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Nuclear gene-inferred phylogenies resolve the relationships of the enigmatic Pygmy Sunfishes, *Elassoma* (Teleostei: Percomorpha)

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ABSTRACT

Elassoma, the Pygmy Sunfishes, has long proven difficult to classify among the more than 15,000 species of percomorph fishes. Hypotheses dating to the 19th Century include *Elassoma* in Centrarchidae or in the monogeneric Elasmomatidae, and more recent phylogenetic hypotheses have classified *Elassoma* in Smegmamorpha that also contained Synbranchiformes, Mugiliformes, Gasterosteiformes, and Atherinomorpha. No published phylogenetic analysis of morphological or molecular data has supported the monophyly of Smegmamorpha, or a consistent resolution of *Elassoma* relationships. In this study, we investigated the phylogenetic relationships of *Elassoma* and test the monophyly of Smegmamorpha with a nucleotide dataset comprising 10 protein-coding nuclear genes sampled from 65 percomorph species. Maximum likelihood analyses of each individual gene and the concatenated 10 genes all result in strong support for a clade composed of *Elassoma* and Centrarchidae, and no analysis supports monophyly of Smegmamorpha. Based on these results, a rank-free phylogenetic definition of Centrarchidae is presented that includes *Elassoma*, and the continued recognition of Smegmamorpha is discouraged. We discuss the implications of these phylogenetic analyses for relationships of several other percomorph lineages, including Kyphosidae, Terapontidae, Kuhlidae, Cheilodactylidae, Percichthyidae, Howellidae, Enoplosidae, Siniperacidae, and Cirrhitidae.

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“The relationships of *Elassoma* remain obscure, and I am not optimistic about the potential for a clear resolution of this problem based on morphological data alone.” Johnson (1993:17)

1. Introduction

As noted by Johnson (1984, 1993), *Elassoma* is one of several species-depauperate percomorph fish clades that have proven difficult to place in a phylogenetic context using morphological data. As progressively larger comparative DNA sequence datasets have been gathered and analyzed to infer the phylogeny of teleost fishes, a few lineages that had proven difficult to integrate morphologically remain problematic across this diversity of molecular

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phylogenetic studies. The phylogenetic affinity of *Elassoma*, or the Pygmy Sunfishes, remains one of the best known, most studied, and most contentious of these small phylogenetically enigmatic percomorph lineages. *Elassoma* is a clade of seven species endemic to freshwater habitats in drainages of the Gulf of Mexico and Atlantic coastal plains, and the Lower Mississippi River Basin (Berra, 2001: pp. 437–439; Nelson, 2006: p. 388; Snelson et al., 2009), and its apparently neotenic nature has confounded efforts to identify a clear sister group for this lineage.

In the original description of *Elassoma*, Jordan (1877: pp. 50–51) noted, “it possibly... belongs to the family Cichlidae... This species seems to bear little relation to any of the genera of Cichlidae... I therefore propose to consider it as forming a distinct subfamily.” Later, *Elassoma* was recognized as an independent monogeneric perch-like fish family, Elasmomidae (=Elasmomatidae) (Hay, 1881; Jordan and Gilbert, 1883: p. 461). Shortly thereafter, it was classified as a genus in the North American endemic Centrarchidae that also contains the sunfishes, blackbasses, rockbasses, and crappies (Boulenger, 1895: p. 34). For the next 90 years, authors variously treated *Elassoma* as either a centrarchid (e.g., Berg, 1947: p. 474)

or its own family that was not believed to be closely related to centrarchids (e.g., Branson and Moore, 1962).

Progress on the phylogenetic placement of *Elassoma* has been, and continues to be hampered by this clade's paedomorphic features that includes the reduction of lateral-line canal bearing bones, reduced size, and loss of late developing bones (Branson and Moore, 1962; Eaton, 1953, 1956; Johnson, 1984; Moore and Sisk, 1963; Wiley and Johnson, 2010). Looking beyond its traditional classifications, Johnson (1984: p. 467, 1993: p. 17) noted that *Elassoma* shares numerous morphological features (e.g., proximal base of the medial half of the uppermost pectoral ray does not extend laterally to form a process for articulation with the scapular condyl and the ossified portion of the ethmoid consists of two, closely applied, disk-like bones) with teleost lineages as diverse as Percopsiformes, Atherinomorpha, and Gasterosteioidei. In a more explicit study, Johnson and Patterson (1993) recognized the clade, Smegmamorpha, containing the Synbranchiformes (swamp and spiny eels), Mugiliformes (mulletts), *Elassoma*, Gasterosteiformes (sticklebacks and sea horses), and Atherinomorpha (silversides, halfbeaks, and killifishes). This species-rich clade was united by the presence of a single morphological synapomorphy, the first epineural inserting on a parapophysis or lateral process of its centrum (Johnson and Patterson, 1993; Wiley and Johnson, 2010). As should be clear from these varying hypotheses, recent phylogenetic studies using morphological data have inconsistently placed *Elassoma* among the more than 15,000 species of percomorphs, although these anatomical studies have been trending toward a phylogenetic resolution of *Elassoma* nearest to either the gasterosteoids or the synbranchiforms, which are often presented as closely related groups (Johnson, 1993; Springer and Orrell, 2004; Wiley and Johnson, 2010).

