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Molecular phylogenetics of Neotropical detritivorous fishes of the family Curimatidae (Teleostei: Characiformes)

Bruno F. Melo[a,b,*], Brian L. Sidlauskas[b,c], Kendra Hoekzema[c], Richard P. Vari[b,1], Casey B. Dillman[b,d], Claudio Oliveira[a]

[a] Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil
[b] Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
[c] Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR, USA
[d] Department of Ecology and Evolutionary Biology, Museum of Vertebrates, Cornell University, Ithaca, NY, USA

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ABSTRACT

Curimatidae, the fourth largest family of detritivorous Neotropical characiform fishes, encompasses eight extant genera and over 110 species dwelling in diverse freshwater habitats from Costa Rica to Argentina. Extensive phylogenetic analyses of soft anatomy and osteology provided evidence for intergeneric and most interspecific relationships, and formed the basis of curimatid taxonomy for nearly 40 years. However, that morphological phylogeny demonstrated incomplete phylogenetic resolution at various scales and has never been tested with extensive molecular data. Herein, we infer molecular phylogenies spanning ~70% of the known species diversity using three nuclear and three mitochondrial loci. Topologies from concatenated likelihood and Bayesian analyses and coalescent Bayesian species trees agree broadly with each other, and with the prior morphological hypothesis in many, but not all respects. All molecular analyses support the monophyly of Curimatidae and of six of its constituent genera, and agree on the placement of *Curimatopsis* as sister to all other curimatids. DNA-based intergeneric relationships differ substantially from prior morphological hypotheses by placing *Curimata* sister to *Potamorhina* and *Psectrogaster* sister to *Pseudocurimata*, rather than in a ladderized arrangement. Our results also resolve a major uncertainty in the morphological tree by revealing *Cyphocharax*, a genus for which no anatomical synapomorphy has ever been proposed, as a paraphyletic assemblage containing a monophyletic *Steindachnerina* and a polyphyletic *Curimatella*. Overall, the phylogeny expands substantially our understanding of the morphology, phylogenetics and evolution of the Curimatidae, and will guide future intrageneric studies by improving precision in the choice of comparative taxa.

1. Introduction

Detritivores of the fish family Curimatidae (Fig. 1) form a ubiquitous and critical component of diverse freshwater ecosystems across the Neotropics. Many curimatid species congregate in large schools (Santos et al., 1985) that constitute a significant portion of the biomass in many major rivers (Lowe-McConnell, 1975) and support regional fisheries (Araujo-Lima and Ruffino, 2003). Species of *Potamorhina*, for example, contribute significantly to landings in Amazonian fish markets in addition to being important components of artisanal fisheries (Araujo-Lima and Ruffino, 2003; Garcia et al., 2009). Ecologically, vast schools of these and other curimatid species of moderate sizes contribute to the carbon flow and nutrient cycling in Neotropical freshwaters (Araujo-Lima et al., 1986) and constitute a major food source for piscivorous fishes, birds and other predators (Kasper et al., 2008; Ferreira et al., 2014).

Curimatids inhabit both sides of the Andean cordilleras. East of those mountains, they range from south of Buenos Aires, Argentina, through Atlantic rivers of cis-Andean South America to the Orinoco basin and Trinidad (Vari, 2003). Major Neotropical basins such as the Paraná-Paraguay, Amazon, Tocantins, Orinoco and the larger drainages of the Guianas contain the greatest curimatid abundance (Vari, 1989a; Sidlauskas and Vari, 2012). In the trans-Andean region, a less speciose curimatid assemblage lives in rivers from northern Peru to southern Costa Rica including the Magdalena and Maracaibo basins (Bussing, 1966; Ortega and Vari, 1986; Vari, 1989a). Few of these western systems harbor more than a single member of the family.

A decade of exhaustive taxonomic revisions by Vari (1982, 1984,

* Corresponding author at: Dept. Morfologia, Instituto de Biociências, Universidade Estadual Paulista, R Prof Dr Antonio C. W. Zanin, s/n, 18618-689 Botucatu, SP, Brazil.
E-mail address: melo@ibb.unesp.br (B.F. Melo).
[1] In memoriam.

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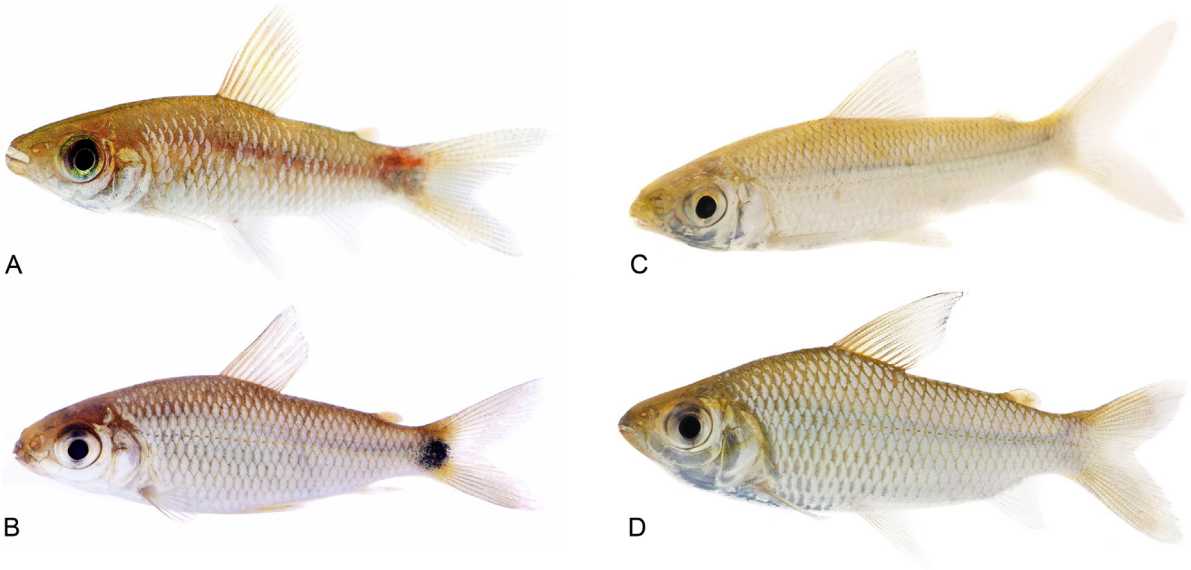

Fig. 1. 1Live specimens of Curimatidae. (A) *Curimatopsis evelynae*, (B) *Cyphocharax spiluropsis*, (C) *Cyphocharax plumbeus*, (D) *Curimatella immaculata.* Photographs by M.I. Taylor.

1989a, 1989b, 1989c, 1989d, 1991, 1992a, 1992b) improved a previously confused taxonomy (e.g. Fernández-Yépez, 1948), clarified species boundaries and provided morphological phylogenies for each curimatid genus with the exception of *Cyphocharax*. As understood in the wake of those revisions, Curimatidae comprises 113 living species (Eschmeyer and Fong, 2018) distributed among eight extant genera (Vari, 2003) plus the fossils †*Plesiocurimata alvarengai* and †*Cyphocharax mosesi* (Malabarba and Malabarba, 2010), making it the fourth largest family of Characiformes. Many species have been recognized quite recently (e.g., Vari et al., 2010, 2012; Melo and Vari, 2014; Melo et al., 2016c; Melo, 2017; Melo and Oliveira, 2017; Dutra et al., 2016, 2018) most often from the Amazon, a region whose ichthyofauna is far from thoroughly sampled (Vari and Malabarba, 1998). These species descriptions have expanded the membership of *Curimatopsis* (Vari, 1982; four species, now 10), *Curimata* (Vari, 1989d; 12 species, now 13), *Steindachnerina* (Vari, 1991; 21 species, now 24), and *Cyphocharax* (Vari, 1992a; 33 species; now 43), while leaving unchanged the membership of *Potamorhina* (Vari, 1984; five species), *Psectrogaster* (Vari, 1989b; eight species), *Pseudocurimata* (Vari, 1989c; six species), and *Curimatella* (Vari, 1992b; five species).

The absence of oral dentition in adults readily distinguishes all curimatid species from all other characiforms except the edentulous hemiodontid *Anodus*, which differs from curimatids in numerous details (Langeani, 1998). The most comprehensive phylogenetic study of the family to date (Vari, 1989a) hypothesized a monophyletic Curimatidae supported by 19 morphological synapomorphies. A recent reanalysis of a supermatrix linking data from that study to data for relationships in the superfamily Anostomoidea expanded that list to 27 synapomorphies (Dillman et al., 2016). The vast majority of these features involve modifications of the branchial apparatus, buccopharyngeal complex, hyoid arch, jaws, palatine arch, and neurocranium, many of which presumably adapt the family to its unusual detritivorous niche. Vari (1989a) further generated a hypothesis of intergeneric relationships for the family (Fig. 2a). Multiple synapomorphies support all genera except *Curimatella*, united only by more extensive caudal fin squamation, and *Cyphocharax*, which remained without any derived delimiting character and of questionable monophyly (Vari, 1992a). That phylogeny resolved as a ladderized topology (Fig. 2a) with a terminal polytomy among *Curimatella*, *Cyphocharax*, *Pseudocurimata* and *Steindachnerina.* Dillman et al. (2016) recovered a similar result, corroborated the non-monophyly of *Cyphocharax* with relationships within that genus as an unresolved comb, and hinted at the possibility of a polyphyletic *Curimatella* in the Bayesian portion of their morphological analysis.

