

Phylogenetic Relationships of the Genera of North American Sunfishes and Basses (Percoidei: Centrarchidae) as Evidenced by the Mitochondrial Cytochrome *b* Gene

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The phylogenetic relationships of genera of the family Centrarchidae and its affinities to the Elasmomatidae were examined using the mitochondrial DNA sequences of the cytochrome *b* gene. A total of 32 individuals representing 16 species from nine genera of centrarchids were sequenced. Outgroup were drawn from other perciform families including several families previously proposed to be closely related to the Centrarchidae. Phylogenetic analysis was conducted using the optimality criteria of maximum parsimony with two weighting schemes and using the maximum likelihood method. A priori and a posteriori alternative hypotheses of relationships within the Centrarchidae were investigated using the Shimodaira and Hasegawa Test. The results of all analyses support the monophyly of the Centrarchidae, although the interrelationships of centrarchid genera differed between analyses. *Elasoma* was never recovered as the sister taxon to the Centrarchidae; however, the sister relationship of these taxa could not be rejected. Additional data are required to unambiguously resolve relationships among centrarchid genera and determine the relationship of the Centrarchidae to other perciforms.

THE Centrarchidae (sunfishes and basses) are a group of 31 species in nine genera endemic to North America (Burr and Mayden, 1992) and constitute a major component of the ichthyofauna in most eastern North American warm-water ecosystems. Internationally, centrarchid fishes are highly prized in recreational fisheries and are the focus of many studies in aquaculture and commercial fisheries. The evolutionary diversity displayed by these fishes has attracted the attention of a diverse array of scientific studies focusing on behavior (e.g., Gross and Charnov, 1980; Dominey, 1981; Lauder, 1986), development (e.g., Mabee, 1988, 1993), ecology (e.g., Werner, 1977; Mittlebach, 1984; Douglas, 1987), evolutionary (Douglas and Avise, 1982; Mayden, 1986) and functional morphology (e.g., Lauder, 1983; Wainwright and Lauder, 1986, 1992), genetic divergence (e.g., Avise and Smith, 1977; Bermingham and Avise, 1986), and host-parasite coevolution (Klassen and Beverley-Burton, 1988).

The family Centrarchidae is currently placed in the Percoidei, one of the largest and most diverse suborders in the Perciformes (Johnson, 1993). Cope first proposed the name Centrarchidae in a series of papers (Cope, 1865, 1868,

1870), although Bailey (1938) was one of the first to use it in its modern sense. Bailey (1938) also proposed a hypothetical phylogeny for the Centrarchidae, although the placement of several genera was ambiguous. More than half a century later, little consensus has been reached regarding the relationships of the family and the interrelationships of the genera and species. Given the extensive basic and applied research interest in these fishes and the interest of recreational anglers in members of the Centrarchidae, it is remarkable that this group of fishes has not received greater systematic attention.

Although several studies have demonstrated the monophyly of the family, phylogenetic affinities of the Centrarchidae remain elusive (Johnson, 1993; Johnson and Patterson, 1993; Mabee, 1993). Centrarchids have been considered to be related to pygmy sunfishes, family Elasmomatidae (Boulenger, 1895); and for many years *Elasoma* was placed in Centrarchidae. Similarities in scale morphology lead McCully (1962) to suggest that centrarchids were derived from an ancestral group that included the Serranidae, a relationship also noted by other workers (Gill, 1861; Bollman, 1891; Bailey, 1938). McCully (1962) also suggested a possible relationship be-

TABLE 1. CLASSIFICATION OF THE CENTRARCHIDAE FOLLOWING BAILEY (1938) AND REVISED CLASSIFICATION RESULTING FROM THIS STUDY.

Bailey (1938)	This study
Centrarchidae	Centrarchidae
Centrarchinae	Centrarchinae
Ambloplitini	Ambloplitini
<i>Ambloplites</i>	<i>Ambloplites</i>
<i>Acantharchus</i>	Archoplitini
Archoplitini	<i>Archoplitites</i>
<i>Archoplitites</i>	<i>Pomoxis</i>
Centrarchini	Centrarchini
<i>Centrarchus</i>	<i>Centrarchus</i>
<i>Pomoxis</i>	Enneacanthini
Lepominae	<i>Enneacanthus</i>
Enneacanthini	Lepominae
<i>Enneacanthus</i>	Lepomini
Lepomini	<i>Lepomis</i>
<i>Chaenobryttus</i>	incertae sedis
<i>Lepomis</i>	<i>Micropterus</i>
Micropterini	<i>Acantharchus</i>
<i>Micropterus</i>	

tween centrarchids and the Percichthyinae, basal members of Johnson's (1984) Percichthyidae. Others have considered centrarchids to be related to members of the Kuhlidae (Jordan and Everman, 1896; Boulenger, 1895; Bailey, 1938).