During the last decade, molecular phylogeneticists have begun to weigh in on the relationships of *Elassoma* with surprising results. Mirroring the morphological hypotheses, where the data point to a handful of possible relationships, molecular and combined morphological and molecular studies (see below) have resulted in just a few possible placements for *Elassoma*: a subset of smegmamorphs, Centrarchidae, Tetraodontiformes and allies, and Labridae. It is critical to note that among all of the published studies including molecular data, only Wiley et al. (2000), Smith and Wheeler (2006), and Li et al. (2009) have included *Elassoma*, Centrarchidae, and representatives of all major components of the Smegmamorpha. Subsequent to Johnson and Patterson (1993), no published phylogenetic analysis of morphological characters (Springer and Orrell, 2004), molecular datasets (e.g., Chen et al., 2003; Dettai and Lecointre, 2008; Miya et al., 2001; Smith and Wheeler, 2006), or combined morphological and molecular datasets (Wiley et al., 2000) have resulted in a monophyletic Smegmamorpha. Despite the extensive and repeated resolution of non-monophyly of Smegmamorpha in analyses of morphology and both mtDNA and nuclear gene-inferred phylogenies, the group continues to be recognized in classifications of teleost fishes as recently as 2010 (Wiley and Johnson, 2010).

Given the persistent non-monophyly of Smegmamorpha and pre-phylogenetic hypotheses that *Elassoma* and Centrarchidae are closely related, it is valuable to review recent efforts that included *Elassoma* in explicit phylogenetic analyses. A parsimony analysis of 55 morphological characters (principally gill arch features) resolved *Elassoma* in a clade containing Gasterosteiformes (*sans* Syngnathiformes, see Kawahara et al., 2008) and Synbranchiformes (Springer and Orrell, 2004). Molecular data has resulted in diverse phylogenetic placements of *Elassoma*. Analyses of whole mtDNA genome sequences resulted in a clade containing a caproid and a single sampled tetraodontiform, plus *Elassoma* (Miya et al., 2001), or a clade containing *Elassoma* and Labridae (Setiamarga et al., 2008). Two analyses of nuclear gene sequences resolved *Elassoma*

as related to a clade composed of sampled Tetraodontiformes, Lophiiformes, Acanthuroidei, and Caproidae (Dettai and Lecointre, 2008; Holcroft, 2004). Interestingly, Elmerot et al. (2002) and Smith and Wheeler (2006) using whole mtDNA genomes, or a combination of mitochondrial and nuclear DNA sequence data, respectively, both resolved *Elassoma* as related to components of the former Smegmamorpha, particularly synbranchiforms. It is similarly noteworthy that three phylogenetic analyses have resulted in the monophyly of Centrarchidae and *Elassoma* that reflects early pre-phylogenetic hypotheses of their relationships (Jones and Quattro, 1999; Li et al., 2009; Santini et al., 2009). The limited taxon sampling (fewer than ten families included), and the study's reliance on less than one kilobase of mtDNA sequence data, limits the confidence in the resolution of Centrarchidae and *Elassoma* as a clade in Jones and Quattro's (1999) molecular study of *Elassoma* phylogeny. Similarly, a weakness of the studies by Santini et al. (2009) and Li et al. (2009) was the sampling of only one and two genes, respectively. Nevertheless, the phylogenies supporting monophyly of *Elassoma* and Centrarchidae are important because both of these studies sampled a large number of percomorph lineages.

In the present study, we investigate the phylogenetic relationships of *Elassoma* using a dataset comprising protein coding exon regions of 10 unlinked nuclear genes sampled across a wide diversity of percomorph teleosts. The taxon sampling includes all lineages that have been hypothesized as closely related to *Elassoma*, particularly centrarchids, cichlids, labrids, tetraodontiforms, and all major lineages of Johnson and Patterson's (1993) Smegmamorpha. With this dataset of more than 9000 base pairs, we provide a multi-locus analysis of *Elassoma* phylogenetic affinities, as well as an assessment of the monophyly of Smegmamorpha. In addition to providing phylogenetic resolution of *Elassoma* and testing the monophyly of Smegmamorpha, the phylogenetic perspective resulting from these analyses provides insights into the relationships of several problematic moronoid/"percid" percomorph lineages whose relationships have typically been unresolved, including Kyphosidae (sea chubs), Terapontidae (grunters), Kuhlidae (flagtails), Cheilodactylidae (morwongs), Percichthyidae (temperate perch), Enoplosidae (oldwife), Siniperidae (Asian basses), and Cirrhitidae (hawkfishes). Thus, our results represent an important step in resolving the phylogenetic relationships of the hyperdiverse percomorph radiation.

2. Materials and methods

The phylogenetic analyses were rooted with two species of non-percomorph acanthomorphs, *Sargocentron vexillarium* (Holo-centridae) and *Percopsis omiscomaycus* (Percopsidae), following the results of phylogenetic analyses using morphological and molecular characters (Johnson and Patterson, 1993; Miya et al., 2003; Smith and Wheeler, 2006). The 68 species sampled for this study are listed in Table 1. We sampled ten of the eleven genes used in Li et al. (2010). Species were selected to include all major lineages of Centrarchidae (Centrarchinae, *Lepomis*, and *Micropterus*), the lineages that comprise Johnson and Patterson's (1993) Smegmamorpha, and percomorph lineages found closely related to Centrarchidae in an earlier analysis of mtDNA and nuclear gene sequences (Smith and Craig, 2007).

Tissues from fish specimens used in DNA extractions were preserved in 70–95% ethanol or were obtained from museum collections and are presented in Table S1 as Supplemental Online Data. Genomic DNA was extracted from muscle or fin clips using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA). The polymerase chain reaction was used to amplify ten PCR fragments with the primers and cycling conditions outlined in Lopez et al. (2004) for

Table 1
Species sampled and their classification.

