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Molecular Phylogenetics of the Chub Suckers (Teleostei: Catostomidae: *Erimyzon*) Inferred from Nuclear and Mitochondrial Loci

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Phylogenetic relationships among the four currently recognized species in the genus *Erimyzon* were reconstructed using sequence data obtained from the mitochondrially encoded COI and the nuclear-encoded IRBP2 genes. Maximum likelihood and Bayesian analyses yielded five groups of *Erimyzon*. Three of the clades corresponded to the species *E. oblongus*, *E. tenuis*, and *E. claviformis*. Individuals identified as *E. sucetta* were split into two separate clades, with one clade more closely related to *E. oblongus* than to the other *E. sucetta* clade in three of four analyses. Two instances of putative hybridization between sympatrically distributed species were also detected, one involving *E. oblongus* and *E. sucetta* and the other involving *E. claviformis* and *E. tenuis*. The results suggest that the currently recognized *E. sucetta* comprises two species, with potentially non-overlapping distributions. Further morphological investigation is warranted to provide additional support for recognizing this putative fifth species of *Erimyzon*.

THE Catostomidae, suckers, is a family of cypriniform fishes distributed throughout much of North American (from Mexico to Canada) and with a restricted and fragmented distribution in eastern Eurasia (China and Russia; Smith, 1992; Chen and Mayden, 2012; Nelson et al., 2016). Currently, 85 catostomid species are recognized as taxonomically valid (Fricke et al., 2021a), of which 83 occur in North America and only two in Eurasia, though several undescribed species from Mexico and the United States await formal description (Jelks et al., 2008). Catostomids are characterized largely by trophic specializations, including (but not restricted to) thick, often papilliated lips; a well-developed palatal organ in the bucco-pharyngeal cavity; comb-like gill rakers in most species; a large and heavily fenestrated basioccipital process; and a single row of at least 16 pharyngeal teeth on the fifth ceratobranchial (Smith, 1992; Conway, 2011; Doosey and Bart, 2011). The common name "sucker" supposedly refers to their large lips and highly protrusible jaws that enable the fishes to suck invertebrates from the benthos (Page and Burr, 2011). Catostomids first appear in the fossil record during the Paleocene (Conway et al., 2010), and extant species are currently organized into four subfamilies—Catostominae, Cycleptinae, Ictiobinae, and Myxocyprininae (Nelson et al., 2016; Bagley et al., 2018).

The Catostominae is currently the largest of the four subfamilies of the Catostomidae, containing ten genera, divided among four tribes: Catostomini (*Castostomus, Chasmistes, Deltistes, Pantosteus,* and *Xyrauchen*), Erimyzontini (*Erimyzon* and *Minytrema*), Moxostomini (*Moxostoma*), and Thoburniini (*Hypentelium* and *Thoburnia*; Bagley et al., 2018). The genus *Erimyzon*, chubsuckers, are found primarily in the United States, with one species present in Ontario, Canada (Mandrak and Crossman, 1996; Hauser et al., 2019). This genus comprises four species (Page and Burr, 2011; Fricke et al., 2021b): *Erimyzon sucetta, Erimyzon oblongus, Erimyzon claviformis*, and *Erimyzon tenuis*. *Erimyzon sucetta* has the widest distribution of the four species and is found along the Gulf slope, from eastern Texas to Florida; throughout the Atlantic slope, from Florida to southern Virginia; and in semi-isolated populations in the Midwest, as far north as the Great Lakes, and Ontario, Canada (Wall and Gilbert, 1980a; Hauser et al., 2019). *Erimyzon sucetta* exhibits a dark lateral stripe that runs the extent of the body onto the caudal fin, but is absent from the snout, a deep body, fewer than 14 predorsal scales, and 34–39 lateral scales (Page and Burr, 2011). *Erimyzon oblongus*, distributed from Georgia to New York and southern New England, and *E. claviformis*, distributed from the Midwest down to eastern Texas and as far east as western Georgia, are almost identical in appearance (Wall and Gilbert, 1980b). The only meristic character that distinguishes these two species is the number of dorsal-fin rays: 9–11 (modally 10) in *E. claviformis* and 11–14 (modally 12) in *E. oblongus* (Bailey et al., 2004). *Erimyzon tenuis* has the smallest distribution of the four species and is restricted to the central Gulf Coast, including eastern Louisiana, southern Mississippi and Alabama, and adjacent areas of the Florida Panhandle (Wall and Gilbert, 1980c). *Erimyzon tenuis* has a pointed dorsal fin (hence the common name Sharpfin Chubsucker), a dark lateral stripe that extends onto the caudal fin as well as around the snout, and black-edged dorsal and anal fins. While these four species are distinguished from one another based on morphology, relationships among all of them, based either on morphological or genetic data, have yet to be investigated.