Hypotheses of relationships within the family have been equally perplexing and have been developed previously to and following the advent of modern phylogenetic theory and methods. Bailey (1938) used meristic, mensural, and color traits to recognize three tribes in two subfamilies, the Centrarchinae and the Lepominae (Table 1). Branson and Moore (1962) examined variation in the acoustico-lateralis system and proposed a substantially different set of relationships (Fig. 1) than those of Bailey (1938). The phenetic analysis of protein variation by Avise et al. (1977) resulted in a phenogram similar to that produced by Branson and Moore (1962; Fig. 1); Avise and Smith (1977) also presented hypotheses for centrarchid relationships based upon phenetic analysis of protein variation. Other studies have used character argumentation and varied morphological datasets to investigate relationships among species and genera of centrarchids. Mok (1981) examined kidney morphology and Chang (1988) examined a suite of osteological characters as did Wainwright and Lauder (1992) and Mabee (1993) (Fig. 1).

Despite these attempts to construct well-supported phylogenetic hypotheses within the family, relationships of genera and species of the

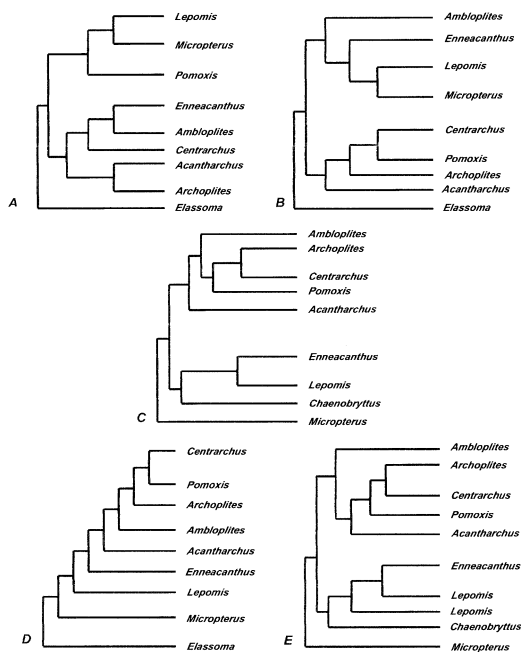


Fig. 1. Alternative hypotheses for relationships among the Centrarchidae. (A) Avise et al. (1977), (B) Branson and Moore (1962), (C) Wainwright and Lauder (1992), (D) Chang (1988), (E) Mabee (1993).

Centrarchidae remain in a state of flux to this day. Similarly, determination of the phylogenetic position of this family relative to other basal perciforms remains unresolved. The development of well-supported hypotheses of relationships of these fishes has ramifications beyond resolving a long-standing systematic question of classification. Our knowledge of the evolutionary relationships of species are prerequisite to evaluating the origin or evolution of their traits and their biogeographic histories (Mayden, 1992). The accurate determination of character polarity in phylogenetic analysis using the outgroup criterion (Wainwright and Wheeler, 1981) is dependent upon inclusion of the sister taxon of the ingroup. Therefore, any examination of the evolution of behavior, functional morphology, or other characters in a phylogenetic context must include the sister taxon to the group of interest.

The objective of this study was to develop phylogenetic hypotheses for relationships among the genera of the Centrarchidae using complete nucleotide sequences of the mitochondrial cytochrome *b* gene and use this phylogeny to assess previously proposed hypotheses of relationships and classifications. The cytochrome *b* gene was chosen because of its utility for investigating phylogenetic relationships at varied hierarchical

levels (Lydeard and Roe, 1997; Song et al., 1998). Furthermore, because cytochrome *b* has been used in a large number of other phylogenetic studies of perciform fishes it offers a variety of taxa for use as outgroups. This offers an opportunity to evaluate the affinities of *Elassoma* to either Centrarchidae or Smegmamorpha as recently proposed by Jones and Quattro (1999) or Johnson (1984) and Johnson and Patterson (1993), respectively.