Beryciformes
Holocentridae
<i>Sargocentron vexillarium</i>
Percopsiformes
Percopsidae
<i>Percopsis omiscomaycus</i>
Percomorpha
Acropomatidae
<i>Acropoma japonicum</i>
Adrianchthyidae
<i>Oryzias latipes</i>
Centrarchidae
<i>Acantharchus pomotis</i>
<i>Elassoma okefenokee</i>
<i>Elassoma zonatum</i>
<i>Lepomis cyanellus</i>
<i>Lepomis gulosus</i>
<i>Micropterus dolomieu</i>
<i>Micropterus salmoides</i>
Centropomidae
<i>Centropomus undecimalis</i>
<i>Lates calcarifer</i>
Channidae
<i>Channa melasoma</i>
Cheilodactylidae
<i>Cheilodactylus pixi</i>
<i>Cheilodactylus variegatus</i>
<i>Chirodactylus brachydactylus</i>
Cichlidae
<i>Cichla temensis</i>
<i>Heros appendiculatus</i>
Cirrhitidae
<i>Amblycirrhitus pinos</i>
<i>Cirrhitichthys falco</i>
<i>Neocirrhites armatus</i>
<i>Paracirrhites arcatus</i>
Enoplosidae
<i>Enoplosus armatus</i>
Gasterosteidae
<i>Gasterosteus aculeatus</i>
Howellidae
<i>Howella brodiei</i>
<i>Howella zina</i>
Kyphosidae
<i>Kyphosus cinerascens</i>
<i>Kyphosus elegans</i>
Labridae
<i>Bodianus rufus</i>
Lateolabracidae
<i>Lateolabrax japonicus</i>
Mastacembelidae
<i>Monopterus albus</i>
Moronidae
<i>Dicentrarchus labrax</i>
<i>Morone chrysops</i>
Mugilidae
<i>Mugil cephalus</i>
Nandidae
<i>Nandus nandus</i>
Oplegnathidae
<i>Oplegnathus punctatus</i>
Percichthyidae
<i>Gadopsis marmoratus</i>
<i>Maccullochella peelii</i>
<i>Macquaria ambigua</i>
<i>Nannoperca australis</i>
<i>Percalates colonorum</i>
<i>Percalates novemaculeata</i>
<i>Percilia irwini</i>
Percidae
<i>Perca flavescens</i>
Priacanthidae
<i>Pristigenys alta</i>
Sciaenidae
<i>Sciaenops ocellatus</i>

Serranidae
<i>Holanthias chrysostictus</i>
Sinipercaidae
<i>Coreoperca whiteheadi</i>
<i>Siniperca chuatsi</i>
<i>Siniperca knerii</i>
<i>Siniperca obscura</i>
<i>Siniperca roulei</i>
<i>Siniperca scherzeri</i>
<i>Siniperca undulata</i>
Terapontidae
<i>Hephaestus fuliginosus</i>
<i>Scortum barcoo</i>
<i>Terapon jarbua</i>
Tetraodontidae
<i>Takifugu rubripes</i>
<i>Tetraodon nigroviridis</i>
Zoarcidae
<i>Lycodes diapterus</i>

RAG1 exon 3 and Li et al., (2007, 2010) for *myh6*, *plagl2*, *Sidkey*, *SLC10A3*, *sreb2*, *UBE3A* (UBE), *UBE*-like (UBEL), *zic1*, and *znf503*. Double-stranded amplifications were performed in a 25 μ L volume containing 1 μ L 25 mM MgCl₂ (Qiagen, Valencia, CA), 2.5 μ L 10 \times CL PCR buffer (Qiagen, Valencia, CA), 2.5 μ L Q solution (Qiagen, Valencia, CA), 0.5 μ L 10 mM dNTP mix, 0.5 μ L of each primer at 10 mM, 0.3 μ L Taq DNA polymerase (Invitrogen Co., Carlsbad, CA), and 2.0 μ L of DNA template.

The double-stranded amplification products were desalted and concentrated using AMPure beads (Agencourt Biosciences, Beverly, MA). Both strands of the purified PCR fragments were used as templates for cycle sequencing and were read using a 3730 \times L DNA analyzer (Applied Biosystems, Foster City, CA). Contiguous sequences were built using Sequencher (Gene Codes, Ann Arbor, MI) from DNA sequences of the complementary heavy and light strands. Heterozygous sites within individual specimens were minimal, scored as "N" and treated as missing data in the phylogenetic analyses. All new DNA sequences were submitted to GenBank.

All of the genes used are protein coding, so the DNA sequence alignments were constructed from alignments of the translated amino acid sequences constructed using the computer program MUSCLE (Edgar, 2004). The concatenated ten gene dataset contained 9150 base pairs. Three data partitions were designated that corresponded to the three separate codon positions among all ten sampled protein coding genes. The optimal evolutionary model for each codon position pooled across the ten genes was selected using the Akaike Information Criterion (AIC) as executed in MrModeltest 2.3 (Nylander, 2004). Optimal models were set in a partitioned maximum likelihood analysis using the computer program RAxML 7.2.6 (Stamatakis, 2006). We used the -D option, which stops the ML searches when they have reached the asymptotic convergence phase. The criterion for stopping the searches is based on computing the Robinson–Foulds (RF) distance (Robinson and Foulds, 1981) between two consecutive intermediate trees and stopping the search when the RF distance is smaller than 1% (Stamatakis, 2011). Support for nodes in the tree inferred with RAxML was assessed using a thorough bootstrap analysis (option -f i) with 500 replicates. In addition to analyzing the concatenated dataset, the phylogenetic resolution of *Elassoma*, the monophyly of Smegmamorpha, and the monophyly and bootstrap support for Centrarchidae were assessed in the separate gene trees inferred from each of the sampled 10 loci.