Despite the exhaustive morphological study, no molecular phylogenetic analysis has tested Vari's (1989) hypotheses of curimatid monophyly and generic relationships, or addressed the problematic four-genus polytomy. Neither has molecular evidence been brought to bear on the entirely unresolved relationships within *Cyphocharax* (Vari, 1992a), or on the incompletely resolved relationships among species of *Curimata* (Vari, 1989d), *Curimatella* (Vari, 1992b), *Curimatopsis* (Vari, 1982), *Psectrogaster* (Vari, 1989b), *Pseudocurimata* (Vari, 1989c) and *Steindachnerina* (Vari, 1991; Netto-Ferreira and Vari, 2011).

Here, we used a large molecular dataset with approximately 70% complete taxon sampling across the family to generate the first multilocus molecular phylogeny of Curimatidae. We test prior morphological hypotheses of intergeneric and interspecific relationships, develop a new and more highly resolved intrafamilial phylogeny, establish a new framework for future taxonomic and evolutionary studies, and identify problematic areas requiring more intense phylogenetic analysis.

2. Material and methods

2.1. Taxon sampling

For our primary analyses, we used 140 ingroup samples representing all eight curimatid genera and spanning 75 species out of 113 valid species (67% species coverage) plus 11 characiform related taxa. These included each of the Anostomidae, Chilodontidae, Prochilodontidae and members of Hemiodontidae, Parodontidae and Serrasalmidae, all of which are characiform families closely related to Anostomoidea (Oliveira et al., 2011; Arcila et al., 2017). We used the more distantly related *Brycon pesu* (Bryconidae) to root the generated trees. Overall, this matrix contains 151 individuals, and is designated hereafter as matrix151 (Supplementary data 1; http://dx.doi.org/10.17632/k8cv4cpmyk.1).

To maximize the ingroup sampling, we constructed a second dataset that includes all data from matrix151, plus 65 barcode sequences (*cytochrome oxidase c subunit I*, *COI*) obtained from public databases (e.g. GenBank, BOLD) or newly generated herein. This larger dataset includes 205 curimatids spanning 83 species (74% species coverage) and the same outgroup taxa, and is designated hereafter as matrix216 (Supplementary data 1). Due to the larger proportion of missing data in

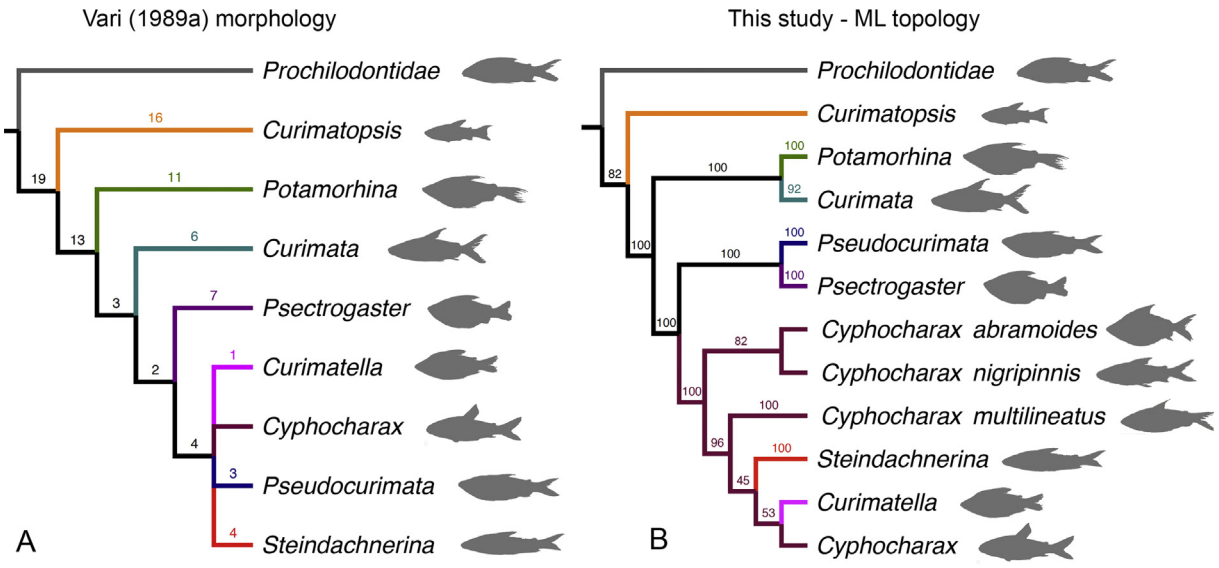

Fig. 2. Phylogenetic hypotheses based on (a) morphological (Vari, 1989a) and (b) molecular data (this study).

the remaining loci, this dataset is less well-suited to infer intrageneric relationships, but does allow verification of the putative generic placement of several otherwise unrepresented species.

2.2. Multilocus sequencing

We used tissues from vouchers available in various fish collections (Table S1; museum abbreviations follow Sabaj, 2016) preserved in 95% ethanol or a saturated DMSO/NaCl solution. We extracted DNA from muscle or fins with using a DNeasy Tissue kit (Qiagen Inc.) following manufacturer's instructions or a modified NaCl extraction protocol (Lopera-Barrero et al., 2008) and amplified partial sequences of the mitochondrial genes *16S rRNA* (*16S*, 495 bp), *cytochrome oxidase C subunit 1* (*COI*, 657 bp) and *cytochrome B* (*Cytb*, 1017 bp) using one round of polymerase chain reaction (PCR). Additionally, we obtained sequences of the nuclear *myosin heavy chain 6 gene* (*Myh6*, 738 bp), *recombination activating gene 1* (*Rag1*, 1452 bp), and *recombination activating gene 2* (*Rag2*, 999 bp) through nested-PCR following Oliveira et al. (2011). Primer sequences for each locus were obtained from the literature (Palumbi, 1996; Lovejoy and Collette, 2001; Li et al., 2007; Melo et al., 2011; Abe et al., 2013) and selected based on those used in previous studies of Anostomoidea (Melo et al., 2014; Melo et al., 2016b; Frable et al., 2016). We used 12.5 µl as a total volume with theoretical mean quantities of 9.075 µl of double-distilled water, 1.25 µl 5× reaction buffer, 0.375 $MgCl_2$, 0.25 µl dNTP mix at 8 mM, 0.25 µl of each primer at 10 µM, 0.05 µl Platinum Taq DNA polymerase enzyme (Invitrogen; www.invitrogen.com) and 1.0 µl genomic DNA (10–50 ng). The PCR consisted of an initial denaturation (4 min at 95 °C) followed by 28–30 cycles of chain denaturation (30 s at 95 °C), primer hybridization (30–60 s at 52–54 °C), and nucleotide extension (30–60 s at 72 °C). We visualized fragments using 1% agarose gel and we cleaned the PCR product using ExoSAP. Sequencing followed using dye terminators (BigDye™ Terminator v 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems) purified again through ethanol precipitation. We then sequenced the samples on an automatic sequencer ABI 3130-Genetic Analyzer (Applied Biosystems) at the Arizona State University (Tucson, Arizona, USA) or the Universidade Estadual Paulista (Botucatu, São Paulo, Brazil). Newly generated sequences have been deposited in GenBank with accession numbers MH537105-MH537592 and MH542680-MH542865 (Table S2).

2.3. Alignment and concatenated analyses

We assembled and edited sequences in Geneious v7.1.7 (Kearse et al., 2012) and applied IUPAC codes where we detected nucleotide uncertainty (low-quality chromatograms: 1.27% of the dataset). We aligned sequences of each gene using the MUSCLE algorithm (Edgar, 2004) implemented on Geneious 7.1.7 and inspected alignments by eye for major misalignments. The index of substitution saturation (Iss) was estimated by DAMBE v5.3.38 (Xia, 2013). We then generated maximum likelihood trees for each gene to check for cross-contamination and to identify incongruences among the gene trees. Because length polymorphism in the loop sites along the *16S* matrix made an unambiguous alignment impossible in those regions, we excised ambiguously aligned regions and included just the unambiguously alignable sites in downstream analyses. Missing data represent 16.1% in the matrix151 and 37.8% in the matrix216. We used PartitionFinder v1.1.1 (Lanfear et al., 2012) to select the best partitioning scheme and the best-fitting models of evolution for each partition using the Bayesian information criterion (BIC).

We used the concatenated matrix to run maximum likelihood (ML) searches for both matrix151 and matrix216 using a random starting tree with GTRGAMMA model (Stamatakis et al., 2008) through RAxML HPC2 on XSEDE v8.2.10 (Stamatakis, 2006) as implemented on the CIPRES portal (Miller et al., 2010). All other parameters were left at default. One thousand bootstrap pseudoreplicates tested the support for each node.

