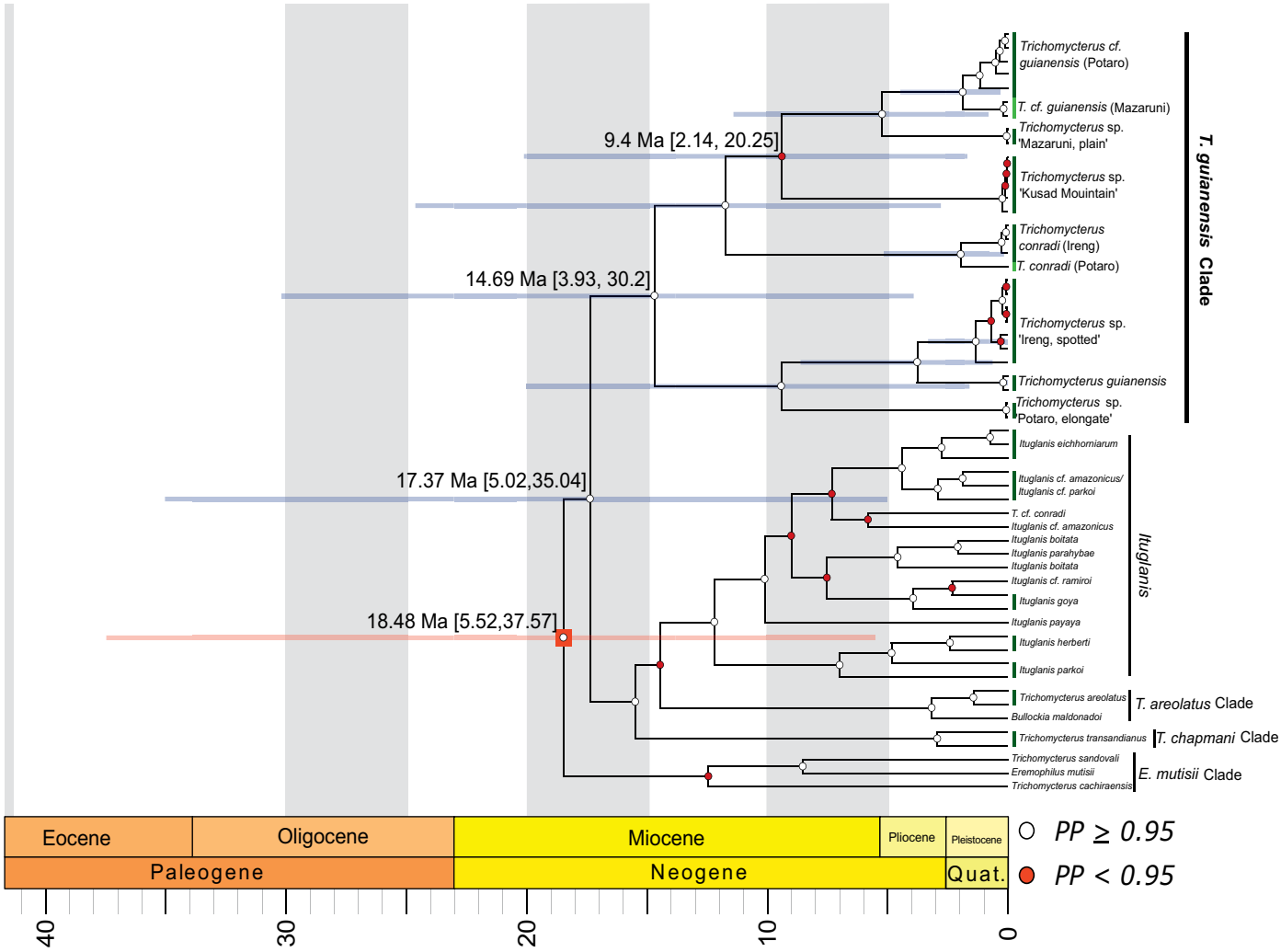

Fig. 5. Time-calibrated tree estimated using BEAST2 of the *Eremophilus* lineage. Red circles represent posterior probabilities less than 75, gray circles represent posterior probabilities from 75–95, and black circles represent posterior probabilities greater than 95; 95% HPDs represented by blue bars, and the red bar represents the 95% HPD for the most recent common ancestor to the *Eremophilus* lineage.

by Costa et al. (2022) was based on an age estimated from Betancur-R et al. (2015) by using mean node ages of a Bayesian tree produced by Betancur-R et al. (2013) as secondary calibration points. In the original time calibration, the HPDs of nodes nearby the Trichomycteridae (e.g., the Callichthyidae–Nematogenyidae node) span 30–40 million years (Betancur-R et al., 2013); therefore, the prior distribution for the Trichomycteridae should certainly be much larger than the roughly six million years of space that was sampled by the model implemented by Costa et al. (2022). Our more relaxed calibration results in a wider range of HPDs of node ages than in Costa et al. (2022). See Supplemental File S5 (see Data Accessibility) for further discussion on the use of time calibrations within the Trichomycterinae.