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The first extensive phylogenetic investigation of the Catostomidae was conducted by Smith (1992), who investigated the relationships among 64 species using a data matrix comprising 157 morphological characters. In that study, Smith (1992) included three species of *Erimyzon* (*E. oblongus*, *E. sucetta*, and *E. tenuis*), which formed a monophyletic group and were placed in the eastern catostomine clade. Within the clade of *Erimyzon* from Smith (1992), *E. tenuis* is the sister taxon of the remaining species (*E. oblongus* + *E. sucetta*). Building on the work of Smith (1992) and Harris and Mayden (2001), Harris et al. (2002) conducted a phylogenetic study of catostomids using cytochrome *b* sequences, with an emphasis on *Moxostoma* and *Scartomyzon*. Three species of *Erimyzon* were included in their study (*E. oblongus*, *E. tenuis*, and *E. sucetta*), but the relationships inferred among the species conflicted with those of Smith (1992) because *E. oblongus* was obtained as the sister taxon to the remaining species (*E. tenuis* + *E. sucetta*). Doosey et al. (2010) investigated the relationships of 60 catostomid species using mitochondrially encoded NADH dehydrogenase subunit 4 and 5 (ND4 and ND5) and recovered *E. oblongus* and *E. sucetta* as monophyletic (these were the only two species of *Erimyzon* included in that study). Chen and Mayden (2012) were the first to use nuclear sequence data (interphotoreceptor retinoid-binding protein gene 2; IRBP2) to investigate the relationships among catostomids. Their study included three species of *Erimyzon* (*E. oblongus*, *E. sucetta*, and *E. tenuis*), and, though the genus was recovered as monophyletic, two species (*E. oblongus* and *E. tenuis*) were recovered as non-monophyletic. Chen and Mayden (2012) noted that the non-monophyly of two species of *Erimyzon* in their study could be explained by specimen misidentification (several sequences they included were obtained from GenBank with no associated vouchers), incomplete lineage sorting and/or lateral gene exchange between individuals of different species, or the existence of undiscovered lineages within the genus, and they noted that further systematic study of all species of *Erimyzon* was needed.

The most recent phylogenetic study on catostomids, conducted by Bagley et al. (2018), used multiple mtDNA loci (cyt*b*, ND2, and COI) and nuclear loci (GHI, IRBP, and RPS7) for 121 individuals representing all genera. This study also included morphological data for 85 taxa (as part of a total-evidence analysis) and used information from the fossil record to investigate divergence times between clades within the family. In their study, Bagley et al. (2018) included three of the four species of *Erimyzon* (*E. oblongus*, *E. sucetta*, and *E. tenuis*) and, congruent with previous work, obtained a monophyletic *Erimyzon*. The relationships inferred among the three species of *Erimyzon* investigated by Bagley et al. (2018) were congruent with those reported by Harris et al. (2002).

While there have been other studies of the phylogenetic relationships of catostomids using various genes (e.g., LSU and SSU rRNA, Harris and Mayden, 2001; ITS1, Sun et al., 2007), these studies only included one species of *Erimyzon* and therefore provide no information on the relationships among members of the genus. No single study conducted to date has been dedicated to investigating the phylogenetic relationships among the species of *Erimyzon*. Given the conflicting hypotheses of relationships among the members of the genus resulting from previous phylogenetic investigations using different character data (i.e., morphological characters, mitochondrial and/or nuclear sequence data) and the potential non-monophyly of several species reported by Chen and Mayden (2012), additional phylogenetic study of the genus is warranted. The purpose of this study is to investigate the phylogenetic relationships among all four members of *Erimyzon* (including samples from across the range of each species) using both mitochondrially encoded COI and nuclear-encoded IRBP2 gene sequence data.

MATERIALS AND METHODS

Tissues, PCR amplifications, and sequencing.—Tissues were obtained from museum-vouchered specimens identified using external morphological characteristics as *E. claviformis*, *E. oblongus*, *E. sucetta*, and *E. tenuis*, from locations across their respective distributions (Fig. 1A, B). Tissues also were obtained from *Minytrema melanops* (Spotted Sucker) for use as an outgroup. A list of all specimens used in this study can be found in Table 1. DNA was extracted from tissues using a modified Chelex extraction protocol (Estoup and Largiader, 1996). Briefly, tissues were cut and placed in 200 µl of 10% Chelex solution and 15 µL of 1% Proteinase K solution. Samples were vortexed, then incubated at 60°C for 90 min, followed by 95°C for 15 minutes to denature the Proteinase K.

The mitochondrially encoded cytochrome *c* oxidase subunit 1 gene (CO1) was amplified using four universal fish primers (Ward et al., 2005): FishF1 (5′–TCAACCAACCACAAAGAGATTGGCAC–3′), FishF2 (5′–TCGACTAATCATAAAGATATCGGCAC–3′) and FishR1 (5′–TAGACTTCTGGGTGGCCAAAGAATCA–3′), FishR2 (5′–ACTTCAGGGTGACCGAAGAATCA GAA–3′). Each 30 µl reaction contained 1X buffer, 1.5 mM $MgCl_2$, 0.20 mM each dNTPs, 0.04% Tween (0.1–1%), 0.25 µM forward and reverse primers each (F_1 and F_2, R_1 and R_2), 0.033 units/µl Taq polymerase, and 1.0 µl of DNA template. PCR amplification was run with an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and elongation at 72°C for 1 min. A final round of elongation was run at 72°C for 10 min.

The nuclear-encoded interphotoreceptor retinoid-binding protein 2 gene (IRBP2) was amplified using primers designed specifically for catostomid fishes (Chen et al., 2008): IRBP 101F (5′–TCMTGGACAAYTACTGCTCACC–3′) and IRBP 1068R (5′–AGATCAKGYTGTATTCCCCACTA–3′). Many standard nuclear genes are not suitable for phylogenetic research on catostomids because suckers have tetraploid genomes, but previous research has shown that IRBP2 does not contain an excessive number of polymorphic sites, which is characteristic of loci with problematic paralogs, and is reliable for inferring species level relationships (Chen et al., 2008; Chen and Mayden, 2012). Each 20 µl reaction contained 1X buffer, 2.5 mM $MgCl_2$, 0.40 mM dNTPs each, 0.20 µM forward primer and reverse primer each, 0.05 units/µl Taq polymerase, and 2.0 µl of DNA template. PCR amplification was run with an initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 2 min. A final round of elongation was run at 72°C for 10 min.