We also performed Bayesian inference (BI) analyses using MrBayes v3.1.2 (Ronquist et al., 2012) as implemented on CIPRES (Miller et al., 2010) using the partitions and models suggested by PartitionFinder (Table S3) for both matrices. We performed two runs of four independent MCMC chains (one cold chain and three heated chains) with 50 million replicates sampling one tree every 5,000 generations. After examining the log likelihood scores and ensuring convergence and stationarity (> 200 ESS) with Tracer v1.5 (Rambaut and Drummond, 2009), we discarded 10% of the trees as burn-in in TreeAnnotator v1.8.4. Then, we used the remaining trees to construct a maximum clade credibility tree (50% majority-rule consensus) in TreeAnnotator. We visualized and edited trees in FigTree v1.4.3 (Rambaut, 2016).

2.4. Coalescent species tree analysis

Finally, we computed a species tree for the data-dense matrix151 using the multispecies coalescent in BEAST2 v2.4.5 (Bouckaert et al.,

2014). The 11 outgroup taxa were removed, and we configured thirteen data partitions following the schema suggested by PartitionFinder (Table S3) in BEAUti v2.4.6 using the STARBEAST2 template. For models calling for estimation of a GAMMA parameter, we used four rate categories. Proportions of invariant sites were estimated for models including an I parameter. For the partitions using a SYM model, we set base frequencies to equality, and otherwise estimated proportions. We configured four tree and clock models: one for the three mitochondrial loci, and one each for the *RAG1*, *RAG2* and *Myh6* partitions. Relative branching times for all four trees were estimated using separate uncorrelated exponential clock models, with N-1 bins. The starting value for the mitochondrial rate was set to one, and the autosomal partitions were set to 0.1 reflecting the expectation of slower evolution in those partitions. The species tree was inferred under a birth-death model with an uninformative (uniform) prior on the extinction fraction.

Each of the 140 ingroup individuals was assigned to one of the 75 species, and three separate analysis chains were run for 150 million generations, logging every 50,000 generations to yield a posterior distribution of 3000 topologies. All three chains reached stationarity after approximately the first 40 million generations, with ESS values for likelihood, prior, posterior and most parameters well above 200. However, the posterior distribution of each chain centered on a slightly different likelihood and occupied a slightly different region of tree-space, implying a lack of complete convergence. Inspection of the topological results from each run revealed disagreement at nodes that also received low support in the concatenated analysis, implying that the dataset contains insufficient information to resolve certain nodes and that longer chains would not likely improve convergence. Thus, rather than combining runs, we removed a 34% burn-in percentage from each run, and computed separate maximum clade credibility species trees for each run in TreeAnnotator v2.4.7. We then took the strict consensus of those three maximum clade credibility trees using the APE package in R (Paradis et al., 2004) as the final result of the species tree analysis.

2.5. Alternative topology analysis

We compared the unconstrained ML concatenated topology to four alternative topologies using the Shimodaira-Hasegawa tests as implemented in RAxML v8.2.10 run through CIPRES, and also as implemented in the Phangorn package in R (Schliep, 2011). For each alternative hypothesis, we built a constraint tree in Mesquite v3.31 (Maddison and Maddison, 2016), and inferred the most likely phylogeny conforming to that constraint in RAxML. The four constraints are: (1) *Potamorhina*_alone, which constrains *Potamorhina* and *Curimata* to the positions hypothesized by Vari (1989a), with *Potamorhina* sister to a clade containing *Curimata*, *Psectrogaster*, *Steindachnerina*, *Pseudocurimata*, *Curimatella* and *Cyphocharax*. The major difference in this test concerns the position of *Curimata*, which is sister to *Potamorhina* in the unconstrained tree. (2) *Psectrogaster*_alone, which constrains *Psectrogaster* and *Pseudocurimata* to the positions hypothesized by Vari (1989a), with *Psectrogaster* sister to a clade containing *Curimatella*, *Cyphocharax*, *Pseudocurimata* and *Steindachnerina*. The major difference in this test concerns the position of *Pseudocurimata*, which is sister to *Psectrogaster* in the unconstrained tree. (3) *Curimatella*_together, in which the species of *Curimatella* are constrained to monophyly, rather than being scattered throughout *Cyphocharax*, and (4) *Cyphocharax*_together: which constrains all the species of *Cyphocharax* and *Curimatella* to joint monophyly, rather than having the species of *Steindachnerina* nested within that clade. *Curimatella* is not constrained to monophyly within that assemblage. The likelihoods of the constrained topologies appear in Table S4. All data appear in Supplementary data 1.

2.6. Morphological character reconstruction

To investigate the evolutionary implications of the molecular phylogeny reconstructed herein, we performed ancestral state reconstructions on nine morphological characters (numbers 28, 37, 193, 194, 241, 329, 346, 406, and 459) extracted from the supermatrix of Dillman et al. (2016). These nine characters represent putative synapomorphies of clades not recovered in the molecular analysis, and thus characters whose interpretation may change. We added data drawn from cleared and stained specimens of three additional species (see Supplementary data 2), fleshed out the missing cells in the sparse matrix of Dillman et al. (2016) with direct morphological observations (see specimen list in the Supplementary data 2) and recoded character 459, dealing with elaboration of the buccopharyngeal complex, such that taxa lacking the complex received a unique character state, rather than being coded as having missing data. We then trimmed the morphological matrix and pruned the maximum likelihood topology to the minimum set of overlapping taxa using Phyutility (Smith and Dunn, 2008). That pruned phylogeny was made ultrametric using the penalized likelihood algorithm chronos in the R package ape (Paradis et al. 2004, Popescu et al. 2012), using the default settings. Ancestral character states were then reconstructed using likelihood and parsimony in MESQUITE v3.5 (Maddison and Maddison, 2018) assuming unordered character states for all multistate characters.

3. Results and discussion

3.1. Overall patterns and areas of agreement with prior morphological hypotheses

Both concatenated matrices contained 5358 bp in their final alignments with the primary matrix151 spanning 75 curimatid species (67%) and 11 outgroup taxa, and the sparser matrix216 adding barcode sequences for additional eight species, which extended the coverage to 83 curimatid species (74%). DAMBE detected no saturation in transitions or transversions in either asymmetrical (Iss.cAsym) or symmetrical (Iss.sSym) topologies. Saturation results and nucleotide frequencies appear in Table S5.

Intrageneric relationships based on ML analysis of the densely sampled matrix151 (Figs. 3 and 4) and the extended matrix216 (Figs. S1–S2) are nearly identical. Bayesian analysis of matrix151 (Figs. S3–S4) and matrix216 (Figs. S5–S6) also yield similar maximum clade credibility trees that diverge mainly in support values. The species tree (Fig. 5) agrees broadly with the concatenated tree about most inter- and intrageneric relationships, but differs with respect to the exact placement of *Steindachnerina* within a paraphyletic *Cyphocharax* (see Section 3.4).

Whether inferred from a concatenated or species-tree approach, the molecular phylogeny of Curimatidae agrees with the morphological hypothesis (Vari, 1989a) in many respects (Fig. 2), and improves resolution within several clades, most notably the species-rich *Cyphocharax* (Figs. 4 and 5). All molecular results herein support the monophyly of Curimatidae (Figs. 2–5), as proposed by Vari's (1983, 1989a) studies. Those papers identified 19 morphological synapomorphies of the branchial apparatus, buccopharyngeal complex, hyoid arch, jaws, palatine arch, and neurocranium, and eight additional synapomorphies for the family were discovered in the synthesis and reanalysis of Vari's data (Dillman et al., 2016). Recent multilocus molecular studies focusing on other characiform lineages (Oliveira et al., 2011; Melo et al., 2014, 2016b; Arcila et al., 2017) have also recovered a monophyletic Curimatidae. Like those earlier molecular studies, results herein place Curimatidae within a strongly supported clade also containing Chilodontidae and Prochilodontidae (Melo et al., 2014, 2016b), but excluding Anostomidae, the fourth anostomoid family. This result conflicts with substantial morphological evidence for a sister relationship between Anostomidae and Chilodontidae (Vari, 1983; Dillman et al., 2016) and is being tested in a densely sampled molecular study of the entire Anostomoidea (Sidlauskas et al., 2018).