Our results support the claim by Ochoa et al. (2017) that the *T. guianensis* clade likely diverged roughly 17.37 Ma, just prior to the uplift of the Northern Andes. Additionally, we recover the oldest common ancestor of the *Eremophilus* lineage at 18.48 Ma. This is concurrent with a geologically busy time in South American history, appearing just after the Incaic phase and diverging during the Quecha phase of the Andean Orogeny, with these events setting the stage for the development of the modern river drainages of South America (Albert et al., 2018). At this time, the major rivers of Guyana were much different, with the Proto-Berbice skirting the southern edge of the Pakaraima Mountains, en route to the mouth of the modern day Berbice River (Lujan and Armbruster, 2011), the Proto-Essequibo occupying much of the Essequibo north of Apoteri, and the proposed Grand Pakaraima River draining higher elevation surfaces and exiting to the north of the Pakaraimas. For the purposes of our discussion, we will refer to populations on the escarpment as upland and below the escarpment as lowland, but note that there are high energy stream segments (riffles and rapids) throughout most of the rivers in the region.

The placement of *T.* sp. 'Kusad Mountain,' a species endemic to Kusad Mountain (Takutu River, Amazon River basin), as sister to *T.* sp. 'Mazaruni, plain' + *T. cf. guianensis*, which are found in the upper Potaro (Essequibo River basin), upper Mazaruni (Essequibo River basin), and upper Caroni (Orinoco River basin) Rivers suggests a complicated evolutionary history for the group. One hypothesis for this set of relationships is that a lowland common ancestor within the Proto-Berbice and/or Proto-Essequibo gave rise to descendant populations in both the Pakaraima plateau and Kusad Mountain, which are separated by the Takutu graben. To further test this hypothesis, more sampling in the intervening region is needed. Most species of *Trichomycterus* in the Guiana Shield have been described in the last 25 years, and significant portions of the highlands of the Guiana Shield (e.g., the Kanuku Mountains, small outcrops and mountains along the borders of Guyana, Venezuela, and Brazil, and small outcrops east of the main Pakaraimas and flanking the Essequibo) remain unsampled. Thus far, no *Trichomycterus* have been found in the Kanuku Mountains, but the only streams potentially elevated enough to support *Trichomycterus* that have been explored are Moco Moco Creek and Kumu Creek (Takutu River drainage), although these collections were likely not high enough upstream based on the species captured (de Souza et al., 2012, 2020; Taphorn et al., 2022).

We interpret our time-calibration results as a geodispersal of a lowland ancestor of the *T. guainensis* clade into the Proto-Berbice and Proto-Essequibo ~17.4 Ma. This would have allowed the range expansion of *Trichomycterus* throughout the lowlands of this region, providing access to the Pakaraima Mountains as well as Kusad Mountain, and this wide-ranging *T. guianensis* clade then diverged into two major subclades ~14.7 Ma. We further interpret two separate geodispersal events into the Grand Pakaraima River, the first being the *T. guianensis* subclade sometime between ~14.7 and ~9.4 Ma, and the second being *T. cf. guianensis* + *T.* 'Mazaruni, plain' sometime between ~14.7 and ~5.2 Ma, as well as a separate geodispersal event by the *T. conradi* lineage into the Proto-Essequibo River ~11.8 Ma.

At approximately 9.4 Ma, concurrent splits within both subclades indicate a potential large-scale event that isolated the uplands, coinciding with the uplift of the Northern Andes and formation of the modern Amazon River. One split was the aforementioned isolation of the *Trichomycterus* sp. 'Kusad Mountain' from the rest of the *T. cf guianensis* subclade. In the *T. guianensis* subclade, *T.* sp. 'Potaro, elongate' is separated from *T. guianensis* + *T.* sp. 'Ireng, spotted.' It is interesting to note that *T.* sp. 'Potaro, elongate' lives in sluggish habitats within the highlands, perhaps preserving a lowland ecotype and morphotype in this otherwise upland clade.