3. Results

The two species of *Elassoma* and seven species of Centrarchidae *sensu stricto* (s.s.) sampled in this study were sequenced for all 10 of

the nuclear genes. As few as 50 (*Sidkey*) and as many as all 68 (*myh6*) sampled specimens were sequenced for each of the 10 genes and all sequences are available on Genbank with accession numbers JQ352809–JQ353202 (Table S1). The inability to sequence a species for a given gene was typically the result of failure to amplify the gene using PCR, or in a very few cases the amplification and sequencing of a genomic region that was different from the targeted gene. The aligned sequences of the 10 genes ranged in size from 672 (*UBE*-like) to 1371 (*RAG1*) base pairs. The optimal evolutionary model for each of the three codon positions across the 10 sampled genes identified using AIC and model fitting was GTR+I+G.

The RAxML phylogeny inferred from the concatenated 10 gene dataset had 53 of the 67 nodes supported with a high bootstrap value ($\geq 75\%$) and 33 of those nodes were supported with a bootstrap value of 100% (Fig. 1). *Elassoma* was resolved as the sister lineage of a monophyletic Centrarchidae s.s. and this node was present in 100% of the bootstrap replicates. Siniperidae was resolved as the sister lineage of the *Elassoma*–Centrarchidae s.s. clade with 93% bootstrap support. There were two nested clades in the phylogeny that contained *Elassoma* and Centrarchidae s.s. The more inclusive of the two clades had a bootstrap score of 99% and contained *Percalates*, Kyphosidae, Kuhliidae, Terapontidae, Enoplosidae, Cheilodactylidae, Percichthyidae, Cirrhitidae, Siniperidae, Centrarchidae s.s., and *Elassoma*. The nested and more exclusive clade was supported with 100% bootstrap support and contained Enoplosidae, Cheilodactylidae, Percichthyidae, Cirrhitidae, Siniperidae, *Elassoma*, and Centrarchidae s.s. (Fig. 1).

Smegmamorpha was not resolved as monophyletic in the phylogeny inferred from the 10 gene dataset. As discussed above, *Elassoma* was resolved as the sister lineage of Centrarchidae s.s. and nested deeply in a clade containing other “percoid” lineages. *Mugil cephalus* (Mugiliformes) and *Oryzias latipes* (Atherinomorpha) were resolved in a clade with the two sampled species of Cichlidae. *Monopterus albus* (Synbranchiformes) was the sister lineage of a clade containing *Channa melasoma* (Channidae) and *Nandus nandus* (Nandidae). *Gasterosteus aculeatus* (Gasterosteiformes) was sister to *Lycodes diapterus* (Zoarcidae) and nested in a clade containing *Holanthias chrysostictus* (Serranidae) and *Perca flavescens* (Percidae). Of the five major lineages comprising Johnson and Patterson's (1993) Smegmamorpha, no two were resolved as sister lineages (Fig. 1).

The RAxML analyses of the individual loci resolved *Elassoma* and Centrarchidae s.s. as sharing a most recent common ancestor in nine of the ten gene trees (Fig. 2), and Smegmamorpha was not monophyletic in any of the ten gene trees (not shown). The *UBE* gene tree was the only one that did not resolve *Elassoma* and Centrarchidae s.s. as monophyletic, but contained a polytomy consisting of *Elassoma*, Centrarchidae s.s., Cheilodactylidae, and Siniperidae. Support for the *Elassoma*–Centrarchidae s.s. clade among the nine gene trees varied from very low bootstrap values (e.g., 22% in the *SLC10A3* gene tree) to strong bootstrap support (e.g., 93% in the *Sidkey* gene tree); however, most of the gene trees exhibited moderate support for this relationship (Fig. 2). Centrarchidae s.s. was monophyletic in nine of the gene trees with the *plagl2* gene tree as the exception (Fig. 2), where *Elassoma* was nested within Centrarchidae s.s.

Given the strong support for the monophyly of the traditional sunfishes, including *Elassoma* (Fig. 1), we provide a formal redefinition of Centrarchidae. We follow those previous authors who concluded that pygmy sunfishes (*Elassoma*) were closely related to other centrarchids (e.g., Berg, 1947; Boulenger, 1895; Nelson, 1976).

Centrarchidae Cope (1868: p. 216) [T.J. Near & W.L. Smith], converted clade name. Comments on name – The name Centrarchidae has long been applied to species classified in *Lepomis*,