MATERIALS AND METHODS

Various species of centrarchid taxa were obtained from throughout their respective ranges. Two individuals of each species were included in this study, and every effort was made to include samples from different localities. Taxonomy follows Robins et al. (1991), with the exception of our use of *Chaenobryttus* Gill 1864 as per Wainwright and Lauder (1992).

Total DNA was isolated from either fresh dead, frozen, or ethanol-preserved specimens of centrarchids and outgroup taxa using digestion with proteinase-K followed by phenol-chloroform extraction. Approximately 100 ng of whole DNA was used as template for polymerase chain reaction amplification (PCR) of the complete mitochondrial cytochrome *b* gene using the forward and reverse primer pair from (Song et al., 1998). Amplification conditions were 95 C for 40 sec, followed by 52 C for 60 sec and 72 C for 90 sec for 35 cycles. PCR products were purified using Millipore Ultrafree-MC filters and used as template for sequencing reactions. Both heavy and light strands were cycle sequenced using Big-Dye dye termination chemistry (Perkin Elmer), using the amplification primers and two additional primers: H15149 (Kocher et al., 1989) and cytb703L (5'-CTACTAATTGCCCTAACTT-3'). The products of all sequencing reactions were visualized using an ABI 3100 automated sequencer.

Additional cytochrome *b* sequences were obtained from GenBank for use as outgroups (see Materials Examined). All cytochrome *b* sequences were aligned by eye using XESEE (Cabot and Beckenbach, 1989). No insertion-deletions were observed in the cytochrome *b* dataset. All sequences were translated into amino acid sequences as an additional check of the alignment using MacClade 4.03 (D. R. Maddison and W. P. Maddison, Sinauer Associates, Inc., Sunderland, MA, 2001). Sequences were examined for the presence of saturation by plotting the number of transitions and transversions against p-distance values (Moritz et al., 1992) and patristic distances (Hassanin et al., 1988). Compari-

sons were made for all taxa and for centrarchids only. Sequences were tested for significant phylogenetic signal using both the g-statistic (Hillis and Huelsenbeck, 1992) and the PTP test as implemented in PAUP* (D. L. Swofford, unpubl.). Homogeneity of base frequencies between taxa was examined using the chi-square test.

All phylogenetic analyses were conducted using PAUP* (D. L. Swofford, unpubl.) using two different methods: the optimality criteria of maximum parsimony (MP) and maximum likelihood (ML). Trees were rooted with a representative syngnathid *Hippocampus zosterae*. For MP analyses, searches were conducted using 100 replicates of the heuristic search option keeping only minimum length trees. Initial trees were obtained via stepwise addition of taxa; branch swapping was conducted using the tree-bisection-reconnection option. The model of sequence evolution for ML analysis was determined using ModelTest 3.0 (Possada and Crandall, 1998). In conjunction with PAUP*, ModelTest calculates the likelihood ratio test statistic and its associated *P*-value using a χ^2 to reject or fail to reject different null hypothesis about the process of DNA substitution. Stability of internal branches was inferred from 1000 bootstrap replicates for MP analyses and 200 replicates for ML using PAUP* and by calculating support/decay indices (Bremer, 1988) as implemented in the program Autodecay 4.02 (T. Errikson, unpubl.) for MP topologies only. Comparisons to alternate topologies as proposed by previous systematic studies was made using the Shimodaira-Hasegawa (SH) test for significant differences in likelihood scores of competing hypotheses (Shimodaira and Hasegawa, 1999) as implemented by the program SHTests v1.0 (A. Rambaut, unpubl.).

To evaluate the previously hypothesized relationship between Centrarchidae and Elassomatidae, we constrained *Elassoma* as the sister group to Centrarchidae but did not constrain relationships within the latter. The resulting topologies were evaluated using the SH test.