The resultant sister relationship of *T.* sp. 'Ireng, spotted' and *T. guianensis* suggests that at this time, the modern upper Ireng River would have joined the modern Potaro as part of the Grand Pakaraima River, while at least the middle Ireng River would have flowed south into the Proto-Berbice (Fig. 1; see Nascimento et al., 2019, but note that the Ireng River is called Rio Maú). The relationship of the upper Ireng and Potaro is also supported by the sister group relationship of the loricariid catfish genera *Yaluwak* (Ireng) and *Corymbophanes* (Potaro) and is possibly corroborated geologically by waterfalls that result from a series of uplifts beginning ~30–15 Ma (Lujan et al., 2020).

We additionally recover three relationships aged roughly 3.8–1.9 Ma that suggest the breakup of the upper Grand Pakaraima River just before the consolidation of the Ireng, Potaro, and Mazaruni Rivers into their modern channels, consistent with the probable Pliocene uplift and tilting of the Rupununi Surface (McConnell [1968] refers to the uplift as end-Tertiary). We discussed above the relationships of *T.* sp. 'Ireng, spotted' + *T. guianensis*, which would indicate a capture of the upper Ireng away from the Grand Pakaraima River approximately 3.8 Ma. This was followed by a potential final consolidation of the major river basins ~1.9 Ma as suggested by the split of *T. cf. guianensis* in the Mazaruni and Potaro as well as the split of the Ireng and Potaro populations of *T. conradi*. This result mirrors the pattern seen in the upland and lowland Inland Guianas Clade of *Gymnotus*, which were cautiously estimated to have diverged ~1.5 Ma (Lehmberg et al., 2018).

After the capture of the upper Ireng evidenced by the split of *T.* sp. 'Ireng, spotted' and *T. guianensis* (~3.8 Ma), the middle Ireng would have likely already flowed into what is now the Rupununi River around Massara as part of the Proto-Essequibo during the breakup of the Proto-Berbice (Fig. 6; Nascimento, 2020). The Ireng River is considered to be the last river to have been captured by the Rio Branco (Lujan and Armbruster, 2011; Nascimento et al., 2019), and