Micropterus, *Acantharchus*, *Archoplites*, *Ambloplites*, *Enneacanthus*, *Centrarchus*, and *Pomoxis*. Definition (node-based) – The least inclusive clade containing *Ambloplites rupestris* (Rafinesque) and *Elassoma zonatum* Jordan. Composition – Includes the species designated in the definition and *Acantharchus pomotis* (Baird), *Ambloplites ariommus* Viosca, *Ambloplites cavifrons* Cope, *Ambloplites constellatus* Cashner and Suttkus, *Archoplites interruptus* (Girard), *Centrarchus macropterus* (Lacepède), *Enneacanthus chaetodon* (Baird), *Enneacanthus gloriosus* (Holbrook), *Enneacanthus obesus* (Girard), *Pomoxis annularis* Rafinesque, *Pomoxis nigromaculatus* (Lesueur), *Lepomis auritus* (Linnaeus), *Lepomis cyanellus* Rafinesque, *Lepomis gibbosus* (Linnaeus), *Lepomis gulosus* (Cuvier), *Lepomis humilis* (Girard), *Lepomis macrochirus* Rafinesque, *Lepomis marginatus* (Holbrook), *Lepomis megalotis* (Rafinesque), *Lepomis microlophus* (Günther), *Lepomis miniatus* (Jordan), *Lepomis peltastes* Cope, *Lepomis punctatus* (Valenciennes), *Lepomis symmetricus* Forbes, *Micropterus catatractae* Williams & Burgess, *Micropterus coosae* Hubbs & Bailey, *Micropterus dolomieu* Lacepède, *Micropterus floridanus* (LeSueur), *Micropterus henshalli* Hubbs & Bailey, *Micropterus notius* Bailey & Hubbs, *Micropterus punctulatus* (Rafinesque), *Micropterus salmoides* (Lacepède), *Micropterus treculi* (Vaillant & Bocourt), *Elassoma alabamiae* Mayden, *Elassoma boehlkei* Rohde & Arndt, *Elassoma evergladei* Jordan, *Elassoma gilberti* Snelson, Krabbenhoft, & Quattro, *Elassoma okatie* Rohde & Arndt, *Elassoma okefenokee* Böhlke. Reference phylogeny – Fig. 1. Synapomorphies – Chang (1988: Fig. 29) identified two putative synapomorphies for Centrarchidae, presence of wing-like transverse processes on the first haemal spine, and more than one anal fin pterygiophore anterior to the first haemal spine.

4. Discussion

Our investigation demonstrates that numerous single copy nuclear genes are effective data sources in resolving the phylogenetic relationships of percomorph fishes, whether they are aimed at the investigating the evolutionary relationships of relatively inclusive clades such as *Elassoma*, or are the basis of testing the monophyly of large groups like Smegmamorpha (Fig. 1). These nuclear genes, and others not used in this study (Li et al., 2009, 2010, 2011), have provided substantial phylogenetic resolution across percomorph lineages, and result in similar inferred relationships across independent loci (e.g., Fig. 2). In addition, a growing number of percomorph species are being sequenced for these genes, offering increased taxonomic sampling for scores of species on Genbank (e.g., Wainwright et al., in preparation). As the international community of ichthyologists attempt to resolve relationships within the most species-rich polytomy in the vertebrate tree of life (i.e., Percomorpha), continued sequencing of the nuclear genes used in this study across a broad sample of percomorph diversity may provide the first specific working strategy in the long history of ichthyology to offer inclusive taxon sampling of character state data that is amenable to data-driven phylogenetic analyses (e.g., Smith and Craig, 2007). Below we discuss specific results from our analyses that include: (1) the phylogenetic relationships of the Centrarchidae, (2) the non-monophyly of Smegmamorpha, (3) the polyphyly of Percichthyidae, (4) the resolution of a novel clade that contains Centrarchidae, Siniperidae, a non-monophyletic Cirrhitidae, a polyphyletic Percichthyidae, Enoplosidae, Kuhliidae, Kyphosidae, Terapontidae, Oplegnathidae, and Girellidae, and (5) biogeographic implications of the clade containing Centrarchidae and Siniperidae.

The phylogenetic analyses presented in this study argue strongly that *Elassoma* shares common ancestry with the