RESULTS

DNA sequences for all taxa included in these analyses (including outgroups) contained 617 (54%) variable sites, and 538 (47%) parsimony informative sites. Preliminary analysis revealed the presence of significant phylogenetic signal as measured by the g-statistic and the PTP test. The distribution of parsimony informative sites by codon position was 117 at the first position, 48 at the second, and 373 at the third. Uncorrected pairwise sequence divergence (p-dis-

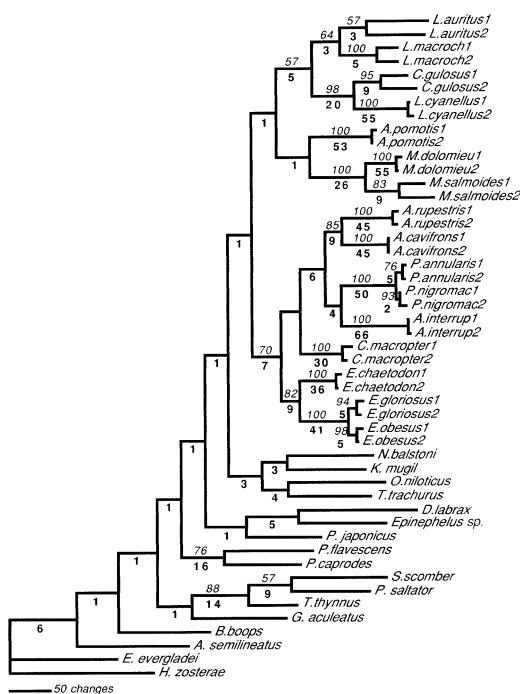


Fig. 2. Single most parsimonious tree resulting from weighting all positions equally. Bootstrap percentages greater than 50% (top) and decay values (bottom) are reported for each node. TL = 4412, CI = 0.254, RC = 0.130.

tance) ranged from 0–29.9% across all taxa, 0–24.8% across centrarchids + *Elassoma*, and 0–20.6% across centrarchids only. Species level differences among the Centrarchidae ranged from 0% for *Ambloplites cavifrons* to 6.8% for *Chaenobryttus gulosus*. Examination of pairwise plots of absolute number of transitions and transversions against p-distance and patristic distance indicated saturation of transitions at the third codon position even for comparisons within the Centrarchidae only (not shown). We explored the effects of multiple hits on the reconstruction of centrarchid phylogeny by conducting phylogenetic searches under parsimony using two weighting schemes: excluding third position transitions and all positions weighted equally. Examination of nucleotide frequencies revealed no significant differences across taxa. Conversion to amino acids revealed a total of 150 variable positions.

The model of sequence evolution determined using hierarchical likelihood ratio tests as implemented in ModelTest 3.0 was the Hasegawa-Kishino-Yano model (HKY 85) and included parameters for the proportion of invariant sites (0.4037) and a gamma shape parameter ($\alpha = 0.7057$). Each phylogenetic analysis result-

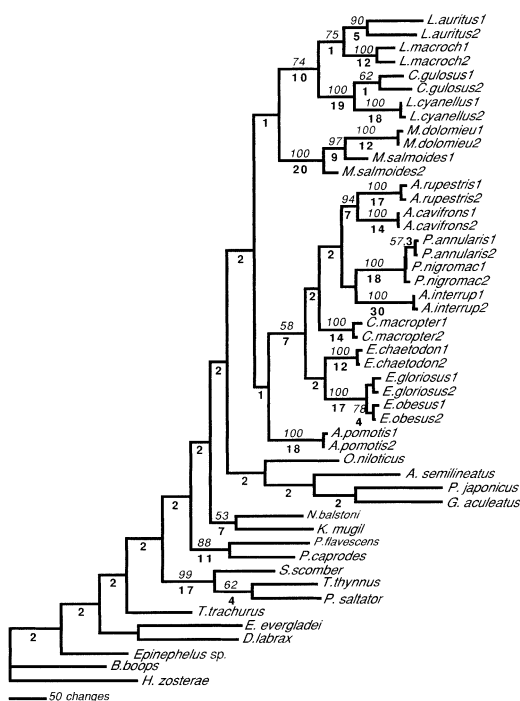


Fig. 3. Single most parsimonious tree derived by excluding third position transitions. Bootstrap percentages greater than 50% (top) and decay values (bottom) are reported for each node. TL = 2114, CI = 0.271, RC = 0.145.