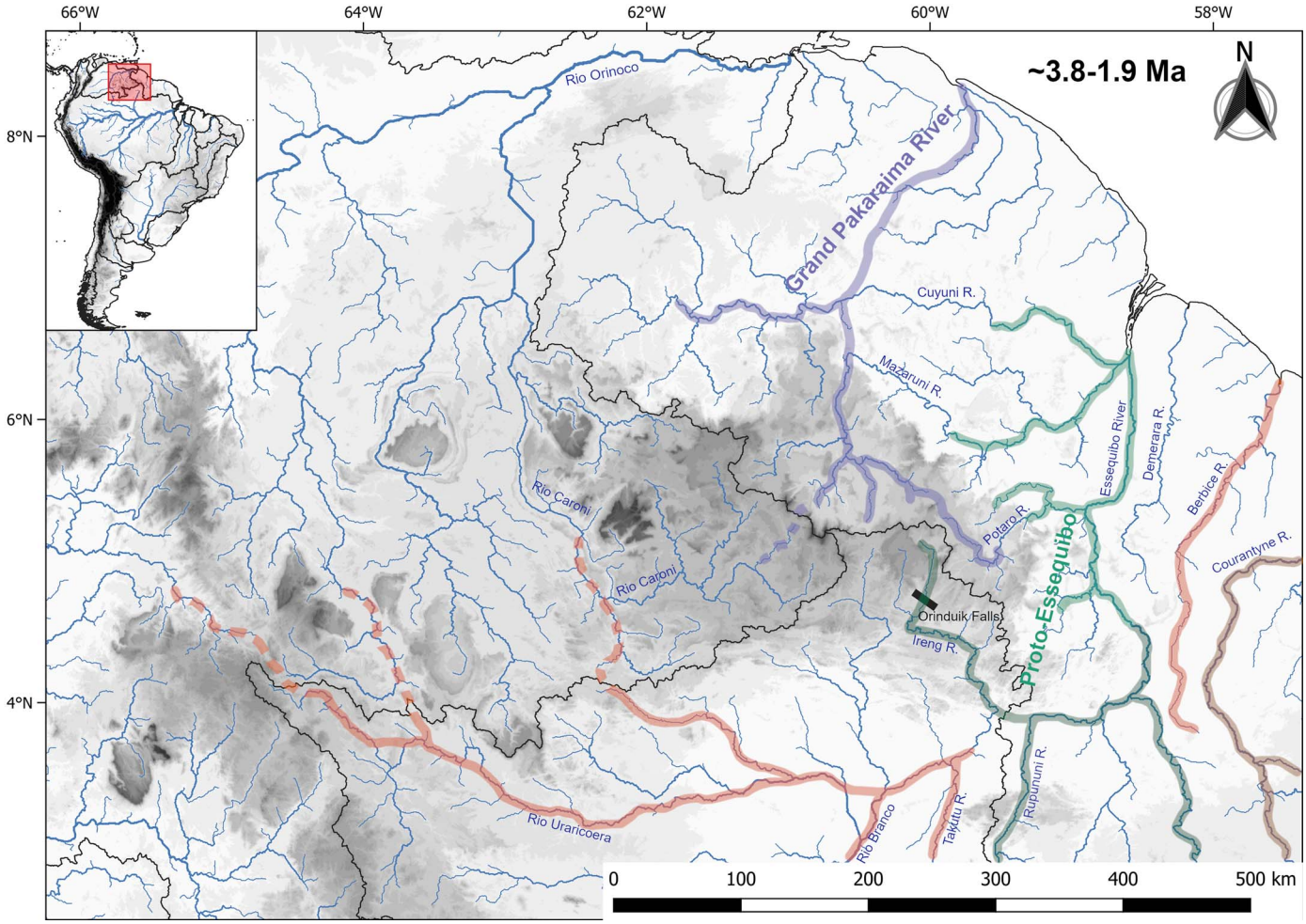

Fig. 6. Map of the rivers of the Guiana Shield with hypothesized routes from ~3.8–1.9 Ma of the remnants of the Proto-Berbice (red), the Proto-Essequibo with captured portions of the former Proto-Berbice (green), and Grand Pakaraima River (purple).

Nascimento (2020) indicates two separate places of possible stream capture: just ENE of Normandia, Brazil and roughly 10 km south of that point. Given the split between Ireng and Potaro *T. conradi* at ~1.9 Ma, we posit that *T. conradi* may have been able to move between the Ireng and Potaro within the Proto-Essequibo following consolidation of the upper and middle Ireng Rivers ~3.8 Ma and prior to the final consolidation into modern channels ~1.9 Ma. In this scenario, the distribution of *T. conradi* in the Ireng and Potaro is due to geodispersal and then vicariance from the capture of the modern Ireng from the Essequibo, whereas its sympatric congeners *T. guianensis* and *T.* sp. 'Ireng, spotted' are the result of vicariance from an earlier capture of the upper Ireng from the Grand Pakaraima River.

Although there is some possible population structure in *T. cf. guianensis* across the Mazaruni and Potaro Rivers, the branch lengths are quite small, suggesting that there may continue to be modern connectivity between these headwaters. There are anecdotal reports of connections during excessively wet seasons, and there are fissures between headwaters across the plateau where the rivers could potentially connect (Hayes et al., 2020). Flow through these interbasin fissures would be consistent with McConnell's (1968) suggestion that the east-flowing portions of the high river basins have not fully consolidated, but there would need to be direct evidence for such connections in the movement of fishes. Alternatively, the branch lengths may be small due to slow evolutionary change between separated populations.

Studies focusing on the deeper biogeographical relationships within the Trichomycterinae remain to be conducted, but we propose here a testable hypothesis for the biogeography of the *Eremophilus* lineage. Ochoa et al. (2020) recovered an undescribed trichomycterid from the Paria Peninsula of northern Venezuela as the sister to all other species of the Trichomycterinae. Similarly, within the *Eremophilus* lineage, a clade endemic to the Magdalena River in Colombia was recovered as sister to the rest of the lineage. Both of these early-branching lineages are restricted north of the Northern Andes. Pairing this geographic data and phylogeny with our time calibration, we hypothesize that the *Eremophilus* lineage rapidly diversified with the uplift of the Andes (i.e., uplands as a cradle *sensu* Rahbek et al. [2019]), but also that any ancestral species that would have inhabited the sub-Andean foreland would have been subjected to drastic habitat changes caused by the rapid transition from Lago Pebas to the modern Amazon River, as well as marine incursions, while those older lineages north of (and on) the Andes were preserved (i.e., uplands as a museum *sensu* Rahbek et al. [2019]). This scenario suggests

that members of the *Eremophilus* lineage may have only recently begun to recolonize and diversify in the lowlands of the Amazon River basin. This scenario may be entirely different from the diversification patterns within the *Trichomycterus sensu stricto* lineage, which are predominately represented in the Brazilian Shield and were subject to a different suite of geologic events. Further investigations of both lineages may lead to a better understanding of the impact of substantial geologic events (such as the Andean Orogeny) on the diversification, origins, and biogeography of major South American fish lineages.