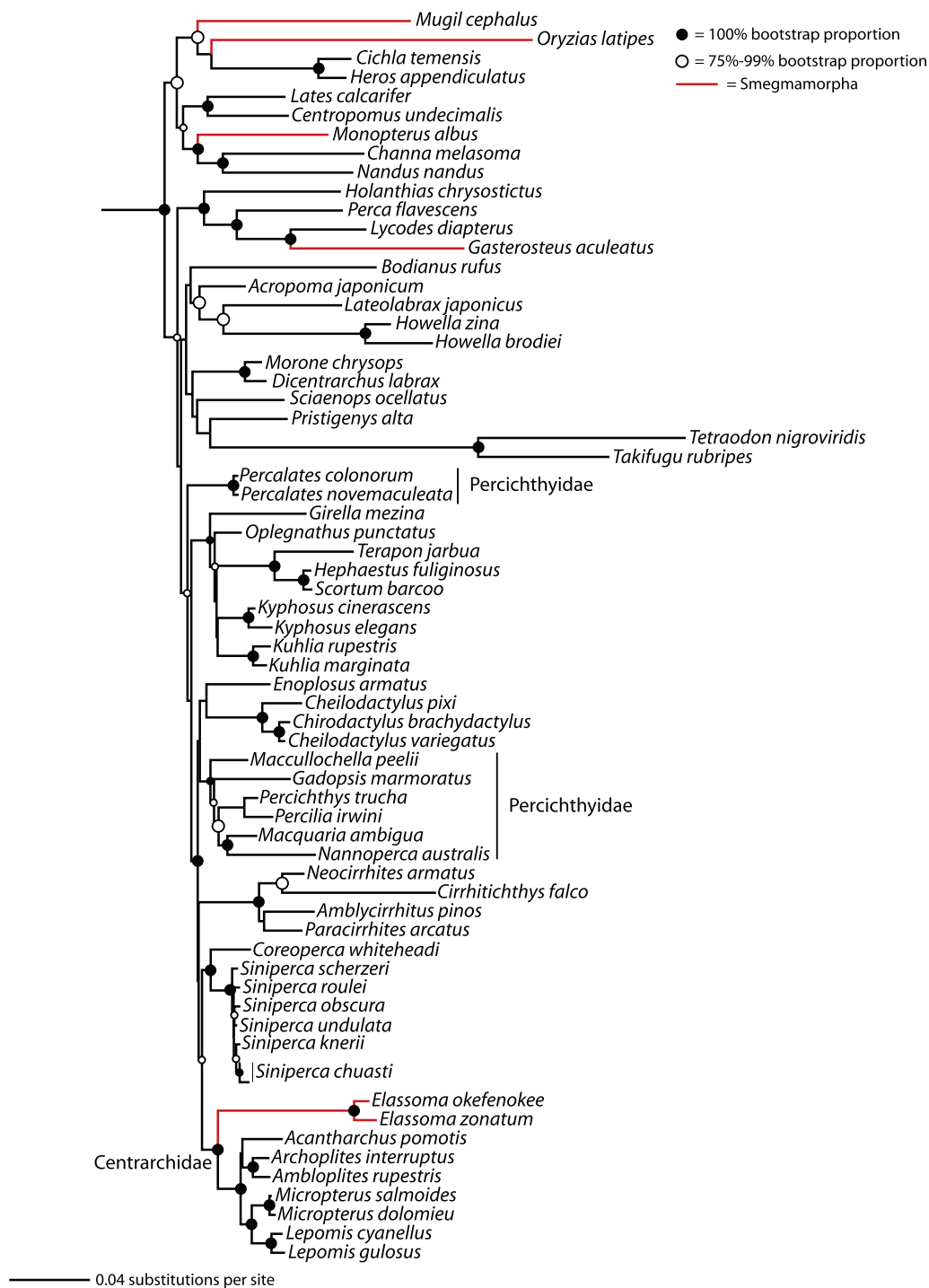


Fig. 1. Phylogeny of 65 species of Percomorpha inferred from a partitioned maximum likelihood analysis of a 10 nuclear gene dataset using RAxML. The phylogeny was rooted with two non-percomorph acanthomorph species, *Percopsis omiscomaycus* and *Sargocentron vexillarium* (not shown). Circles at nodes report percent presence in a bootstrap analysis with 500 replicates. Bootstrap values less than 75% are not shown. Red branches in the phylogeny highlight lineages assigned to Smegmamorpha. Lineages assigned to Percichthyidae and Centrarchidae are marked on the phylogeny.

remainder of the centrarchids (Figs. 1 and 2). In addition to reducing redundancy of taxonomic names that apply to the same clade (*Elassoma* and *Elassomatidae* would both delimit the same set of species and their common ancestor), the recognition of this relationship in the taxonomy of percomorphs will highlight the substantive morphological differentiation exhibited by *Elassoma*, relative to other centrarchids and the closely related siniperids (Fig. 1). The paedomorphic morphology exhibited by *Elassoma*, relative to other percomorphs, was recognized in the first proposal

that *Elassoma* and Centrarchidae were closely related by Boulenger (1895: p. 34), who stated that *Elassoma* “appears to represent a dwarfed form of the *Centrarchidae*, with which it agrees in essential respects.” The only specific anatomical feature cited by Boulenger (1895) as evidence for the relationships of *Elassoma* was that the vertebral column is similar to that observed in all other centrarchids. Jordan and Evermann (1896: p. 982) agreed that *Elassoma* were “dwarfed sunfish” and found “no serious objection” with Boulenger’s (1895) classification of *Elassoma* as a centrarchid, but