ed in a single topology (Figs. 2–4), each supporting the monophyly of the Centrarchidae and the constituent genera. In general, relationships between genera differed by analyses, although some consistency was observed. All analyses supported *Enneacanthus gloriosus* as sister to *Enneacanthus obesus* and this clade as sister to *Enneacanthus chaetodon*. Similarly, all analyses supported the relationship of *Pomoxis* sister to *Archoplites*, and this clade sister to *Ambloplites*. *Chaenobryttus gulosus* was always resolved as sister to *Lepomis cyanellus*, and these two taxa were always sister to a *L. auritus* + *L. macrochirus* clade. Branch support indices revealed substantial support for most terminal nodes (species) and sub-terminal nodes (genera). However, nodes supporting relationships between some genera received substantially less support. For example, under MP analysis bootstrap and decay support for the *Lepomis* clade was generally higher when third position transitions were excluded, whereas other clades (e.g., *Ambloplites*) decreased and still others (e.g., *Pomoxis*) maintained high bootstrap support with a decrease in decay values. In general, compared to the equally weighted MP analysis, decay values decreased when third

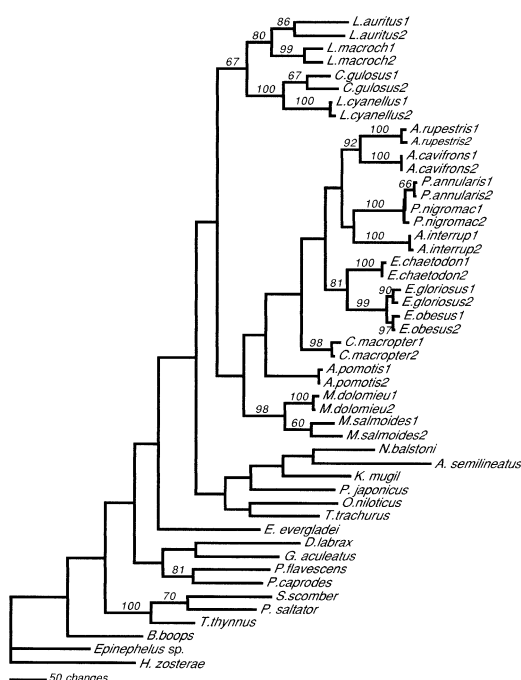


Fig. 4. Maximum likelihood tree generated using the HKY85 + Γ + I model of sequence evolution. $LnL = -18365.01$, $\alpha = 0.7057$ and $I = 0.4037$. Bootstrap percentages greater than 50% are reported for each node.

position transitions were excluded. Bootstrap values for ML analysis were somewhat intermediate to those observed in both MP analyses. In none of the analyses was *Elassoma* (Elassomatiidae) identified as the sister group to Centrarchidae.

The SH tests of alternative topologies revealed that of the alternative hypotheses compared, both of the MP trees and the tree constraining the sister relationship of *Elassoma* and the Centrarchidae could not be rejected as being significantly worse than the ML tree. However, all other phylogenetic hypotheses (Fig. 1) were rejected (Table 2).

DISCUSSION

Relationships within the Centrarchidae.—Earlier systematic treatments of the family identified two or three subfamilies and nine or more genera. Jordan's (1877) evaluation of the Centrarchidae identified three subfamilies and nine genera: the Micropterinae—*Micropterus*, the Centrarchinae—*Centrarchus* and *Pomoxis*, and the Lepominae—*Chaenobryttus*, *Ambloplites*, *Archoplites*, *Acantharchus*, *Lepomis*, and *Enneacanthus*. On the basis of the number of anal fin spines, Bailey

TABLE 2. SUMMARY OF SHIMODAIRA-HASEGAWA TEST OF ALTERNATE HYPOTHESES. *Elassoma* REFERS TO TREES RESULTING FROM CONSTRAINING THE SISTER RELATIONSHIP OF *Elassoma* AND THE CENTRARCHIDAE.

Tree	lnL	ln diff.	Significantly worse	P
Fig. 1A	-18469.56	104.55	Yes	0.001
Fig. 1B	-18480.93	115.92	Yes	<0.001
Fig. 1C	-18604.28	239.27	Yes	<0.001
Fig. 1D	-18432.61	67.59	Yes	0.022
Fig. 1E	-18581.07	216.06	Yes	<0.001
Fig. 2	-18406.99	41.99	No	0.272
Fig. 3	-18415.22	50.21	No	0.132
Fig. 4	-18365.01	—	Best	—
<i>Elassoma</i>	-18398.24	33.24	No	0.352

(1938) divided the Centrarchidae into two subfamilies: the Lepominae—*Lepomis*, *Enneacanthus*, and *Micropterus*, those possessing three anal spines, and the Centrarchinae, including all remaining genera possessing four or more anal spines.