Our phylogeny corroborates prior hypotheses of the monophyly of

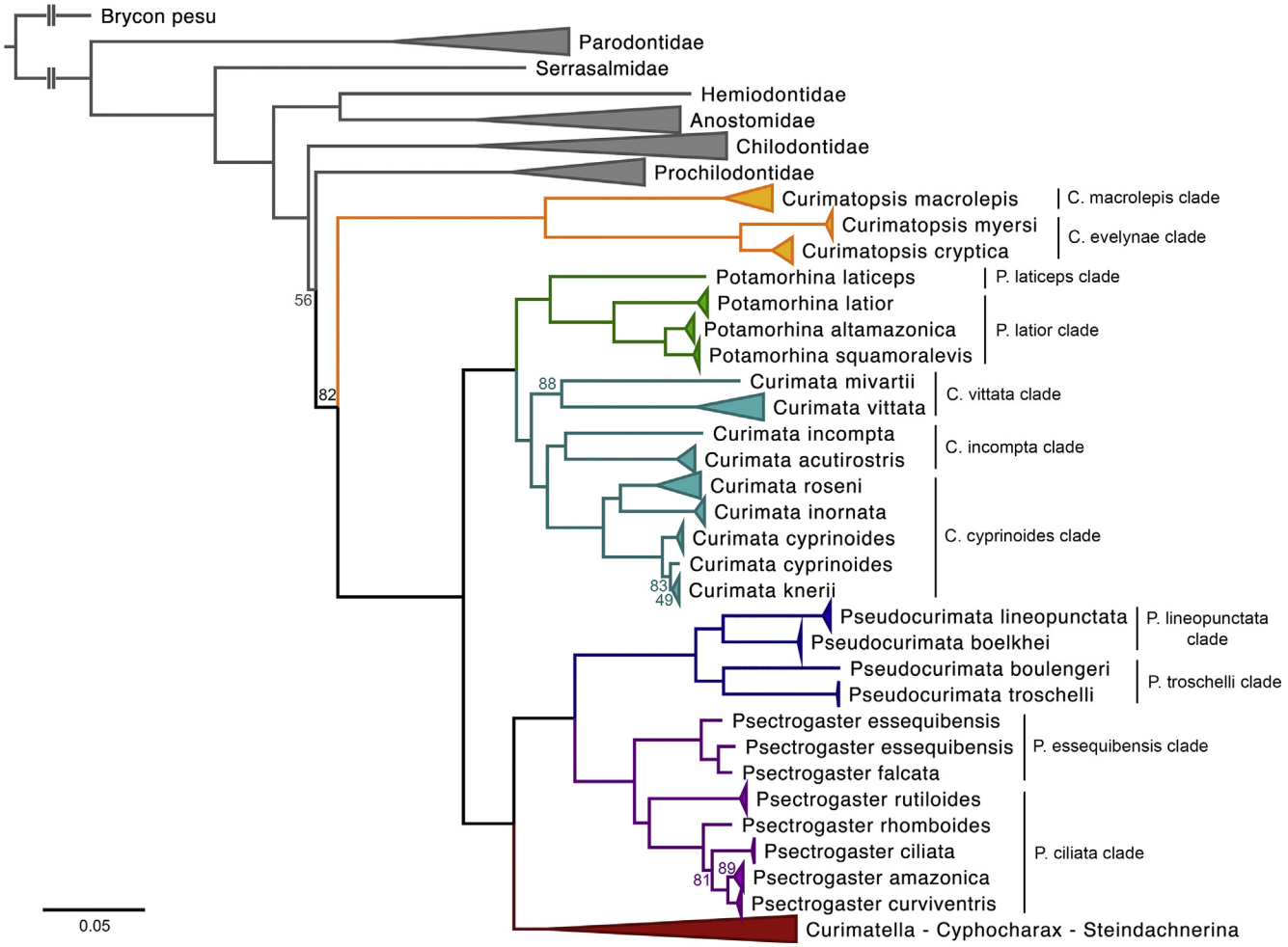

Fig. 3. Phylogenetic relationships within *Curimatopsis*, *Potamorhina*, *Curimata*, *Psectrogaster* and *Pseudocurimata* based on the best maximum likelihood tree of the concatenated molecular dataset. Bootstrap values ≥90% are not shown.

Curimatopsis (Fig. 2) which is unsurprising due to the strong morphological support of 16 synapomorphies (Vari, 1982; 1989a), and corroborates interspecific resolution in two well-established subclades: the *C. macrolepis* clade and the *C. evelynae* clade (Vari, 1982; Melo et al., 2016a; Melo and Oliveira, 2017). We likewise confirm the monophyly of its sister clade containing the remainder of the Curimatidae (Fig. 2), which received prior support from 13 morphological synapomorphies (Vari, 1989a). Our results also corroborate the monophyly of *Curimata*, *Potamorhina*, *Psectrogaster*, and *Pseudocurimata* (Fig. 2) and propose the recognition of intrageneric subclades within all curimatid genera (Figs. 3 and 4). *Steindachnerina* resolved as polyphyletic (Fig. 4) due to the placement of a single species (*S. corumbae* Pavanelli and Britski, 1999) assigned to the genus after the most recent taxonomic revision (Vari, 1991). That species clearly lies within the broad assemblage of species currently assigned to *Cyphocharax* and *Curimatella* (Figs. 4 and 5 and comments in Section 3.4). With that species reassigned, the molecular resolution of *Steindachnerina* matches the morphological concept exactly.

Despite the broad congruence outlined above, the molecular results differ from the prior morphological hypothesis in some important respects. Most notably, *Curimata* is resolved as sister to *Potamorhina*, and *Pseudocurimata* as sister to *Psectrogaster*, rather than in a ladderized arrangement (Vari, 1989a) (Fig. 2). The molecular analysis also resolves Vari's polytomy among *Curimatella*, *Cyphocharax* and *Steindachnerina*, and reconstructs the first hypothesis of relationships among the species of *Cyphocharax* (Figs. 4 and 5). We discuss each of these results in greater detail below.

3.2. *Curimata* as sister to *Potamorhina*

Curimata, the third-largest curimatid genus, comprises thirteen species with diverse fusiform to compressiform body shapes, and includes some of the most morphologically divergent members of the family, such as the complexly pigmented *C. vittata* and the highly elongated *C. ocellata*. Despite their morphological disparity, many synapomorphies unite them as a natural group, including most prominently the presence of numerous fleshy folds entirely covering the roof of the mouth (Vari, 1989a). *Curimata* has a well-studied taxonomy and reasonably resolved morphology-based interrelationships (Vari, 1989d), and has long been held to be sister to a clade containing *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax* and *Steindachnerina* (Fig. 2a). As such, its placement as sister to *Potamorhina* in the molecular results herein is surprising (Fig. 2b). The five species of *Potamorhina* achieve the largest body sizes within the Curimatidae and comprise one of the most ecomorphologically distinctive groups within the family on account of their tiny scales, large heads, pronounced migratory tendencies and numerous other synapomorphies, many of which deal with a pronounced lengthening of the gill arches and posterior portion of the splanchnocranium (Vari, 1984, 1989a).

The novel sister-group relationship between these two genera receives very high support in the molecular results with a bootstrap of 100 in the ML analysis (Fig. 3) and posterior probabilities exceeding 99% in both concatenated (Fig. S3) and species tree (Fig. 5) Bayesian approaches. The Shimodaira-Hasegawa test determined that the best molecular tree conforming to the morphological hypothesis is