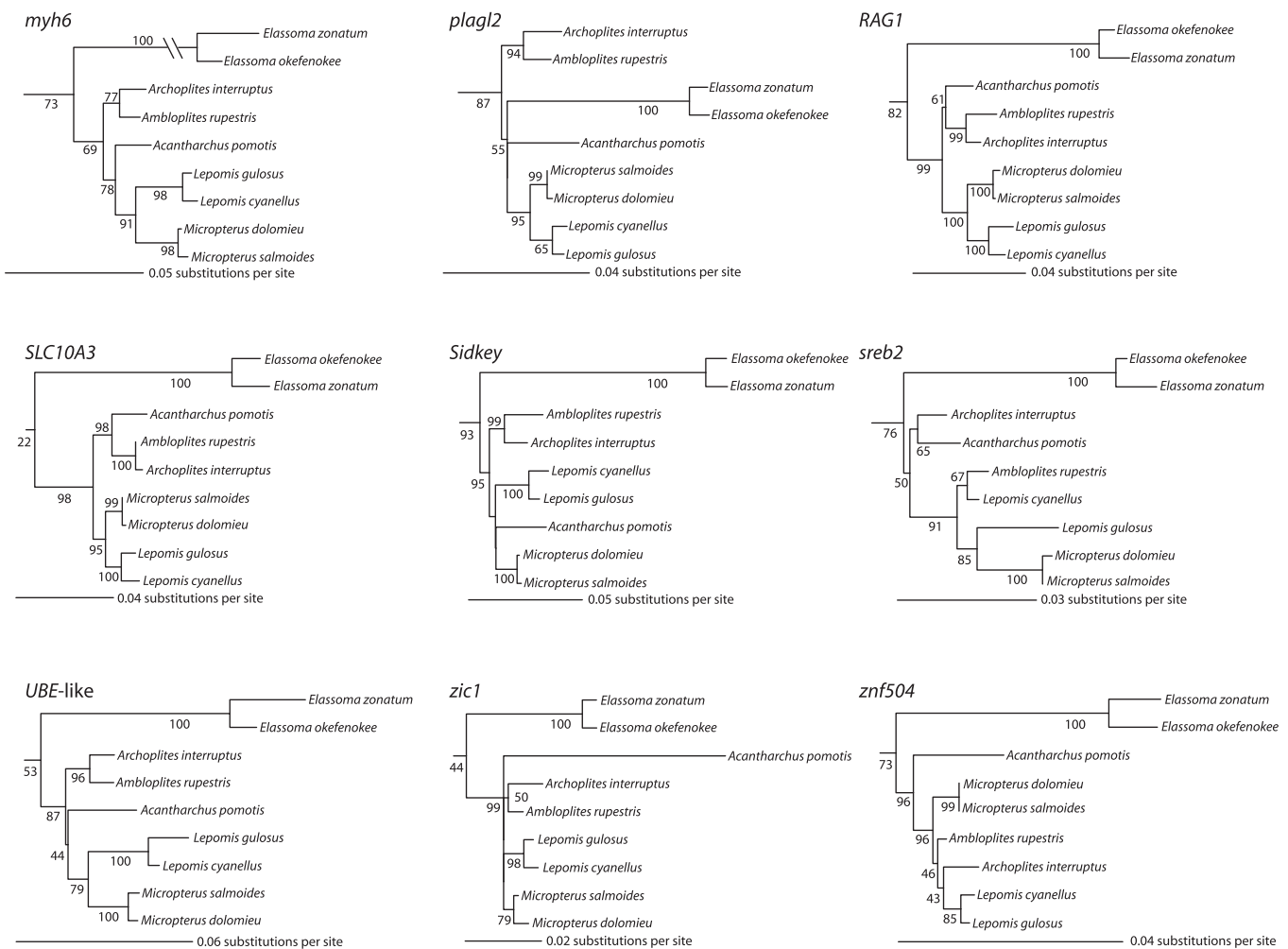


Fig. 2. Gene trees inferred from nine separate loci using partitioned maximum likelihood analyses in RAxML. Numbers at nodes report percent presence in a bootstrap analysis with 500 replicates. Only the relationships among sampled species of Centrarchidae (including *Ellassoma zonatum* and *E. okefenokee*) are shown.

they did classify them in separate family, Ellassomidae (=Ellassomatidae). Subsequent authors noted morphological differences between *Ellassoma* and other centrarchids in the olfactory organs (Eaton, 1956), eyes (Moore and Sisk, 1963), dorsal-fin spines and their associated osteological support structures (Smith and Bailey, 1961), and acustico-lateralis system (Branson and Moore, 1962), culminating in discussions that *Ellassoma* represents a neotenic lineage of Centrarchidae characterized by a suite of reduced morphological traits (Eaton, 1953). However, Johnson (1993) highlighted morphological character states, such as presence of a full neural spine on the second preural centrum, fused haemal spines on the second and third preural centra, a fused neural spine on the first centrum, and the mesethmoid with two discoidal ossifications, which he had not observed at any developmental stage in other “percoid” lineage. The new, well-resolved phylogenetic perspective resulting from the analysis of ten nuclear genes (Fig. 1), coupled with descriptions of the unique morphology in *Ellassoma* provide an interesting system to examine the mechanisms of pedomorphosis from a developmental genetic perspective, as has been investigated in other lineages of percomorphs (e.g., Albertson et al., 2010).

Several anatomical trends and one unique morphological trait in *Ellassoma* prompted Johnson and Patterson (1993) to propose a heterogeneous grouping of percomorph lineages, which included *Ellassoma*, called Smegmamorpha. Our phylogenetic analyses agree with recent morphological and molecular analyses that refute the

monophyly of Smegmamorpha (e.g., Chen et al., 2003; Dettai and Lecointre, 2008; Miya et al., 2001; Smith and Wheeler, 2006; Springer and Orrell, 2004; Wiley et al., 2000), and, in our limited taxon sampling, no two smegmamorph lineages shared common ancestry (Fig. 1). Smegmamorpha has been characterized as a “minimally supported hypothesis” (Springer and Orrell, 2004: p. 247) and has not been accepted in the most widely cited compendiums of ray-finned fish classification (Nelson, 1994, 2006). Johnson and Patterson’s (1993) Smegmamorpha was a novel grouping, informed primarily from one feature discovered in the course of a then ongoing study of intermuscular bones and ligaments (Patterson and Johnson, 1995). Smegmamorpha has not withstood scrutiny from subsequent phylogenetic analyses of either morphological or molecular characters, but the hypothesis, as a whole, was important for developments in percomorph systematics over the past two decades. Johnson and Patterson’s (1993) provocative proposal challenged the prevailing hypothesis that Atherinomorpha was the sister of a Percomorpha that included Beryciformes, Lampriformes, and Zeiformes (e.g., Rosen, 1973), and it forced scientists investigating the phylogenetics of teleosts to look more broadly when exploring relationships among all spiny-rayed fishes.