Amplicons were cleaned using 0.7X Mag-Bind® Total Pure NGS beads (Omega Bio-Tek) and sequenced at the Genomics Core Lab at Texas A&M University–Corpus Christi or at Retrogen, Inc. (San Diego, CA). Chromatographs were edited

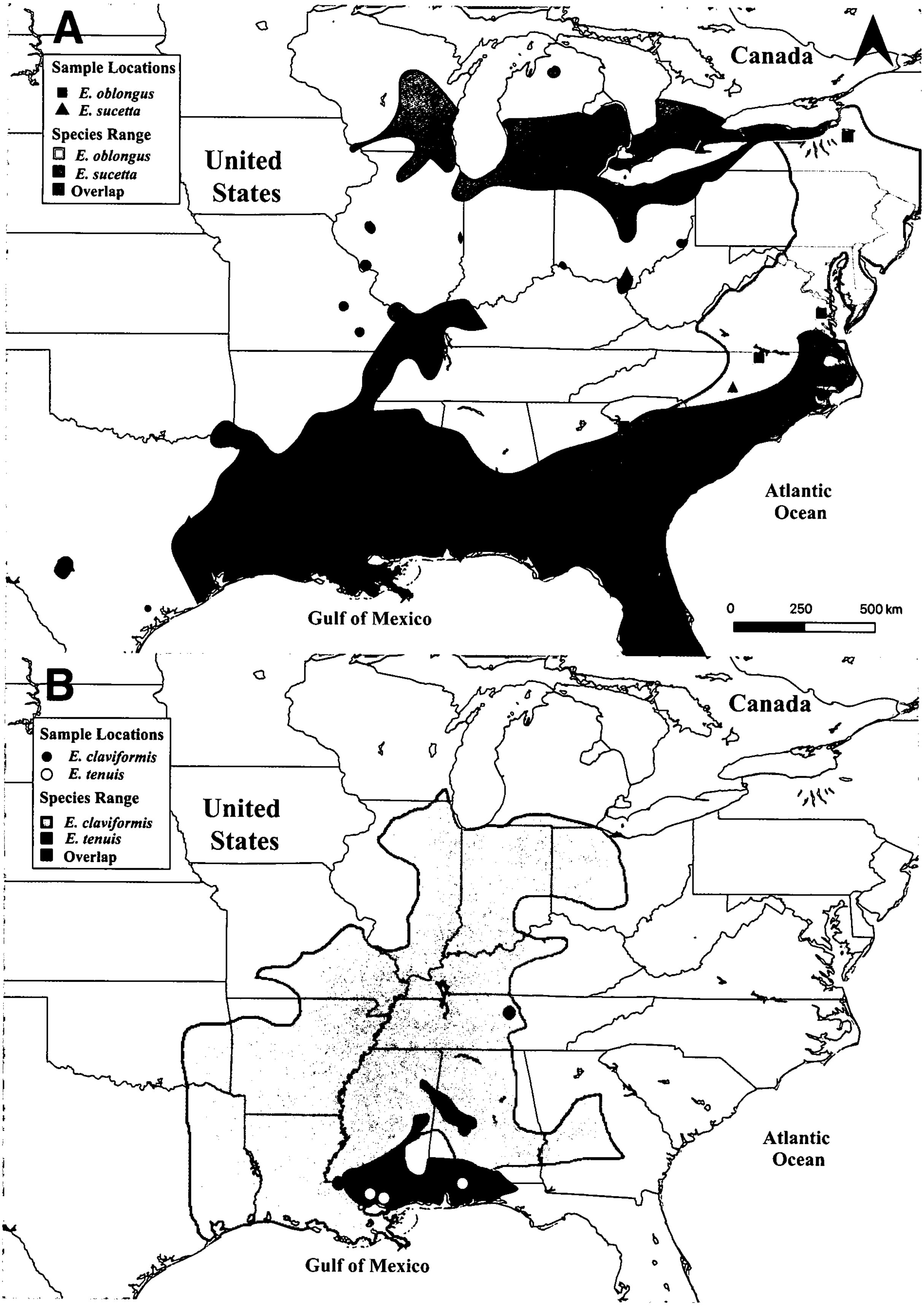

Fig. 1. Maps showing locations where tissue samples from each species (circle *E. claviformis*, square *E. oblongus*, triangle *E. sucetta*, open circle *E. tenuis*) were acquired and the described distribution of each species (modified from Page and Burr, 2011; map A: *E. sucetta* and *E. oblongus*, map B: *E. claviformis* and *E. tenuis*).

Table 1. GenBank accession numbers and available museum voucher numbers for samples used in the study. Museum collection abbreviations follow Sabaj (2020).