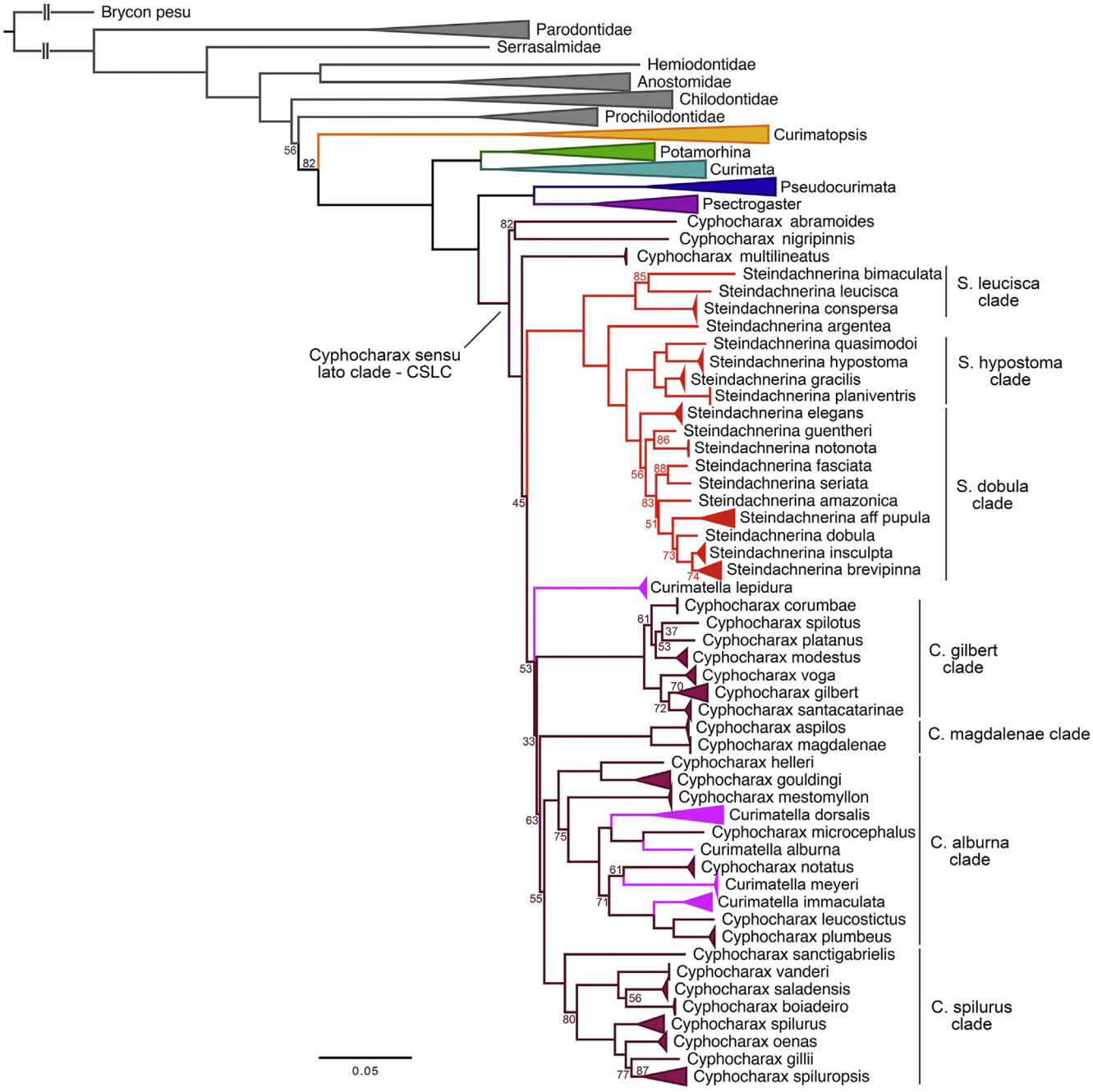

Fig. 4. Phylogenetic relationships within *Curimatella*, *Cyphocharax* and *Steindachnerina* based on the best maximum likelihood tree of the concatenated molecular dataset. Bootstrap values ≥90% are not shown.

significantly less likely than the unconstrained tree ($P < 0.001$). In that light, the strength of the morphological support for this placement bears re-examination.

Vari (1989a) inferred the placement of *Curimata* as sister to a large clade containing *Psectrogaster*, *Curimatella*, *Cyphocharax*, *Pseudocurimata* and *Steindachnerina* on the basis of only three characters: the elimination of a gap between the anterior articular cartilages on the second hypobranchial, the presence of a ridge on the medial surface of the metapterygoid, and the presence of three or more fleshy longitudinal folds on the roof of the mouth (Vari, 1989a). In all three cases, *Potamorhina* appears to possesses the primitive state of the character in question (a gap between the cartilages, an unelaborated upper palate, and only moderate development of the ridge), whereas *Curimata* possesses a derived condition. However, the morphology encoded in the first case is clearly evolutionary labile and in the second case, the evolutionary reconstruction depends strongly on the algorithm chosen and whether one recognizes two or three distinct character states. In the third case, reasons exist to question the homologization of the derived condition in *Curimata* with that present in *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax* and *Steindachnerina*.

Vari (1989a: Fig. 22) illustrated four distinct conditions of the cartilages associated with the second hypobranchial and hypothesized a transitional homology among them, with the primitively large gap between the anterior cartilages present in *Curimatopsis* being first reduced to the condition still apparent in *Potamorhina* and then eliminated, with an additional fission of the remaining cartilage then evolving in the lineage leading to *Pseudocurimata*. Reconstruction of this character (#329 of Dillman et al., 2016) on the molecular tree, suggests instead a

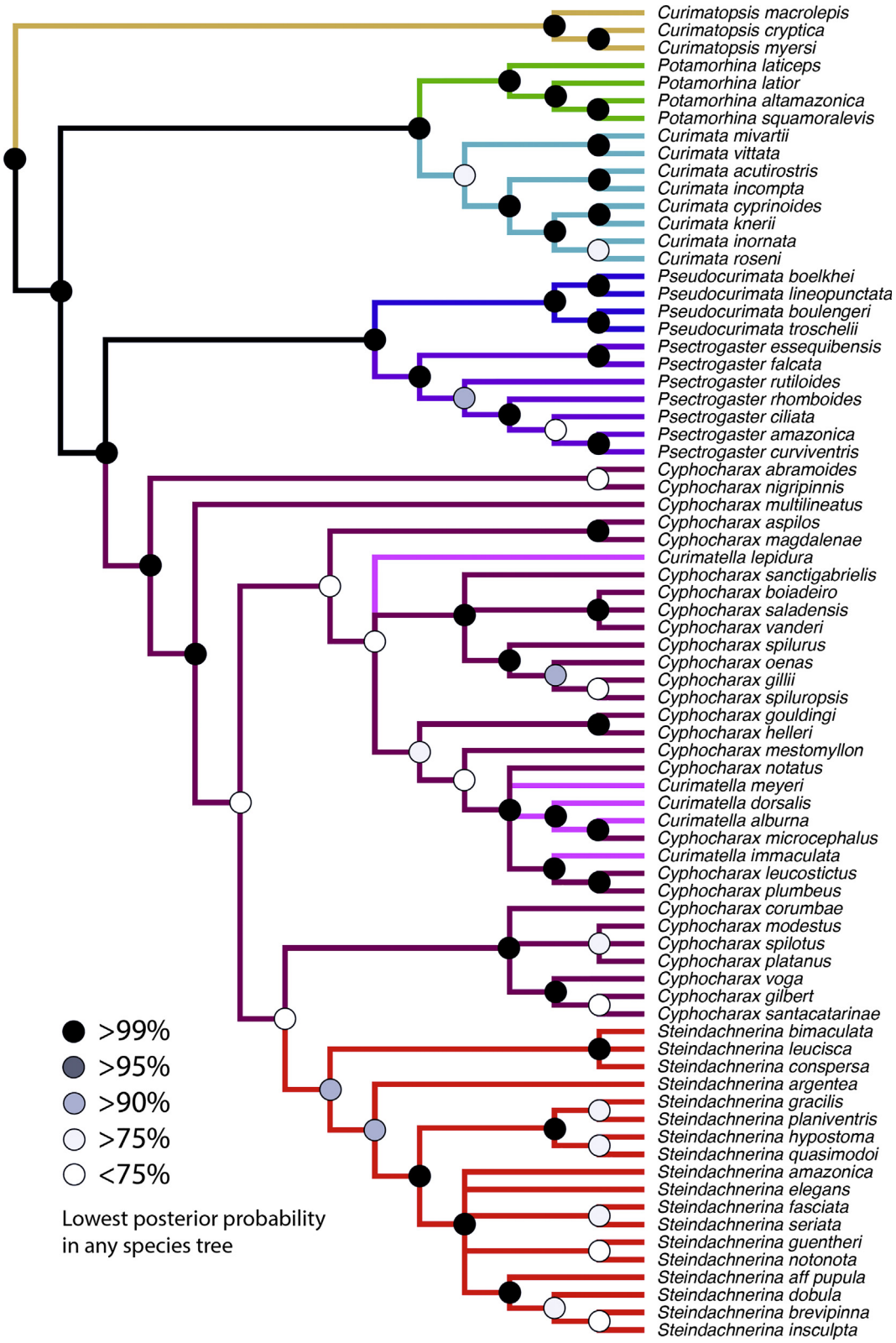

Fig. 5. Coalescent species tree of Curimatidae inferred by *BEAST without outgroup taxa.

single instance of fusion of these cartilages, followed by slight separation in *Potamorhina*. Such a scenario implies that the apparently transitional morphology in *Potamorhina* is instead a partial reversion, but given that the morphology is labile even on the morphological tree, the slightly less intuitive evolutionary history implied by the molecular tree is certainly reasonable.

Vari (1989a) indicated the presence of a partial or complete ridge on the medial surface of the metapterygoid (character 193 of Dillman et al., 2016), as one of the clear synapomorphies for Curimatidae. He interpreted the partial ridge possessed by *Curimatopsis* and *Potamorhina* as the morphology possessed by the most recent common ancestor of the family, and used the possession of a complete ridge running