The use of molecular data to investigate the phylogenetic relationships of percomorphs has resulted in the discovery of several clades that were never suggested or hypothesized from the study of traditional morphological characters. For example, analyses of

both whole mtDNA genomes and nuclear genes consistently resolve a clade that contains Tetraodontiformes (pufferfishes, triggerfishes and relatives) and Lophiiformes (anglerfishes and relatives) (Dettai and Lecointre, 2005; Miya et al., 2003). Our analyses resolve a previously unrecognized clade with 100% bootstrap support that we informally refer to as “centrarchiforms.” It contains Centrarchidae, Cheilodactylidae, Cirrhitidae, Enoplosidae, Girellidae, Kuhliidae, Kyphosidae, Oplegnathidae, Siniperccidae, Terapontidae, and a polyphyletic Percichthyidae (Fig. 1). A similar clade was resolved in a phylogenetic analysis of DNA sequences of two mtDNA rRNA genes and two nuclear genes (histone H3 and 28S rRNA), but the study did not include *Elassoma* or Enoplosidae (Smith and Craig, 2007).

Our analysis did not support monophyly of the included species of Percichthyidae as presented in Nelson (2006). Earlier examination of morphological characters led to the conclusion that the delimitation of Percichthyidae outlined in Gosline (1966) was polyphyletic and should be restricted to *Bostockia*, *Edelia* (= *Nannoperca*), *Gadopsis*, *Maccullochella*, *Macquaria*, *Nannatherina*, *Nannoperca*, *Percalates*, *Percichthys*, *Percilia*, and *Plectroplites* (= *Macquaria*) (Johnson, 1984). The species of Nelson's (2006) Percichthyidae that we sampled were resolved into three clades, (1) the two sampled species of *Howella* were monophyletic, outside of the centrarchiform clade, and the sister lineage of *Lateolabrax* (Lateolabracidae), a result that was observed in the molecular phylogenies presented in Smith and Craig (2007) and Li et al. (2009), which supports the recognition of Howellidae as a lineage distinct from Percichthyidae (Prokofiev, 2007a,b), (2) the two species of *Percalates* were the sister lineage of all other centrarchiforms, and (3) a well-supported clade containing *Maccullochella*, *Gadopsis*, *Percichthys*, *Percilia* (Perciliidae), *Macquaria*, and *Nannoperca*. Despite being synonymized with *Macquaria* based on allozyme and morphological analyses (MacDonald, 1978), *Percalates* was distantly related to the clade of core Percichthyidae containing freshwater species from Australia and South America. Previous phylogenetic analysis of mtDNA sequenced data also found that *Percalates novemaculeata* and *P. colonorum* and other *Macquaria* species were not monophyletic (Jerry et al., 2001). The phylogenetic nesting of *Percilia irwini* in the clade of core Percichthyidae necessitates the synonymization of Perciliidae with Percichthyidae (Fig. 1), a result intimated from analysis of morphology (Arratia, 2003). Future investigation of relationships in the newly discovered centrarchiform clade needs to focus on more complete sampling of lineages that comprise the Cirrhitidae (Burrige and Smolenski, 2004; Greenwood, 1995; Regan, 1911), and the potential inclusion of species classified in Dichistiidae, Microcanthidae, and Scorpididae (Smith and Craig, 2007; Smith and Wheeler, 2006; Yagishita et al., 2009).

The clade containing the entirely freshwater Centrarchidae that are distributed primarily in eastern North America and Siniperccidae that are endemic to eastern Asia exhibits a disjunct geographic distribution between these two areas (Near and Koppelman, 2009; Snelson et al., 2009; Zhou et al., 1988), which is a common pattern in plants (Donoghue and Smith, 2004). The ostariophysan teleost clade Catostomidae is distributed in east Asia and North America, but a substantial number of species are also distributed in western North America (Smith, 1992). The only other freshwater ray-finned fish lineage that exhibits an exclusively disjunct east Asia-eastern North America geographic distribution similar to Centrarchidae and Siniperccidae is the ancient clade Polyodontidae, or paddlefishes (Grande and Bemis, 1991). It is tempting to speculate on the role of features like the Bering Land Bridge as a mechanism connecting areas occupied in the present day by Centrarchidae and Siniperccidae, and paleogeographic changes severing connections between Asia and North America that served as a vicariant mechanism (e.g., Tiffney and Manchester, 2001). However, the Centrarchidae–Siniperccidae clade may not have been entirely

freshwater throughout their history as the Miocene aged siniperccid fossil taxon †*Inabaperca taniurai* is from marine deposits (Yabumoto and Uyeno, 2000). The entire centrarchiform clade contains an interesting mixture of marine and freshwater lineages and increased taxon sampling and finer phylogenetic resolution among clades will provide a historical perspective to investigate the historical biogeography of these lineages and efforts to reconstruct the history of transitions between marine and freshwater habitats in percomorph teleosts.

5. Conclusions

Phylogenetic analyses of exon regions from ten nuclear genes provide very strong resolution of the sister-group relationship between *Elassoma* and centrarchids, a long-standing problem in the systematics of teleost fishes (e.g., Boulenger, 1895; Branson and Moore, 1962; Johnson and Patterson, 1993; Jones and Quattro, 1999). Support for monophyly of Centrarchidae that includes *Elassoma* is strong (Fig. 1), and this clade is present in nine of the ten gene trees (Fig. 2). Our analyses also reject a monophyletic Smegmamorpha, which was diagnosed with a single morphological character state that appears to have a convergent origin across disparate percomorph lineages. Any continued recognition of Smegmamorpha seems unwarranted. The resolution of a newly discovered centrarchiform clade from phylogenetic analysis of the ten nuclear gene dataset highlights the potential for continued clade discovery that exists in the future of percomorph and broader teleost phylogenetics. These newly discovered lineages would certainly provide novel perspectives in studying the evolution and diversification of the most phylogenetically unresolved and species-rich clade of vertebrates.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2012.01.011.

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