Specimen	Voucher ID	Location	COI	IRBP2
Erimyzon claviformis 1	NYSM 68828	Tennessee	MT019584	MT019587
Erimyzon claviformis 2	NYSM 68878	Alabama	MT019585	MT019600
Erimyzon claviformis 3	LSUMZ 14307	Louisiana	MT019583	MT019586
Erimyzon oblongus 1	SLU uncat.	South Carolina	MT019580	MT019588
Erimyzon oblongus 2	NYSM 76911	New York	MT019578	MT019601
Erimyzon oblongus 3	NYSM 70745	North Carolina	MT019579	MT019589
Erimyzon oblongus 4	VIMS 12453	Virginia	MT019577	MT019590
Erimyzon oblongus 5	TCWC 20312.01	South Carolina	MT019570	MT019595
Erimyzon sucetta 1	OSUM 114024C	Ohio	MT019573	MT019598
Erimyzon sucetta 2	OSUM 114024A	Ohio	MT019574	MT019597
Erimyzon sucetta 3	TCWC 17553.03	Texas	MT019576	MT019596
Erimyzon sucetta 4	TCWC 19709.01	Texas	MT019567	MT019591
Erimyzon sucetta 5	TCWC 19709.01	Texas	MT019568	MT019602
Erimyzon sucetta 6	UF 147862	Texas	MT019569	MT019592
Erimyzon sucetta 7	GenBank	Ontario, Canada	KX145453.1	-
Erimyzon sucetta 8	NYSM 72573	North Carolina	MT019575	MT019603
Erimyzon sucetta 9	AUM 2658	Florida	MT019566	MT795953
Erimyzon sucetta 10	UF 171742	Florida	MT019572	MT019593
Erimyzon tenuis 1	SLU uncat.	Louisiana	MT019581	MT019599
Erimyzon tenuis 2	SLU uncat.	Louisiana	MT019582	MT019604
Erimyzon tenuis 3	UF 237618	Florida	MT019571	MT019594
Minytrema melanops	SLU uncat.	South Carolina	MT019565	MT019605

by eye using Sequencher v.5.4.6 (GeneCodes Corporation) or Geneious v.7.1 (Kearse et al., 2012). One GenBank sequence was included in the COI data set, from *E. sucetta* collected in Ontario, Canada, to incorporate a sequence from the northern edge of the species range (GenBank accession number KX145453.1). The resulting alignments for COI and IRBP2 contained a total of 607 bp and 792 bp, across 21 and 20 individuals, respectively. All sequences were submitted to GenBank and their accession numbers are provided in Table 1.

Phylogenetic analysis.—Maximum-likelihood analysis was carried out in RAxML v.8.02 (Stamatakis, 2014) for both genes. Branch support was assessed by employing the bootstopping option in RAxML, which computes bootstrap thresholds during analysis and determines that enough bootstrap replicates have been generated when the difference in branch support is smaller than 3% between 99% of the permutations (Pattengale et al., 2010; Stamatakis, 2014). Bayesian analysis was carried out in BEAST v.2.5 (Drummond et al., 2012), with models of nucleotide evolution selected for each gene by assessing the corrected Akaike information criterion (AICc), Bayesian information criterion (BIC), and decision theory (DT) outputs from jModelTest v.2.1.4 (Darriba et al., 2012). Independent BEAST runs under a strict molecular clock and a relaxed molecular clock were performed to determine which model was most appropriate for each gene. For both COI and IRBP2, runs consisted of 10 million steps after a burn-in of 1 million steps. The sampling interval for both COI and IRBP2 was set to 1,000 trees. To confirm adequate run length, log files were viewed in Tracer v.1.7 (Rambaut et al., 2018) and the effective sample size was assessed. Model (relaxed vs. strict) fit was then assessed by comparing AIC values based on Monte Carlo sampling (AICM) using 100 bootstrap replicates (Baele et al., 2012). For each analysis, TreeAnnotator v.2.5 was used to obtain the maximum clade credibility tree and estimate posterior probabilities for each clade.

RESULTS

The final COI alignment consisted of 607 bp without gaps, and there were 104 variable and 51 parsimony-informative sites. The mean nucleotide composition for COI was as follows: T = 30.8%, C = 26.4%, A = 26.4%, and G = 16.3%. The final alignment for IRBP2 consisted of 792 bp without gaps, and there were 38 variable and 17 parsimony-informative sites. The mean nucleotide composition for IRBP2 was as follows: T = 25.2%, C = 23.8%, A = 27.8%, and G = 23.3%.

Estimation of models of nucleotide evolution from jModelTest resulted in the K80+G and K80+I models for COI and IRBP2, respectively. Additionally, model comparison using AICM showed that a relaxed molecular clock was preferred for COI and a strict molecular clock for IRBP2 in Bayesian analysis. Inferred phylogenetic relationships for COI were congruent between methodologies (maximum-likelihood vs. Bayesian) and resolved the same clades, though Bayesian analysis produced higher support values for most nodes (Figs. 2, 3). Both analyses obtained five major clades (A1–D) of *Erimyzon.* Clade A1 contained individuals of *E. sucetta* from Ontario, Ohio, and Texas. Clade A2 mostly comprised individuals identified as *E. sucetta* from Florida and North Carolina. Clade B contained *E. oblongus,* clade C contained *E. tenuis,* and clade D contained *E. claviformis.* Both phylogenetic hypotheses generated using COI sequence data recovered clade A2 (*E. sucetta* from eastern part of the range) as more closely related to clade B (*E. oblongus*), than to clade A1 (*E. sucetta* from the western part of the range).