Despite the absence of substantial branch support, analysis of the cytochrome *b* dataset supports the monophyly of Centrarchidae regardless of the phylogenetic analyses employed. Generic and species relationships did differ between analyses (Figs. 2–4). We attribute the lack of stability observed regarding these relationships to the relatively short branches uniting the various centrarchid genera as evidenced by the low bootstrap and decay values. The placement of *C. gulosus* in a genus separate from *Lepomis* as proposed by Wainwright and Lauder (1992) and Mabee (1993) is not supported by our phylogenetic analysis of cytochrome *b* sequence variation. All analyses placed *C. gulosus* as the sister taxon to *L. cyanellus*, a clade that was consistently identified as the sister group to a clade inclusive of the sister species *Lepomis macrochirus* and *Lepomis auritus*.

The sister-group relationship of *Lepomis* and *Micropterus* as proposed by Branson and Moore (1962) and Avise et al. (1977) cannot be rejected in our analysis of cytochrome *b* variability, because one of the three topologies from this study supported this relationship. The affinities of the monotypic genus *Acantharchus* has, according to earlier studies, resided with *Archoplites*, *Pomoxis*, and *Centrarchus* (Branson and Moore, 1962; Avise et al., 1977; Wainwright and Lauder, 1992). Two of the three topologies resulting from our analysis support this affinity, although the parsimony analysis weighting all substitutions equally placed *Acantharchus* as sister to *Micropterus*. Results of the SH test support

the refutation of the sister relationship of *Enneacanthus* and *Lepomis* proposed by Wainwright and Lauder (1992) and Mabee (1993), and the sister relationships of *Centrarchus* and *Pomoxis* (Branson and Moore, 1962; Mok, 1981; Chang, 1988).

Affinities of Ellassoma.—In an extensive review of the acoustico-lateralis system, Branson and Moore (1962) removed *Ellassoma* from the Centrarchidae and placed it in a separate, albeit closely related family, Ellassomatidae. Roberts (1964) examined chromosomal variation and also concluded that *Ellassoma* was not a member of the Centrarchidae. Greenwood et al. (1966) included *Ellassoma* in the Centrarchidae but provided no morphological evidence for the monophyly of this group. Johnson (1984) and Johnson and Patterson (1993) provided morphological evidence supporting the hypothesis that Ellassomatidae is not closely related to the Centrarchidae and instead placed them in the newly formed taxon Smegmamorpha together with mugilids, atherinimorphs, gasterosteiforms, synbranchiforms, and several other taxa. Later, G. D. Johnson and V. G. Springer (1997, unpubl.) proposed, on the basis of morphological data that ellassomatids were most closely associated with the gasterosteiforms. Recently, Jones and Quattro (1999) investigated the affinities of the Ellassomatidae using partial 12S and 16S rRNA gene sequences. Their analyses produced several sets of relationships, including one (Jones and Quattro, 1999:fig. 1) which supported a sister group relationship between Ellassomatidae and Centrarchidae, with this clade sister to Moronidae (sensu Johnson, 1984). Chang (1988) also proposed that the Centrarchidae and Ellassomatidae formed a monophyletic group supported by two morphological characters.

Several of the alternative hypotheses of centrarchid relationships (Branson and Moore, 1962; Avise et al., 1977; Chang, 1988) tested in our paper indicate that *Ellassoma* is the sister taxon to the Centrarchidae. All of these topologies were found to be significantly longer than the most parsimonious solutions (Table 2); however, topologies derived when only the sister group relationship of Ellassomatidae and Centrarchidae was constrained were not significantly different than the shortest trees. Interestingly, the equally weighted MP analysis (with no constraints) placed *Ellassoma* in a trichotomy with a representative smegmamorph (seahorse) and the remaining taxa in our analysis. All other analyses (Figs. 3–4) placed *Ellassoma* as sister to the Moronidae, or as the sister group to a large clade inclusive of Centrarchidae and many oth-

er perciform families. Regardless, *Ellassoma* was never identified as the sister group to either Centrarchidae or *Gasterosteus* in any unconstrained analyses.