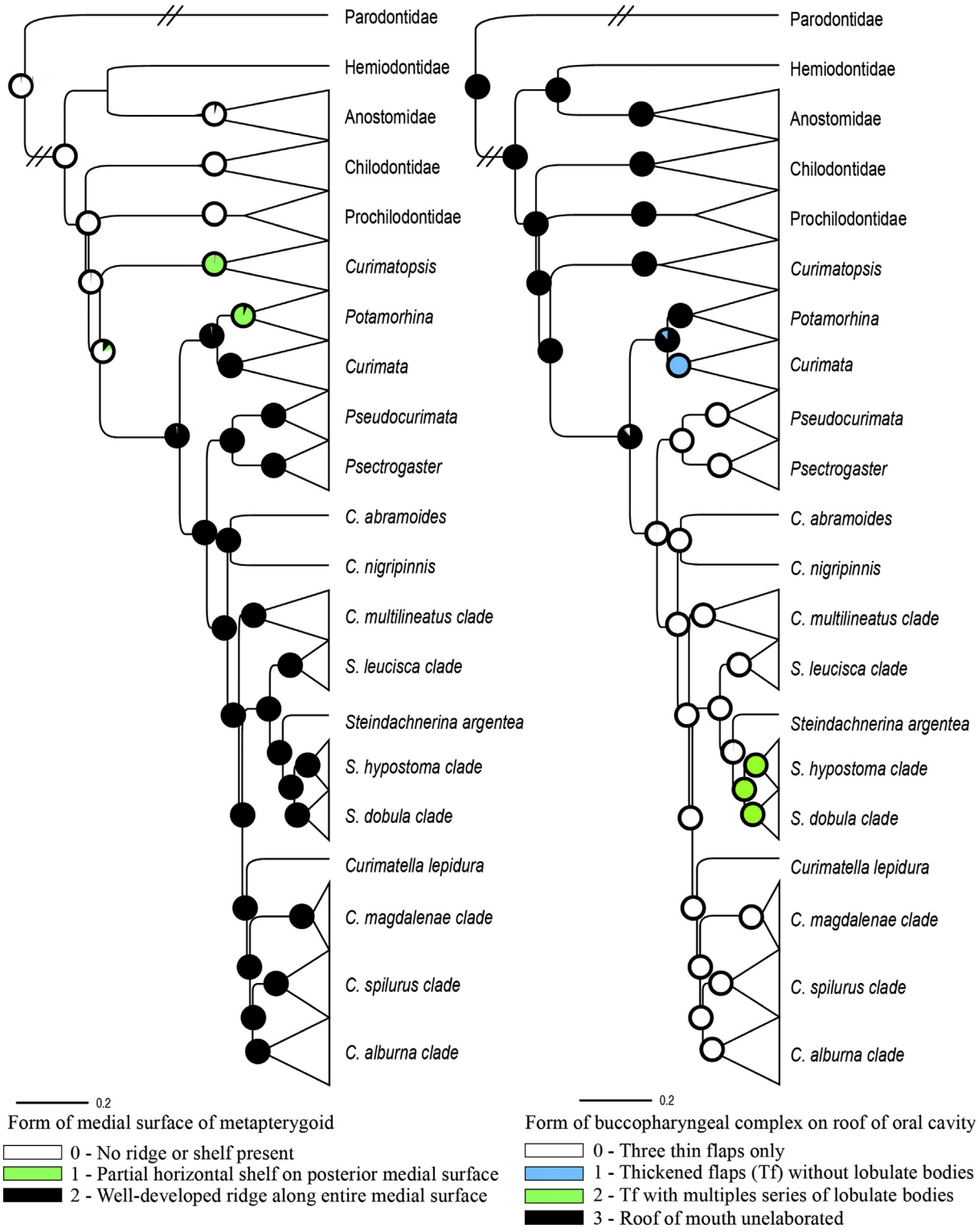

Fig. 6. Maximum Likelihood ancestral character state reconstructions for medial surface of the metapterygoid (left) and the buccopharyngeal complex (right) in Curimatidae and related species. Parallel hash marks indicate branches that were reduced in length to fit the figure to the space available.

between the bone's joint with the mesopterygoid anteriorly and the hyomandibular posteriorly to hypothesize a clade containing all other curimatid genera. However, he also noted that the morphology of that ridge reaches its greatest development in *Curimata*, in which a greatly thickened anterior portion of the metapterygoid's ridge articulates with an expanded medial projection of the mesopterygoid, which is also unique to *Curimata*.

Likelihood reconstruction of this character's history (Fig. 6; left panel) suggests a more complicated scenario, in which the common ancestor of the family probably lacked the ridge, the common ancestor of all genera except *Curimatopsis* possessed a well-developed ridge, and in which the partial ridges of *Curimatopsis* and *Potamorhina* are derived independently. However, the parsimony approach (figure not shown) finds an equally parsimonious scenario in which the greatly thickened

ridge possessed by *Curimata* and the lesser degree of expansion possessed by members of *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax* and *Steindachnerina,* represent independent, homoplastic elaborations of a partial ridge present in the most recent common ancestor of all curimatids. That interpretation is consistent with Vari's (1989a) inference that the presence of the ridge is a synapomorphy for the family. Because of the alternative reconstructions, it may not be possible to infer the evolutionary history of this character with certainty. However, it is worth noting that if this multistate character were treated as a binary character encoding only the presence or absence of the ridge in any form, then all approaches would agree that the most recent common ancestor of Curimatidae possessed the ridge in question.

The final character aligning *Curimata* with *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax*, and *Steindachnerina* in Vari's (1989a) reconstruction involves the presence of at least three fleshy folds or lobes on the upper roof of the mouth. Since publication of that work, the presence of the complex and various degrees of its elaboration have held substantial taxonomic relevance, and helped to distinguish numerous species and clades otherwise only separable by internal characteristics. In that light, the sister-group relationship between *Potamorhina* (which lacks the folds entirely) and *Curimata* (which possesses the most elaborately developed folds of any curimatid genus) is quite surprising, because it implies either repeated evolution of these fleshy folds, or loss of these folds in the lineage leading to *Potamorhina.* Either of these scenarios is plausible. Likelihood (Fig. 6; right panel) and parsimony reconstructions of the character as encoded in Dillman et al. (2016; character 459) indicate a dual origin of the complex, with the extreme development of the complex and presence of numerous secondary folds in *Curimata* non-homologous with the three thin flaps seen in *Pseudocurimata*, *Psectrogaster*, *Curimatella*, *Cyphocharax* and some *Steindachnerina*, or with the unusual lobulate morphology that characterizes a subclade within *Steindachnerina*. However, that interpretation depends strongly on the multistate coding of the character. When the character is simplified to reflect mere presence or absence of any form of elaboration on the roof of the mouth (analysis not shown), then both approaches reconstruct a single origin of the elaboration, followed by a loss of such elaboration in *Potamorhina.* Given the strong dependence of this reconstruction on the granularity of character encoding, the morphology possessed by the most recent common ancestor of all curimatids excluding *Curimatopsis* should be considered equivocal.

Overall, the placement of *Curimata* as sister to *Potamorhina* involves only a slightly less parsimonious reconstruction of morphological evolution, with a single additional state change required in character 193, dealing with the morphology of the ridge on the medial surface of the metapterygoid. Because of the multistate coding of characters 329 and 459, the reconstruction of the history of the hypobranchial cartilages and buccopharyngeal complex implied by the molecular and morphological topologies is equally parsimonious, though we acknowledge that a binary coding for these characters would result in two extra character state transitions. Thus, rather than revealing major conflict between the morphological and molecular topologies, the analysis reveals potential evolutionary lability at the base of Curimatidae, strong dependence of inference on the number of distinct character states recognized, and an instance in which the morphology possessed by *Curimata* represents an extreme, and potentially non-homologous version of the morphology demonstrated by *Psectrogaster*, *Pseudocurimata*, *Curimatella*, *Cyphocharax* and *Steindachnerina.* As discussed below, the novel placement of *Pseudocurimata* also prompts re-evaluation of the evolutionary history of the fourth ceratobranchial, with a process on that bone now optimizing as a synapomorphy uniting *Curimata* and *Potamorhina*. Although we acknowledge surprise at the potential non-homology of the buccopharyngeal complex implied by the molecular results, the overall tree lengths of the two reconstructions are similar and the conflict minor. Overall, we view the molecular signal as stronger than the morphological signal with respect to this area of conflict, and posit that *Curimata* and *Potamorhina* are indeed each other's closest living relatives.

3.3. Pseudocurimata as sister to Psectrogaster

Pseudocurimata is a small group of six species notable as the only uniquely trans-Andean genus of Curimatidae. Our results reveal a novel, strongly supported relationship of *Pseudocurimata* sister to the widely distributed cis-Andean genus *Psectrogaster* (Figs. 2 and 3) that contrasts with Vari's (1989a) placement of *Pseudocurimata* within a polytomy containing *Curimatella*, *Cyphocharax*, and *Steindachnerina,* with that assemblage in turn sister to *Psectrogaster* (Fig. 2). Shimodaira-Hasegawa tests (Table S4) strongly prefer the unconstrained tree to any topology separating *Psectrogaster* and *Pseudocurimata*, and suggest a re-examination of the morphological support underlying the earlier hypothesis.

The morphological revision implied by this molecular arrangement is less profound than that implied by *Curimata*'s relocation. Vari (1989a) identified four putative synapomorphies linking *Pseudocurimata* to *Curimatella*, *Cyphocharax* and *Steindachnerina*: loss of the ventral process on the fourth ceratobranchial; presence of a basihyal tooth plate; reorientation of the canals in the fourth and fifth infraorbitals; and reduction of the laterosensory canal system in the sixth infraorbital. The first (character 346 of Dillman et al., 2016) is equally homoplastic on either the morphological or molecular tree, and involves a process found only in *Potamorhina*, *Curimata* and *Psectrogaster*. Vari originally conceived this process as being lost in members of the terminal polytomy, while the molecular tree instead implies two independent origins, once in *Psectrogaster*, and again in the lineage leading to *Curimata* and *Potamorhina*. In that light, this character provides a morphological synapomorphy supporting the novel combination of the latter two genera.

The second relevant character here, the presence or absence of the basihyal tooth plate (character 241 of Dillman et al., 2016), is highly labile within Curimatidae. It is plesiomorphically present across Anostomoidea, but universally absent in *Curimatopsis*, *Potamorhina* and *Psectrogaster*, while variably present or absent in *Curimata*, *Pseudocurimata*, *Curimatella*, *Cyphocharax* and *Steindachnerina* (Vari 1989a). Although relocation of *Pseudocurimata* (which generally possesses the tooth plate) implies an additional transition in this character, the overall signal is neither strong nor compelling.