Phylogenetic hypotheses generated using IRBP2 sequence data differed in tree topology between methodologies (Figs. 4, 5). The maximum-likelihood analysis (Fig. 4) resulted in a

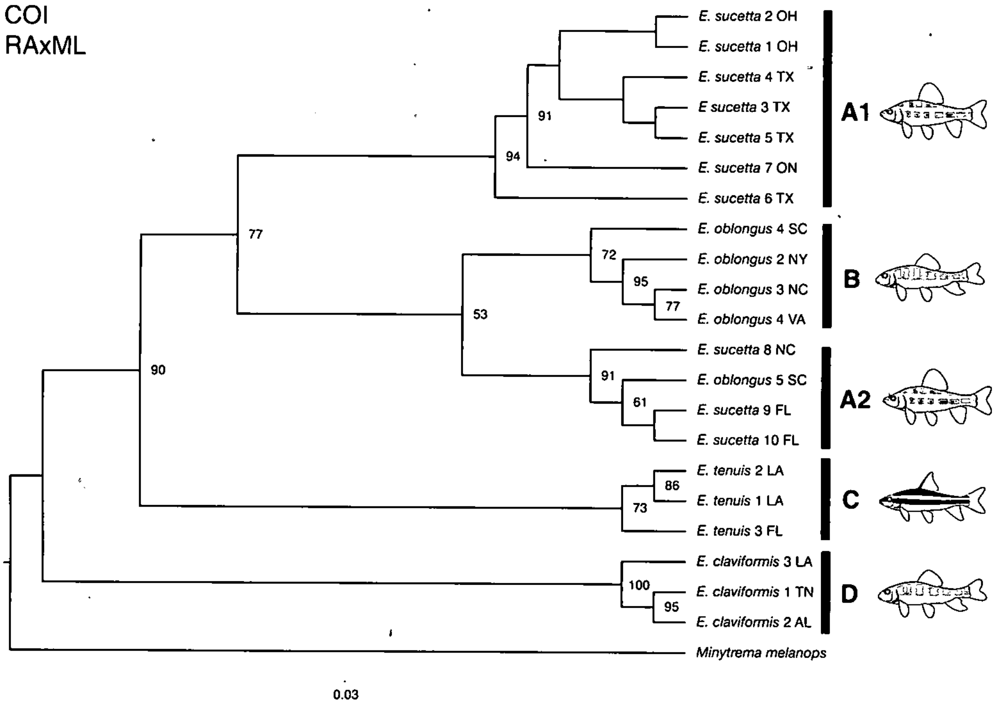

Fig. 2. COI genetree of *Erimyzon* inferred from maximum-likelihood analysis using RAxML v.8.02. Values at nodes represent bootstrap support. State abbreviations: AL, Alabama; FL, Florida; LA, Louisiana; NC, North Carolina; NY, New York; OH, Ohio; ON, Ontario, Canada; SC, South Carolina; TN, Tennessee; TX, Texas; VA, Virginia. See Data Accessibility for tree file.

topology that included clade D as the sister taxon to all other remaining taxa. The maximum-likelihood analysis also recovered clade A2 as paraphyletic with respect to clades B plus C. In this analysis, clade A1 was the sister taxon to the clade comprising clades A2, B, and C. The Bayesian analysis (Fig. 5) obtained clade A1 plus A2 in a sister-group relationship, with clade D as the sister taxon to A1 and A2. Clades B and C were obtained in a sister-group relationship and together this clade represents the sister taxon to the clade comprising clades A1, A2, and D. Support values for relationships resulting from the analyses of the IRBP2 dataset were generally lower than those resulting from the analyses of the COI dataset.

There was a discrepancy in the placement of two individuals between the different gene trees. In the COI analyses, one individual of *E. oblongus* (*E. oblongus* 5) was obtained as a member of clade A2, whereas in the IRBP2 analyses it was obtained as a member of clade B with all other *E. oblongus*. One individual of *E. claviformis* (*E. claviformis* 3) was obtained as a member of clade D with all other *E.*