Conclusions and taxonomic recommendations.—Although analysis of the cytochrome *b* dataset did not produce a single unambiguous answer regarding the relationships within the Centrarchidae, several taxonomic changes can be suggested with confidence (Table 1). Based on these results *Enneacanthus* should be placed in the subfamily Centrarchinae with *Ambloplites*, *Archoplites*, *Centrarchus*, and *Pomoxis*. The affinities of *Acantharchus* do not lie with *Ambloplites*; and therefore, it should be removed from the *Ambloplitini* and considered *incertae sedis* until its affinities can be determined with certainty. Similarly the ML tree does not support the placement of *Micropterus* in the Lepominae, and we, therefore, refrain from making any formal assignment of this genus at this time. Finally, *C. gulosus* should revert to *L. gulosus* on the basis of priority of *Lepomis* Rafinesque 1819 over *Chaenobryttus* Gill 1864.

When the true phylogeny is unknown, perhaps the best estimator of phylogenetic accuracy is a strongly supported set of relationships. Adding characters has been shown to produce more strongly supported topologies in several cases (e.g., Bremer et al., 1999; Poe and Swoford, 1999; but see Graybeal, 1998). It is apparent from these analyses that the cytochrome *b* gene alone is insufficient to robustly resolve relationships within the Perciformes and that the eventual resolution of the affinities of *Ellassoma* will likely require the use of additional data. We are currently assembling a larger dataset inclusive of morphological characters used in previous examinations of the Centrarchidae as well as additional DNA sequence data from both mitochondrial and nuclear loci. It is hoped that this larger dataset will provide a more robust solution to the question for relationships within the Centrarchidae as well as aid in answering the question of the affinities of *Ellassoma*.

MATERIAL EXAMINED

Institutional abbreviations follow Leviton et al. (1995). KJR tissue extraction numbers, collection localities, voucher specimen collection numbers, and GenBank accession numbers are as follows: *Lepomis auritus* 1 (KJR-18)—Town Ck. at MD Rt. 51, 3.7 mi. ESE Old Town, Potomac River Dr., Allegheny Co., MD. UAIC 12287.01, AY115969; *Lepomis auritus* 2 (KJR-94)—Middle Fork of the Broad River at GA Hwy. 57, WNW