Today, many of the rivers of Guyana are being dramatically degraded due to gold mining (mostly through sedimentation and mercury pollution from gold amalgamation) and will be impacted by regional drilling as oil interests increase in the region (Panelli, 2019; Montaña et al., 2021). Insights into present biogeographic patterns and the ability to predict future river connections may help guide policy makers in making difficult decisions pertaining to irrigation, plumbing, hydroelectric, and the regulation of fisheries (Winemiller et al., 2016). *Trichomycterus* of the Guiana Shield are found in high elevation headwaters with few other fishes, and many of these upland tributaries have yet to be sampled. The trichomycterids yet to be discovered here may shed light on the complex biogeographical story of the ancient Pakaraima Mountains, and those distributed throughout all of South America may lend insights into the constant reshuffling of the headwaters of major South American rivers. The rapid and recent diversification of this family makes it ideal to aid in the study of the processes and patterns of evolution in Neotropical freshwater fishes and provides ample opportunities to build upon evolutionary ecology hypotheses related to factors such as stream gradient, elevation, and river chemistry.

DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyologyandherpetology.org/i2024002. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

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LITERATURE CITED

Akin, D. R. 2022. Descriptions and phylogenetic relationships of the Guiana Shield Pencil Catfishes. Unpubl. M.S. thesis, Auburn University, Auburn, Alabama. Available at https://etd.auburn.edu//handle/10415/8300

Albert, J. S., P. Val, and C. Hoorn. 2018. The changing course of the Amazon River in the Neogene: center stage for Neotropical diversification. Neotropical Ichthyology 16:e180033.

Armbruster, J. W. 2011. Global catfish biodiversity. American Fisheries Society Symposium 77:15–37.

Armbruster, J. W., N. K. Lujan, and D. D. Bloom. 2021. Redescription of the Guiana Shield darter species *Characidium crandellii* and *C. declivirostre* (Crenuchidae) with descriptions of two new species. Ichthyology & Herpetology 109:102–122.

Arratia, G. 1998. *Silvinichthys*, a new genus of trichomycterid catfishes from the Argentinian Andes, with redescription of *Trichomycterus nigricans*. Ichthyological Exploration of Freshwaters 9:347–370.

Arratia F., G., A. Chang G., S. Menu-Marque, and G. Rojas M. 1978. About *Bullockia* gen. nov., *Trichomycterus mendozensis* n. sp. and revision of the family Trichomycteridae (Pisces, Siluriformes). Studies on Neotropical Fauna and Environment 13:157–194.

Barido-Sottani, J., V. Bošková, L. D. Plessis, D. Kühnert, C. Magnus, V. Mitov, N. F. Müller, J. PečErska, D. A. Rasmussen, C. Zhang, A. J. Drummond, T. A. Heath, O. G. Pybus, T. G. Vaughan, and T. Stadler. 2018. Taming the BEAST—a community teaching material resource for BEAST 2. Systematic Biology 67:170–174.

Baskin, J. N. 1973. Structure and relationships of the Trichomycteridae. Unpubl. Ph.D. diss., City University of New York, New York.

Bell, M. A., and G. T. Lloyd. 2015. strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. Palaeontology 58:379–389.

Berrangé, J. P. 1975. The geomorphology of southern Guyana with special reference to the development of planation surfaces. Anais Décima Conferência Geológica Interguianas 1:804–824.

Betancur-R, R., R. E. Broughton, E. O. Wiley, K. Carpenter, J. A. López, C. Li, N. I. Holcroft, D. Arcila, M. Sanciangco, J. C. Cureton II, F. Zhang, T. Buser, M. A. Campbell, J. A. Ballesteros ... **G. Ortí.** 2013. The tree of life and a new classification of bony fishes. PLoS Currents Tree of Life. 2013 Apr 18. Edition 1.

Betancur-R, R., G. Ortí, and R. A. Pyron. 2015. Fossil-based comparative analyses reveal ancient marine ancestry erased by extinction in ray-finned fishes. Ecology Letters 18:441–450.

Bogan, S., and F. L. Agnolin. 2009. Primer registro fósil de la familia Trichomycteridae (Teleostei: Siluriformes; Plioceno) en la Formación Monte Hermoso, Argentina. Revista del Museo Argentino de Ciencias Naturales 11:193–198.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10:e1003537.