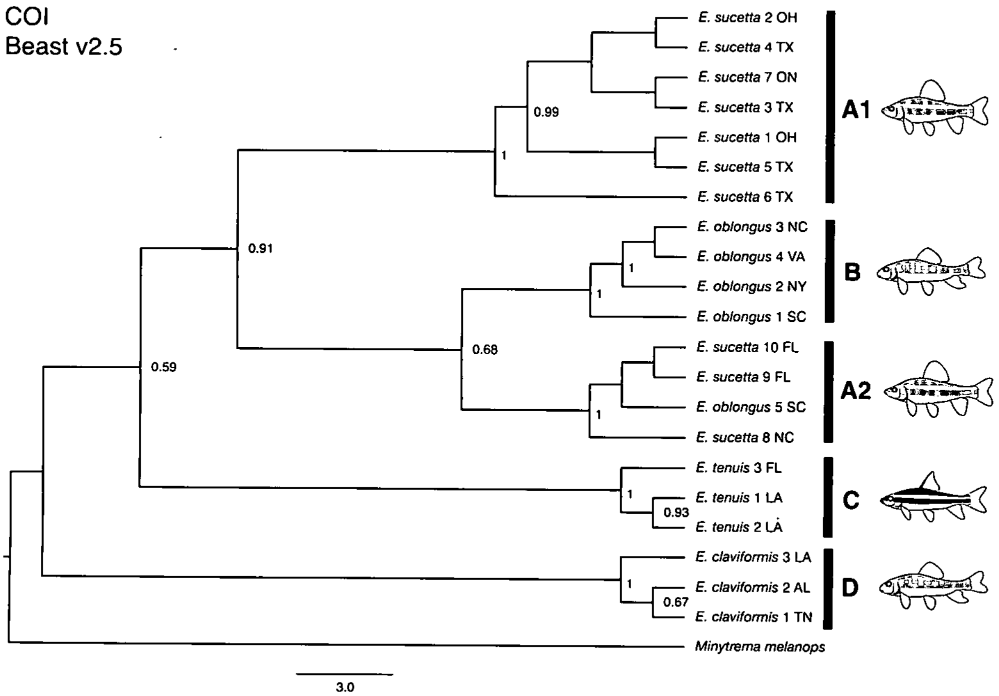

Fig. 3. COI genetree of *Erimyzon* inferred from Bayesian analysis, using Beast v2.5. Values at nodes represent posterior probability support. State abbreviations: AL, Alabama; FL, Florida; LA, Louisiana; NC, North Carolina; NY, New York; OH, Ohio; ON, Ontario, Canada; SC, South Carolina; TN, Tennessee; TX, Texas; VA, Virginia. See Data Accessibility for tree file.

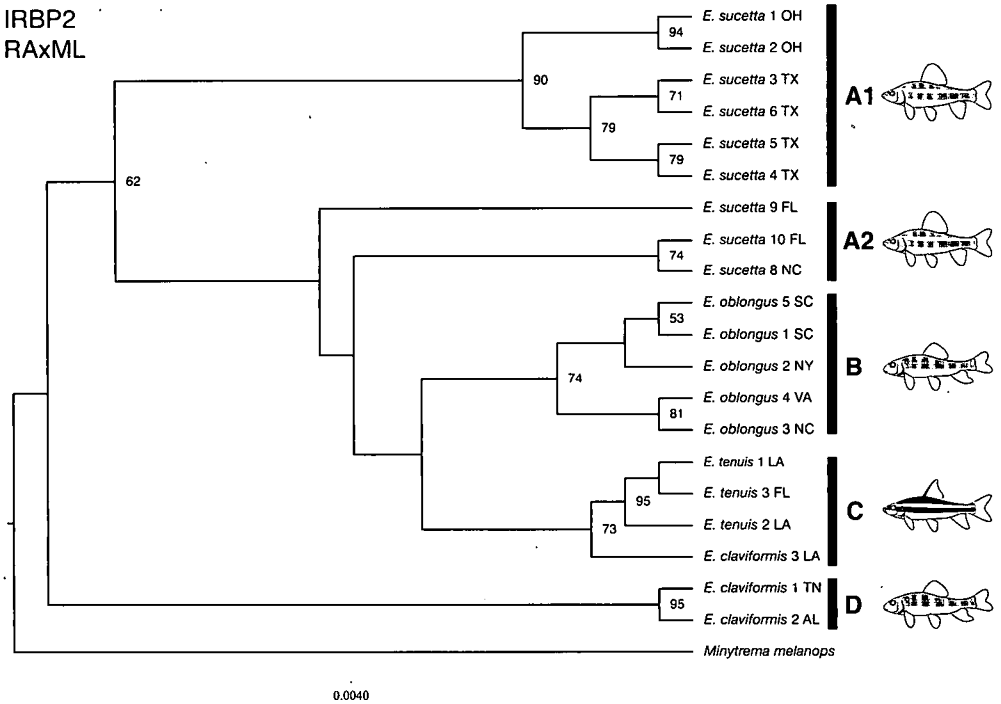

Fig. 4. IRBP2 genetree of *Erimyzon* inferred from maximum-likelihood analysis, using RAxML v.8.02. Values at nodes represent bootstrap support. State abbreviations: AL, Alabama; FL, Florida; LA, Louisiana; NC, North Carolina; NY, New York; OH, Ohio; ON, Ontario, Canada; SC, South Carolina; TN, Tennessee; TX, Texas; VA, Virginia. See Data Accessibility for tree file.

claviformis in the COI analyses, but as a member of clade C in the IRBP2 analyses.

DISCUSSION

The genus *Erimyzon* has been resolved as monophyletic in all previous phylogenetic investigations, although hypotheses of relationships between the species included in these previous studies have been inconsistent (Smith, 1992; Harris and Mayden, 2001; Doosey et al., 2010; Chen and Mayden, 2012; Bagley et al., 2018). Three of the four phylogenetic hypotheses generated herein using both maximum-likelihood and Bayesian approaches resolved five clades within the genus *Erimyzon*. Individuals identified as *E. sucetta* were recovered in two clades, with clade A1 comprising individuals from Ontario, Ohio, and Texas, and clade A2 comprising individuals from North Carolina and Florida. Further, *E. sucetta*, as currently recognized, was recovered as paraphyletic in three out of four analyses.