The final two character states suggesting a closer affinity of *Pseudocurimata* to *Curimatella*, *Cyphocharax* and *Steindachnerina* involve subtleties of the infraorbital canal system posterior to the eye. In these four genera, the angle between the canals in the fourth and fifth infraorbitals is slightly more acute than in the plesiomorphic condition (character 28 of Dillman et al., 2016), and the sixth infraorbital contains a simple unbranched sensory canal, rather than the plesiomorphically tripartite canal of that bone (character 37 of Dillman et al., 2016). Placement of *Pseudocurimata* as sister to *Psectrogaster* implies independent sharpening of that angle and loss of the canal segment in *Pseudocurimata* and in the most recent common ancestor of *Curimatella*, *Cyphocharax* and *Steindachnerina*, or slightly earlier evolution of these derived states with reversion in *Psectrogaster*.

Overall, the morphological support for a union of *Pseudocurimata* with *Curimatella*, *Cyphocharax* and *Steindachnerina* rests on the evolutionarily labile presence of the basibranchial tooth plate, a shift in the angle between two infraorbital canal segments, and the loss of canal branches in the sixth infraorbital. While such subtle characters can and do sometimes provide clear synapomorphies, this particular suite of characters does not seem to provide sufficient evidence to overrule the very strong molecular signal supporting a sister relationship between *Pseudocurimata* and *Psectrogaster*.

Interestingly, both the molecular and morphological phylogenies are congruent with Vari's (1989c) biogeographic hypothesis for species

of *Pseudocurimata*, in which initial diversification of *Pseudocurimata* in the north of its range was followed by southward colonization through various central trans-Andean drainages and then diversification along their allopatric distributions. Of the four species that we sequenced, *P. lineopunctata* occurs at the northern edge of the genus' distribution, and *P. troschelii* at the southern extreme, with the other two species lying geographically intermediate, but closest in space to their closest genetic relatives.

3.4. Resolution of the Cyphocharax, Curimatella and Steindachnerina polytomy

Our results cast substantial light on the resolution of the terminal polytomy in Vari's (1989a) hypothesis (Fig. 2). Aside from relocating *Pseudocurimata* to a position sister to *Psectrogaster*, the phylogeny reveals the speciose clade remaining (herein named *Cyphocharax sensu lato* clade – CSLC) to encompass a paraphyletic *Cyphocharax*, a polyphyletic *Curimatella*, and a monophyletic *Steindachnerina* (Figs. 4 and 5). The Shimodaira-Hasegawa tests strongly rejected the possibility of a monophyletic *Curimatella*, and although the tree constraining all species of *Cyphocharax* and *Curimatella* to joint monophyly (exclusive of *Steindachnerina*) was the most likely of the constrained topologies we tested, it still differed significantly from the unconstrained topology ($P < 0.05$) (Table S4). Specialists on the group have long suspected *Cyphocharax* to represent an artificial group as it lacks any identified synapomorphy (Vari, 1989a; Dillman et al., 2016). Indeed, its most recent definition simply hinges on the absence of the features defining *Curimatella*, *Pseudocurimata* and *Steindachnerina*. As such, its molecular resolution as paraphyletic into which the latter two genera nest (Figs. 4 and 5) accords with the original morphological hypothesis (Vari, 1989a) and with recent reanalysis (Dillman et al., 2016). It also provides the first reconstruction of relationships among its species (Fig. 4), which defied Vari's (1992a) best attempts to solve the problem using anatomical data.

The first splits within the CSLC receives strong statistical support in all molecular reconstructions, and reveals *Cyphocharax abramoides* and *C. nigripinnis* to fall outside a clade containing all other members. *Cyphocharax abramoides* from the Orinoco and Amazon is one of the most distinctive members of the family, with a very deep body, a prominent predorsal spine and numerous minuscule scales over its body (Vari, 1992a). *Cyphocharax nigripinnis* from the Amazon basin has a less extreme morphology, despite an autapomorphic densely pigmented adipose fin. Long branches subtend both species, and while they appear as sisters in all analyses (Figs. 4, 5, S2, S4 and S6), that arrangement receives a bootstrap support of only 82% and a posterior probability lower than 75% in the Bayesian species-tree analysis. As such, it is possible that one or the other is more closely related to the remainder of the CSLC. No morphological synapomorphy has been proposed to support any of these possible arrangements.

Although the monophyly of the remainder of the CSLC received universal molecular support, the first split within that clade is equivocal. The second split in the Bayesian species tree (Fig. 5) and likelihood analyses (Figs. 4, S2) separates *Cyphocharax multilineatus* from the remaining species in the CSLC, while the concatenated Bayesian approach disagrees, and places *C. multilineatus* sister to a well-supported clade containing *C. gilbert* and several other species, which we have marked as the "*C. gilbert* clade" (Figs. S4 and S6). Support for any of these placements is weak, with posterior probabilities and bootstrap support in the vicinity of 50%, and it is important to not over interpret the placement of *C. multilineatus*. That said, this species possesses distinctive longitudinal dark stripes on the trunk and is more densely pigmented than most other *Cyphocharax*, with only *C. pantostictos* (not analyzed), and *C. helleri* possessing comparable morphologies. Our analysis reconstructed *C. helleri* from Suriname (which has the faintest stripes of the three species) as sister to *C. gouldingi* from French Guyana and Rio Araguaia in a more nested position always distant from *C. multilineatus*, implying that the stripes have evolved at least twice. Only molecular data from *C. pantostictus* will be able to reveal whether that species represents a third evolution of this color pattern, or whether it is closely related to one or another striped species.

Similar to the uncertainty inherent in the placement of *Cyphocharax multilineatus*, the next several bifurcations within the CSLC received low statistical support, very short internodes, and discrepancies in topologies of the three main constructions (Figs. 4, 5 and S4). Evidently, this dataset lacks sufficient signal to unequivocally resolve relationships at this level. This is intriguingly the same area in which Vari (1992a) failed to discover morphological synapomorphies, although not for lack of trying (see discussion and hints of his frustration on his pages 123–126). The molecular results do identify certain well-supported subclades (Fig. 4), which we discuss briefly below in order to provide a framework for finer scale investigations and revisions within this large clade in the future. It seems possible that these lineages separated from one another very quickly, leaving few opportunities for molecular or morphological synapomorphies to accumulate on the short internodes. If so, it may require a phylogenomic approach to uncover sufficient variation to resolve the relationships among and within these subclades.

We designate the most clearly supported subclade within the CSLC as the *Cyphocharax gilbert* clade, which also includes *C. modestus* from the upper Rio Paraná, *C. naegelii* from the Rio Paraná, *C. platanus* from the Rio Uruguay, *C. santacatarinae* from the Rio Ribeira de Iguape, *C. spilotus* and *C. voga* from coastal rivers of southern Brazil and *Cyphocharax corumbae* (new combination, see Section 3.5) from the Rio Corumbá, upper Rio Paraná (Figs. 4 and S2). In combination with *C. gilbert*, *C. voga* and *C. santacatarinae*, all with distributions in the coastal drainages of southeastern and southern Brazil, this is a geographically limited clade containing some of the most southerly-distributed members of the family, and likely a ripe prospect for future phylogeographic analysis to help elucidate the history of Atlantic coastal river systems.

The well-supported *Cyphocharax magdalenae* clade is also geographically restricted, but falls at the opposite extreme of the family's range. The single included sister-species pair involves *C. magdalenae*, which ranges from Costa Rica (the source of our tissues) south to the Río Magdalena in Colombia and *C. aspilos* from Lago Maracaibo in Venezuela. These two very similar species are the only trans-Andean species of *Cyphocharax*, and our results strongly support their sister relationship (Figs. 4 and 5). These distinctive forms possess a deep caudal peduncle without any dark pigmentation, a unique combination within *Cyphocharax* (Vari, 1992a).

The monophyletic *Cyphocharax spilurus* clade (Fig. 4) is one of the most complex groups within the Curimatidae and includes eight analyzed species, all of which possess a dark, horizontally elongate to circular spot of pigmentation on the caudal peduncle. Relationships within this clade vary in degree of support, with some smaller subclades receiving high support, such as the grouping of *C. vanderi* (upper Rio Paraná), *C. saladensis* (Río Uruguay) and *C. boiadeiro* (Rio Araguaia). Shared features such as a truncated laterosensory system and reduced number of lateral line scales might represent synapomorphic conditions supporting this clade (Melo, 2017), although resolving their actual relationships will likely require the inclusion of more species.

A second well-supported component of the *Cyphocharax spilurus* clade contains several species widely distributed along the Amazon/Guianas/Orinoco system. *Cyphocharax spilurus* (Guianas) appears sister to a clade containing *C. oenas* (Río Orinoco) which is, in turn, sister to a more restricted clade of *C. gillii* (Río Paraguay) and *C. spiluropsis* (Araguaia and Tapajós rivers). These species demonstrate progressive increases in overall morphological similarity, albeit differing in details of the dark caudal-peduncle spot and multiple meristic features (Vari, 1992a). This is the first hypothesis proposing monophyly and interspecific relationships for this clade and it certainly will help delineate future taxonomic projects.