Bouckaert, R. R., and A. J. Drummond. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evolutionary Biology 17:42.

Costa, W. J. E. M., and F. A. Bockmann. 1993. Un nouveau genre néotropical de la famille des Trichomycteridae

(Siluriformes: Loricarioidei). Revue Française d'Aquariologie et Herpetologie 20:43–46.

Costa, W. J. E. M., J. L. O. Mattos, W. M. S. Sampaio, P. Giongo, F. B. de Almeida, and A. M. Katz. 2022. Phylogenetic relationships of a new catfish of the genus *Trichomycterus* (Siluriformes, Trichomycteridae) from the Brazilian Cerrado, and the role of Cenozoic events in the diversification of mountain catfishes. Zoosystematics and Evolution 98:151–164.

Crawford, F. D., C. E. Szelewski, and G. D. Alvey. 1985. Geology and exploration in the Takutu Graben of Guyana Brazil. Journal of Petroleum Geology 8:5–36.

Eigenmann, C. H. 1909. The Fresh Water Fishes of Patagonia and an Examination of the Archiplata-Archhelonis Theory. The University, Princeton, New Jersey.

Eigenmann, C. H. 1917. Descriptions of sixteen new species of Pygidiidæ. Proceedings of the American Philosophical Society 56:690–703.

Fernández, L. 2017. Family Trichomycteridae—pencil catfishes, torrent catfishes, and parasitic catfishes (Candirus), p. 311–322. *In*: Field Guide to the Fishes of the Amazon, Orinoco and Guianas. P. van der Sleen and J. S. Albert (eds.). Princeton University Press, Princeton, New Jersey.

Fernández, L., J. Arroyave, and S. A. Schaefer. 2021. Emerging patterns in phylogenetic studies of trichomycterid catfishes (Teleostei, Siluriformes) and the contribution of Andean diversity. Zoologica Scripta 50:318–336.

Fricke, R., W. N. Eschmeyer, and J. D. Fong. 2023. Eschmeyer's Catalog of Fishes: Genera/Species by Family/Subfamily. Available at https://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp

Hartmann, L. A. 2002. The Mesoproterozoic Supercontinent Atlantica in the Brazilian Shield—review of geological and U-Pb Zircon and Sm-Nd isotopic evidence. Gondwana Research 5:157–163.

Hayes, M. M., H. J. Paz, C. C. Stout, D. C. Werneke, and J. W. Armbruster. 2020. A hotspot atop: rivers of the Guyana Highlands hold high diversity of endemic pencil catfish (Teleostei: Ostariophysi: Siluriformes). Biological Journal of the Linnean Society 129:862–874.

Heath, T. A., J. P. Huelsenbeck, and T. Stadler. 2014. The fossilized birth–death process for coherent calibration of divergence-time estimates. Proceedings of the National Academy of Sciences of the United States of America 111: E2957–E2966.

Hubert, N., and J.-F. Renno. 2006. Historical biogeography of South American freshwater fishes. Journal of Biogeography 33:1414–1436.

Huelsenbeck, J. P., and F. Ronquist. 2005. Bayesian analysis of molecular evolution using MrBayes, p. 183–226. *In*: Statistical Methods in Molecular Evolution. Statistics for Biology and Health. R. Nielsen (ed.). Springer, New York.

von Humboldt, A. 1805. Mémoire sur l'*Eremophilus* et *Astroblepus*, deux nouveaux genres de l'ordre des apodes. Voyage de Humboldt et Bonpland, Deuxième partie. Observations de Zoologie et d'Anatomie Comparée. F. Schell, G. Dufour et Companie, Paris 1:17–20.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30:3059–3066.

Katz, A. M., M. A. Barbosa, J. L. de O. Mattos, and W. J. E. M. da Costa. 2018. Multigene analysis of the catfish genus *Trichomycterus* and description of a new South American trichomycterine genus (Siluriformes, Trichomycteridae). Zoosystematics and Evolution 94:557–566.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Lehmberg, E. S., A. A. Elbassiouny, D. D. Bloom, H. López-Fernández, W. G. Crampton, and N. R. Lovejoy. 2018. Fish biogeography in the "Lost World" of the Guiana Shield: phylogeography of the weakly electric knifefish *Gymnotus carapo* (Teleostei: Gymnotidae). Journal of Biogeography 45:815–825.

Lovejoy, N. R., and B. B. Collette. 2001. Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. Copeia 2001:324–338.