The results of the COI analysis in this study are similar to those of Smith (1992) in placing *E. oblongus* and *E. sucetta* as sister groups, contrary to the findings of Bagley et al. (2018), in which *E. sucetta* and *E. tenuis* were sister groups. The results of our IRBP2 analyses are less clear, as the topologies obtained using different methodologies were incongruent. These

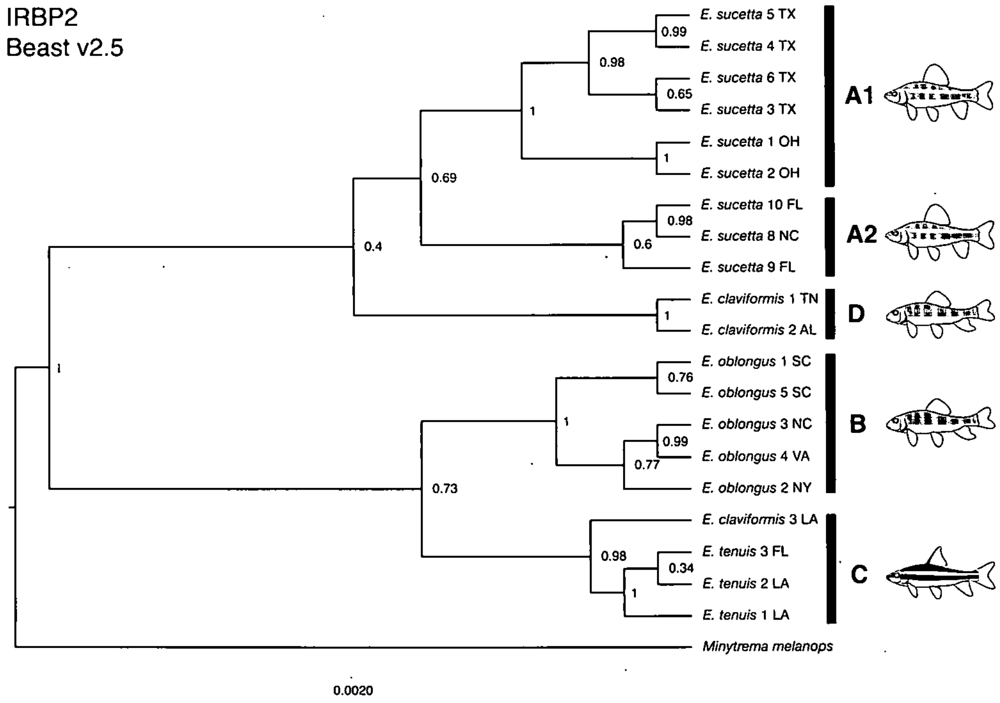

Fig. 5. IRBP2 genetree of *Erimyzon* inferred from Bayesian analysis, using Beast v2.5. Values at nodes represent posterior probability support. State abbreviations: AL, Alabama; FL, Florida; LA, Louisiana; NC, North Carolina; NY, New York; OH, Ohio; ON, Ontario, Canada; SC, South Carolina; TN, Tennessee; TX, Texas; VA, Virginia. See Data Accessibility for tree file.

results are somewhat similar to those of Chen and Mayden (2012), as our BEAST analysis yielded *E. oblongus* and *E. tenuis* as sister species, though the monophyly of *E. oblongus* and *E. tenuis* are uncertain in their results. In contrast to previous work, our study included *E. claviformis,* and in three of the four analyses, this species was recovered as the sister group to all other species of *Erimyzon*. Many previous phylogenetic studies do not provide a list of voucher specimens from which morphological characters or DNA sequences were obtained (e.g., Smith, 1992; Chen and Mayden, 2012), and this makes it difficult to compare results between studies, especially given the relatively recent removal of *E. claviformis* from the synonymy of *E. oblongus* (Bailey et al., 2004). For example, it is unclear whether the material of *E. oblongus* included in the study of Smith (1992) would represent true *E. oblongus* or specimens that now would be identified as *E. claviformis*. The potential non-monophyly of *E. oblongus,* as reported by Chen and Mayden (2012), could also be an artifact of the taxonomic decision of Bailey et al. (2004) to recognize *E. claviformis,* especially if the sequences of *E. oblongus* used were from individuals collected within the range of *E. claviformis*. As Chen and Mayden (2012) do not reference museum voucher specimens or provide general localities for the source of specimens/sequences used in their study, the reported non-monophyly of *E. oblongus* (and also *E. tenuis*) by those authors is difficult to reconcile.

When comparing topologies generated using COI and IRBP2, two individuals were recovered as members of different clades: *E. oblongus* individual 5 (obtained as a member of clade A2 in COI analyses and clade B in IRBP2 analyses) and *E. claviformis* individual 3 (obtained as a member of clade D in COI analyses and clade C in IRBP2 analyses). Because specimens in both cases were obtained from areas of sympatry, this likely indicates current or past hybridization between species of *Erimyzon* that overlap in distribution. Fishes hybridize more frequently than any other vertebrate group, with hybridization occurring more frequently between freshwater fishes than marine fishes (Hubbs, 1955; Campton, 1987). Multiple factors influence the rate of hybridization including unequal density of parental species and increased encounters in areas of secondary contact, to name but two (Hubbs, 1955; Campton, 1987), and anthropogenic habitat loss or alteration of stream systems has likely increased hybridization rates by bringing species into contact that may have otherwise been separated (Scribner et al., 2000). Hybridization has been documented in many different groups of North American freshwater fishes including centrarchids (reviewed by Bolnick, 2009), darters (Keck and Near, 2009), cyprinids (Broughton et al., 2011), and catostomids (Bart et al., 2010; Harris et al., 2014), but not in the genus *Erimyzon* (though see Chen and Mayden, 2012). Gene flow between evolutionarily independent lineages appears to play an important role in the evolution of freshwater fishes, but it also makes species delimitation difficult (Dowling and DeMarias, 1993; Willis et al., 2012). In the context of this study, it is unclear whether contemporary or historical hybridization are driving the results, but a more thorough sampling of the species of *Erimyzon* is needed, especially in putative areas of overlap, to estimate the frequency and timing of such events and to understand how gene flow among putative species has shaped contemporary patterns of diversity.