The remaining species of *Cyphocharax* appear intermingled with

species traditionally recognized in *Curimatella*, which clearly resolved as polyphyletic in our phylogeny (Fig. 4) with the type species, *C. lepidura* falling phylogenetically distant from its nominal congeners. *Curimatella*'s current definition rests on a single proposed synapomorphy (Vari, 1989a; 1992b), the extensive squamation on the caudal fin. This character state appears in distinct clades across the Characiformes (e.g. Anostomidae, Characidae) (Sidlauskas and Vari, 2008; Mirande, 2010) and is clearly evolutionarily labile. Given the wide separation between *C. lepidura* and the other four species in the genus, that pattern of squamation appears to have evolved at least twice in Curimatidae, likely with a complicated and equivocally reconstructed pattern of gains and losses. For example, even if one disregards *C. lepidura*, the clade containing the remaining four species with extensively scaled caudal fins also includes at least four species lacking such extensive squamation: *Cyphocharax notatus*, *Cy. microcephalus*, *Cy. leucostictus* and *Cy. plumbeus*. Some of these species (*Cy. microcephalus*) as well as others not sampled (*Cy. pinnilepis*, *Cy. derhami*), have scales over a portion of their caudal fins, albeit never to the extent seen in *Curimatella* (Vari et al., 2010). Clearly, this character evolves quickly, and the presence of caudal-fin scales does not effectively diagnose a subclade of curimatid fishes.

In contrast to *Cyphocharax* and *Curimatella*, the monophyly of *Steindachnerina* as conceived by Vari (1989a) on the basis of four synapomorphies associated with restructurings of the branchial and hyoid arches has never been questioned. Leaving aside *Cyphocharax corumbae* (see Section 3.5), our phylogeny also returns a monophyletic *Steindachnerina* with highest support in the concatenated ML analysis (100% bootstrap, Figs. 4, S2) and over 90% posterior probability in all three Bayesian reconstructions (Figs. 5, S4 and S6).

The two approaches do, however, differ in the overall placement of *Steindachnerina*. The concatenated results infer a placement as sister to a clade containing all species currently assigned to *Curimatella* and the majority of species in *Cyphocharax* (Figs. 4, S4), while the species-tree approach nests *Steindachnerina* more deeply, placing it as sister to the *Cyphocharax gilbert* clade (Fig. 5). In either case, the placement of *Steindachnerina* receives low statistical support (45% bootstrap in the concatenated tree and < 75% in the species tree, Figs. 4 and 5), and falls into a region of extremely short internodes along the backbone of the phylogeny, suggesting that this multilocus dataset does not possess many informative characters at this level of the phylogeny. While the monophyly of *Steindachnerina* seems certain, resolution of its precise location within the CSLC will require a larger dataset.

The dataset more effectively resolves relationships within *Steindachnerina*, and identifies three major subclades also hypothesized by Vari (1991) (Fig. 4). The first is the *S. leucisca* clade, also containing *S. conspersa* and *S. bimaculata*. Vari (1991) recognized these three species along with *S. binotata* as a distinct lineage supported by six synapomorphies including the presence of diagnostic spots of dark pigment on the dorsal midline and details of the infraorbital bones ringing the eyes. Together with *S. argentea*, this clade contains the only members of *Steindachnerina* with a relatively simple form of the buccopharyngeal apparatus involving three simple primary folds on the roof of the mouth, plesiomorphically widespread among species of *Cyphocharax* and *Curimatella*.

All other members of *Steindachnerina* possess hypertrophied lobulate bodies covering all or part of the upper surface of their palate, and these species form a well-supported clade in the morphological hypothesis (Vari, 1991) and all molecular reconstructions herein (Figs. 4 and 5). Vari also recognized the major subclades discovered by the molecular analysis (the *S. dobula* and *S. hypostoma* clades in Fig. 4), on the basis of differences in the arrangement of the lobulate bodies and other aspects of the osteology of the splanchnocranium. Given the broad agreement between both datasets and the strong statistical support in the molecular phylogeny, the reality of these three major subclades within *Steindachnerina* (with *S. argentea* representing a fourth monotypic lineage) seems certain. Relationships within each of these subclades are much less certain, with both Vari's (1991) reconstruction and our results containing numerous polytomies or weakly-supported nodes (Figs. 4 and 5). Future studies should delve more deeply into the nuances of these interspecific relationships.

3.5. Nomenclature

Our results clearly support the long-suspected paraphyly of *Cyphocharax*, and also reveal the polyphyly of *Curimatella* (Figs. 4 and 5). Two options exist as to how to address these problems. One could assign a single genus name to the entire CSLC, or recognize a series of monophyletic components of the CSLC as successive genera. Unfortunately, both options pose substantial nomenclatural problems of their own.

The first option would require assigning the oldest available genus name to all species currently assigned to *Curimatella*, *Cyphocharax* and *Steindachnerina*. *Curimatella* Eigenmann and Eigenmann, 1889 is the oldest name, but has been applied historically to only five species, whereas *Cyphocharax* Fowler (1906) and *Steindachnerina* Fowler (1906) as currently defined include the majority of curimatid species. Thus, assigning all these species to *Curimatella* would create substantial taxonomic instability by changing the generic assignment of more than 50% of the species and radically changing the definition of *Curimatella*. This would result in an oversize genus containing a disproportionate number (~60%) of the ~110 valid species in the Curimatidae, thereby obscuring much of the evolutionary history that a system of nomenclature is intended to index.

The seemingly preferable alternative to an unwieldy *Curimatella* involves the recognition of numerous genera within the CSLC. Such an action could preserve all three-genera names and would result in a stable *Steindachnerina*. It would also require erection of several new genera, some of which would recognize morphologically or geographically distinctive subclades within the current concept of *Cyphocharax*, such as the southerly *C. gilbert* clade, the caudal-peduncle spotted *C. spilurus* clade, the unpigmented species of the *Curimatella alburna* clade or the easily diagnosable *C. abramoides*, *C. nigripinnis* and *C. multilineatus*.

Despite the advantages of this second option, we opine that erection of the requisite new genera is still premature. Despite our extensive taxonomic sampling, our analysis still lacks about 30% of the known curimatid species. These could not be reassigned to a new genus on the basis of molecular information, at least not without guessing as to their correct placement. Other species, including the highly divergent *Cyphocharax abramoides* and *C. nigripinnis,* were incorporated into the phylogenetic analysis on the basis of a single sequenced specimen. The addition of more specimens may adjust their phylogenetic position, or increase the confidence in their placement as sister to the remainder of the CSLC. And, due to the phylogenetic uncertainty in the relationships among subclades within that large group, some species may shift from one subclade to another in future analyses of an expanded dataset. As such, we prefer to leave the assignment of species to genera unchanged for the present, with the exception of *Steindachnerina corumbae*, which is clearly not a member of *Steindachnerina*.

Pavanelli and Britski (1999) described this species after Vari's (1991) revision and assigned it to *Steindachnerina* without a phylogenetic analysis or detailed examination of osteology, and its generic placement remained untested until now. Our analysis included two specimens from the Rio Corumbá (upper Rio Paraná) proximate to the type locality and revealed that *S. corumbae* is instead a member of the well-supported *Cyphocharax gilbert* clade (Figs. 4 and 5). The expanded dataset further confirmed the novel phylogenetic position (Figs. S2 and S6). One of us (BLS) has also examined two cleared and stained specimens of the species (USNM 350156) from the Rio Corumbá and determined that they do not possess the synapomorphies of *Steindachnerina* (Vari, 1991). Most notably and obviously, they lack the prominent lateral expansions to the anterior portion of the basihyal and

the basihyal tooth-plate that typify all members of *Steindachnerina*. Therefore, we transfer *S. corumbae* to *Cyphocharax* as a new combination, *Cyphocharax corumbae* (Pavanelli and Britski, 1999).

3.6. Future directions

Using a species-dense dataset, we present the first hypothesis of interspecific relationships within *Cyphocharax*, the most speciose curimatid genus, and provide a multilocus perspective on intergeneric and interspecific relationships in Curimatidae. Future directions will involve efforts to formally describe new genera and to address the generic level assignments of *Cyphocharax abramoides*, *C. nigripinnis*, *C. multilineatus* (and possibly *C. pantostictus*), the *C. gilbert* clade and others subclades of the CSLC, and to resolve the placement of species not currently represented in the dataset. The subclades propositioned by our phylogeny will help future species descriptions by improving precision in the choice of comparative taxa to be further analyzed. Recent genetic and taxonomic studies have also revealed the existence of several cryptic genetic lineages that may represent undescribed curimatid species (Melo et al. 2016a) and further genetic and morphological studies should explore the status of these biological entities. Phylogenomic approaches provide powerful resolution and are underway to address problematic areas of the phylogeny as indicated herein. Overall, the phylogeny herein provides a framework for the studies of the evolutionary processes involved in the species diversification of curimatids, and expands substantially our understanding of the morphology, phylogenetics and evolution of the family Curimatidae.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ympev.2018.06.027.

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