Lujan, N. K., and J. W. Armbruster. 2011. The Guiana Shield, p. 211–224. *In*: Historical Biogeography of Neotropical Freshwater Fishes. J. S. Albert and R. E. Reis (eds.). University of California Press, Berkeley, California.

Lujan, N. K., J. W. Armbruster, D. C. Werneke, T. F. Teixeira, and N. R. Lovejoy. 2020. Phylogeny and biogeography of the Brazilian–Guiana Shield endemic *Corymbophanes* clade of armoured catfishes (Loricariidae). Zoological Journal of the Linnean Society 188:1213–1235.

Lujan, N. K., C. A. Cramer, R. Covain, S. Fisch-Muller, and H. López-Fernández. 2017. Multilocus molecular phylogeny of the ornamental wood-eating catfishes (Siluriformes, Loricariidae, Panaqolus and Panaque) reveals undescribed diversity and parapatric clades. Molecular Phylogenetics and Evolution 109:321–336.

Lundberg, J. G., L. G. Marshall, and J. Guerrero. 1998. The stage for Neotropical fish diversification: a history of tropical South American rivers, p. 13–48. *In*: Phylogeny and Classification of Neotropical Fishes. L. R. Malabara, R. E. Reis, R. P. Vari, Z. M. S. Lucena, and C. A. S. Lucena (eds.). EDIPUCRS, Porto Alegre, Brazil.

McConnell, R. B. 1968. Planation surfaces in Guyana. The Geographical Journal 134:506–520.

Méndez-Camacho, K., O. Leon-Alvarado, and D. R. Miranda-Esquivel. 2021. Biogeographic evidence supports the Old Amazon hypothesis for the formation of the Amazon fluvial system. PeerJ 9:e12533.

Miles, C. 1943. On three recently described species and a new genus of pygidiid fishes from Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales 5:367–369.

Miller, E. C., and C. Román-Palacios. 2021. Evolutionary time best explains the latitudinal diversity gradient of living freshwater fish diversity. Global Ecology and Biogeography 30:749–763.

Miller, M., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, p. 1–8. *In*: Proceedings of the Gateway Computing Environments Workshop, New Orleans, Louisiana.

Montaña, C. G., E. Liverpool, D. C. Taphorn, and C. M. Schalk. 2021. The cost of gold: mercury contamination of fishes in a Neotropical river food web. Neotropical Ichthyology 19:e200155.

Nascimento, F. A. 2020. O papel dos processos de rearranjo de drenagem na morfogênese neógena da alta/média bacia hidrográfica do rio Branco. Unpubl. Ph.D. diss., Universidade Federal de Minas Gerais, Brasil. https://repositorio.ufmg.br/handle/1843/46817

Nascimento, F. A., A. A. T. Gomes, A. A. R. Salgado, and S. S. Tavares Júnior. 2019. Evidências de rearranjo de drenagem na Bacia do Alto rio Branco, Amazónia, p. 83–86. *In*: Atas do IX Congresso Nacional de Geomorfologia: Gomorfologia 2019: Que Futuro para a Geomorfologia?

Ochoa, L. E., A. Datovo, C. DoNascimiento, F. F. Roxo, M. H. Sabaj, J. Chang, B. F. Melo, G. S. C. Silva, F. Foresti, M. Alfaro, and C. Oliveira. 2020. Phylogenomic analysis of trichomycterid catfishes (Teleostei: Siluriformes) inferred from ultraconserved elements. Scientific Reports 10:2697.

Ochoa, L. E., F. F. Roxo, C. DoNascimiento, M. H. Sabaj, A. Datovo, M. Alfaro, and C. Oliveira. 2017. Multilocus analysis of the catfish family Trichomycteridae (Teleostei: Ostariophysi: Siluriformes) supporting a monophyletic Trichomycterinae. Molecular Phylogenetics and Evolution 115:71–81.

Oliveira, C., G. S. Avelino, K. T. Abe, T. C. Mariguela, R. C. Benine, G. Ortí, R. Vari, and R. Corrêa e Castro. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolutionary Biology 11:275.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction, p. 205–247. *In*: Molecular Systematics. D. Hillis, C. Moritz, and B. Mable (eds.). Sinauer, Sunderland, Massachusetts.

Panelli, L. F. 2019. Is Guyana a new oil El Dorado? The Journal of World Energy Law & Business 12:365–368.

de Pinna, M. C. C. 1989. A new sarcoglanidine catfish, phylogeny of its subfamily, and an appraisal of the phyletic status of the Trichomycterinae (Teleostei, Trichomycteridae). American Museum Novitates 2950:1–39.

R Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rahbek, C., M. K. Borregaard, A. Antonelli, R. K. Colwell, B. G. Holt, D. Nogues-Bravo, C. M. Rasmussen, K. Richardson, M. T. Rosing, R. J. Whittaker, and J. Fjeldså. 2019. Building mountain biodiversity: geological and evolutionary processes. Science 365:1114–1119.

Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Reis, V., and M. de Pinna. 2023. Diversity and systematics of *Trichomycterus* Valenciennes 1832 (Siluriformes: Trichomycteridae) in the Rio Doce Basin: iterating DNA, phylogeny and classical taxonomy. Zoological Journal of the Linnean Society 197:344–441.

Santos, J. O. S., P. E. Potter, N. J. Reis, L. A. Hartmann, I. R. Fletcher, and N. J. McNaughton. 2003. Age, source, and regional stratigraphy of the Roraima Supergroup and Roraima-like outliers in northern South America based on U-Pb geochronology. GSA Bulletin 115:331–348.

de Souza, L. S., J. W. Armbruster, and D. C. Werneke. 2012. The influence of the Rupununi portal on distribution of freshwater fish in the Rupununi district, Guyana/L'influence du portail du Rupununi sur la distribution des poissons dulcaquicoles dans le district du Rupununi, Guyana. Cybium, International Journal of Ichthyology 36:31–44.

de Souza, L. S., J. W. Armbruster, and P. W. Willink. 2020. Connectivity of Neotropical river basins in the central Guiana Shield based on fish distributions. Frontiers in Forests and Global Change 3:8.

Stadler, T. 2009. On incomplete sampling under birth–death models and connections to the sampling-based coalescent. Journal of Theoretical Biology 261:58–66.

Suchard, M. A., P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evolution 4:vey016.

Taphorn, D. C., J. W. Armbruster, H. López-Fernández, and C. R. Bernard. 2010. Description of *Neblinichthys brevibracchium* and *N. echinasus* from the upper Mazaruni River, Guyana (Siluriformes: Loricariidae), and recognition of *N. roraima* and *N. yaravi* as distinct species. Neotropical Ichthyology 8:615–624.

Taphorn, D. C., E. Liverpool, N. K. Lujan, C. DoNascimiento, D. D. Hemraj, W. G. R. Crampton, M. A. Kolmann, J. P. Fontenelle, L. S. de Souza, D. C. Werneke, M. Ram, D. D. Bloom, B. L. Sidlauskas, E. Holm … **H. López-Fernández.** 2022. Annotated checklist of the primarily freshwater fishes of Guyana. Proceedings of the Academy of Natural Sciences of Philadelphia 168:1–95.

Tomassini, R. L., C. I. Montalvo, C. M. Deschamps, and T. Manera. 2013. Biostratigraphy and biochronology of the Monte Hermoso Formation (early Pliocene) at its type locality, Buenos Aires Province, Argentina. Journal of South American Earth Sciences 48:31–42.

Valenciennes, A. 1832. Nouvelles observations sur le Capitan de Bogota, *Eremophilus mutisii*. *In*: Recueil d'Observations de Zoologie et d'Anatomie Comparée: Faites dans l'Océan Atlantique, dans l'Interieur du Nouveau Continent et dans la Mer du Sud, pendant les Années 1799, 1800, 1801, 1802 et 1803. F. Schoell et G. Dufour.

Villa-Verde, L., H. Lazzarotto, and S. M. Lima. 2012. A new glanapterygine catfish of the genus *Listrura* (Siluriformes: Trichomycteridae) from southeastern Brazil, corroborated by morphological and molecular data. Neotropical Ichthyology 10:527–538.

Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. N. Hebert. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B 360:1847–1857.

Winemiller, K. O., H. López-Fernández, D. C. Taphorn, L. G. Nico, and A. B. Duque. 2008. Fish assemblages of the Casiquiare River, a corridor and zoogeographical filter for dispersal between the Orinoco and Amazon basins. Journal of Biogeography 35:1551–1563.

Winemiller, K. O., P. B. McIntyre, L. Castello, E. Fluet-Chouinard, T. Giarrizzo, S. Nam, I. G. Baird, W. Darwall, N. K. Lujan, I. Harrison, M. L. J. Stiassny, R. A. M. Silvano, D. B. Fitzgerald, F. M. Pelicice … **L. Sáenz.** 2016. Balancing hydropower and biodiversity in the Amazon, Congo, and Mekong. Science 351:128–129.