Overall, the results of the phylogenetic analyses are indicative of five evolutionarily independent groups within the genus *Erimyzon,* rather than four, suggesting species designations within the genus need to be re-visited. Clade A2 most likely represents *E. sucetta,* because the species was described based on specimens obtained from "the rivers of the Carolinas" (Lacepède, 1803), though no type material is extant (Gilbert, 1998). Clade A1, also composed of individuals identified as *E. sucetta,* was distinct from clade A2 in all analyses and likely represents an additional, currently unrecognized species

The geographic distributions of two clades recovered within *E. sucetta*, east and west of the Appalachians, appear consistent with the biogeographic break described between Apalachicola, Florida, and Mobile Bay in Alabama (Wiley and Mayden, 1985). Swift et al. (1986) summarized the distributions of over 200 freshwater fishes in the southeastern United States and found that the majority of species boundaries were centered roughly on this area. This biogeographic break does not just encompass strict species boundaries, concurrent patterns in population structure of *Amia calva, Lepomis punctatus, L. gulosus*, and *L. microlophus* are consistent with a break in that area also (Bermingham and Avise, 1986). Many species of freshwater fishes have a similarly "U-shaped" distribution centered on the Atlantic slope, to the east and the Mississippi River basin to the west, with a gap in their range within the Appalachians. A notable example is *Aphredoderus sayanus*, which has been described as having three groups based on morphology: one from the Atlantic slope (*A. s. sayanus*), one from the Mississippi River basin to the Great Lakes (*A. s. gibbosus*), and an intermediate form from the southern Atlantic slope and eastern Gulf slope (Boltz and Stauffer, 1993). Preliminary genetic data show high variability among localities with little overlap among ddRAD sequenced genomic fragments (C. B. Dillman, pers. comm., unpubl. data), but more data in terms of geographic sampling and genome coverage are necessary to test this hypothesis. While there are indeed common, repeating biogeographic patterns for different species and species-pairs inhabiting the freshwaters of the Atlantic and Gulf slopes, there are also likely species-specific evolutionary responses to coastline changes and other climatic events (Bagley et al., 2013). Further exploration of the population structure of fishes and other freshwater organisms with largely congruent distributions is needed to better define the timing and frequency of vicariance and/or dispersal events that result in contemporary biogeographic patterns.

Several hypotheses have been put forth to explain the contemporary biogeographic pattern observed in freshwater fishes of the southeastern United States. Bermingham and Avise (1986) suggested that heightened sea levels during the Pliocene interglacial period (2–5 mya) would have pushed freshwater species into refugia above the fall line. Once sea levels began to recede, individuals were able to colonize newly exposed coastal habitats from highland habitats associated with Gulf and Atlantic drainages. Wiley and Mayden (1985) suggested that patterns of species distributions of fishes in the Gulf drainages were likely the result of vicariance events associated with the Mobile Bay and/or the Mississippi River drainages. In particular, during the Pliocene, the Tennessee River was connected to the upper Mobile Bay basin, forming the "Appalachian River," which shaped the contemporary distribution of species that lie to the east of

Mobile Bay (are similar to those in the Tennessee River drainage) or to the west (are similar to those in the Mississippi drainage) of Mobile Bay (Swift et al., 1986; Mayden, 1988). There is also evidence that contemporary ichthyofaunal distributions may have been shaped by multiple events through geologic history, allowing for concurrent distributional patterns of species and populations over various time scales (Bermingham and Avise, 1986). These past geologic events are likely responsible for the present distribution of species of *Erimyzon*. While less supported in the nuclear data, the five clades recovered show multiple eastern and western splitting events between clades, with further diversification along the Atlantic slope drainages between *E. oblongus* and clade A2 of *E. sucetta*. Further investigation with more nuclear data (e.g., internal transcribed spacer 1, nuclear ribosomal protein S7) and a greater geographic sampling is needed to better understand patterns of diversity within *Erimyzon*. Furthermore, it may be that other cryptic genetic lineages are present but not sampled in this study.

Though the current study cannot resolve the species status of individuals in the western clade of *E. sucetta*, the genetic distinction observed between the two clades of *E. sucetta* and their apparent disjunct geographic distributions are consistent with the presence of a fifth species in the genus *Erimyzon*. The name *Moxostoma kennerlii*, based on material collected from near Victoria, Texas, and currently in the synonymy of *E. sucetta*, is available and could be reinstated for the western clade (clade A1 herein). The current study lays an initial framework for a taxonomic revision of *Erimyzon*, although examination of the morphology of specimens exhaustively sampled from throughout the currently accepted range of *E. sucetta* (in particular, but the other nominal species as well) is necessary prior to revising the taxonomy of the genus and will be paramount to understanding the distribution and number of species in the genus. This step will be crucial to characterize both the geographic distribution and the morphologically distinguishing characters of the two putative species currently confused under the name *E. sucetta*, as well as detecting and describing other potential instances of cryptic diversity.

DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyologyandherpetology.org/i2020115. 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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