Franklin Springs, Savannah River Dr. Franklin Co., GA. UAIC 12376.02, AY115970; *Chaenobryttus gulosus* 1 (KJR-14)—Hatchet Ck. at FL Hwy. 222, Alachua Co., FL. UAIC 12286.02, AY115971; *Chaenobryttus gulosus* 2 (KJR-107)—Easley Ck. at Clark/Hot Springs Co. line, just east of Witherspoon, Ouachita River Dr., AR. UAIC 12420.01, AY115972; *Lepomis cyanellus* 1 (KJR-8)—Horseshoe Run, at CR. 7, Monongahela River Dr., Tucker Co., W.V. UAIC 12253.07, AY115973; *Lepomis cyanellus* 2 (KJR-146)—Whiteside Ck. at WI. Hwy. 78, 4 mi. NE of Wiota, Pecatonica River Dr. LaFayette Co., WI. UAIC 12528.01, AY115974; *Lepomis macrochirus* 1 (KJR-19)—Orange Ck. at HWY 21, St. Johns River Dr., Putnam Co., FL. UAIC 12290.02, AY115975; *Lepomis macrochirus* 2 (KJR-109)—Easley Ck. at Clark/Hot Springs Co. line, just east of Witherspoon, Ouachita River Dr., AR. UAIC 12420.03, AY115976; *Ambloplites rupestris* 1 (KJR-12)—Horseshoe Run, at CR. 7, Monongahela River Dr., Tucker Co., W.V. UAIC 12253.06, AY115977; *Ambloplites rupestris* 2 (KJR-115)—Indian Ck., bridge at TN Hwy. 64 near Olive Hill, Tennessee River Dr., Hardin Co., TN. SIUC 37911, AY115978; *Ambloplites cavifrons* 1 (KJR-7)—Town Ck. at CR 674 in Philpott, Henry Co., VA. UAIC 12285.02, AY115979; *Ambloplites cavifrons* 2 (KJR-182)—Tar River, Franklin Co., N.C. UAIC 13074.02, AY115980; *Centrarchus macropterus* 1 (KJR-35)—Barnishee Bayou, Tennessee River Dr., Shelby Co., TN. UAIC 11865.02, AY115981; *Centrarchus macropterus* 2 (KJR-165)—Black River at Oak Grove Church Rd. downstream of Pope's Lake, Cape Fear Dr., Harnett Co., N.C. UAIC 13070.07, AY115982; *Enneacanthus chaetodon* 1 (KJR-103)—Gum Swamp Ck., Scotland Co., N.C. UAIC 13139.04, AY115983; *Enneacanthus chaetodon* 2 (KJR-84)—Clark Branch, Mullica River, Camden Co., N.J. UAIC 11844.03, AY115984; *Enneacanthus gloriosus* 1 (KJR-1)—Cedar Ck. at US hwy. 43, Mobile Co., AL. UAIC 11704.14, AY115985; *Enneacanthus gloriosus* 2 (KJR-63)—Everglades canal L-31W, south of FL Hwy. 9336 bridge, Dade Co., FL. UAIC 12367.01, AY115986; *Enneacanthus obesus* 1,2 (KJR-85, 159)—Clark Branch, Mullica River, Camden Co., N.J. UAIC 11844.04, AY115987, AY115988; *Pomoxis annularis* 1 (KJR-157)—Piney Ck., Limestone Co., AL. UAIC 11821.02, AY115989; *Pomoxis annularis* 2 (KJR-166)—Big Muddy River at state hwy. bridge 3 area, Mississippi River Dr., Union Co., IL. UAIC 12610.08, AY115990; *Pomoxis nigromaculatus* 1 (KJR-6)—Beaver Ck., at CR 21, NW of Ottery, Coosa River Dr., St. Clair Co., AL. UAIC 12309.04, AY115991; *Pomoxis nigromaculatus* 2 (KJR-138)—Oxbow near Little River at Dead Mans Point, 4 mi.

south of Broken Bow, McCurtain Co., OK. OSUS 27536, AY115992; *Acantharchus pomotis* 1 (KJR-56)—Sand River, west of Aiken city limit, Savannah River Dr., Aiken Co., S.C. INHS 45049, AY115993; *Acantharchus pomotis* 2 (KJR-82)—Clark Branch, Mullica River, Camden Co., N.J. UAIC 11844.02, AY115994; *Archoplites interruptus* 1,2 (KJR-61)—Lagoon Valley Reservoir, Solano Co., CA., AY115995, AY115996; *Micropterus dolomieu* 1 (KJR-15)—Horseshoe Run, at CR. 7, Monongahela River Dr., Tucker Co., W.V. UAIC 12253.08, AY115997; *Micropterus dolomieu* 2 (KJR-96)—Rockcastle River, ca. 9 km. SSW of Livingston at I-75 Laurel/Rockcastle Counties KY. UAIC 12354.05, AY115998; *Micropterus salmoides* 1 (KJR-133)—Spring Ck. at Rocky Ford State Park, Cherokee Co., OK. OSUS 27528, AY115999; *Micropterus salmoides* 2 (KJR-189)—Jacks Ck. at Hwy. 26 Santee-Cooper Dr. Clarendon Co., S.C. UAIC 12590.09, AY116000; *Perca flavescens*, AF045357; *Percina caprodes*, AF045354; *Epinephelus* sp., AF143193; *Nanatherina balstoni* 1,2 Margaret River, Western Australia, AY116002; *Dicentrarchus labrax*, X81566; *Kuhlia mugil* (KJR-70)—USNM 336646, AY116003; *Apogon semilineatus*, AB018995; *Boops boops*, X81567; *Pomatomus saltator*, AF143199; *Trachurus trachurus*, X81568; *Elassoma evergladei* 1,2 (KJR-54,55)—Haw Ck. at Sweetwater bridge, St. Johns River Dr., 5.5 mi. SSW Bunnell, Flagler Co., FL. INHS 45005, AY116001; *Pseudolabrus japonicus*, AB018993; *Oreochromis niloticus*, AB018989; *Scomber scombrus*, X81564; *Thunnus thynnus*, X81563; *Gasterosteus aculeatus* 1,2 (KJR-100, 101)—Kings River, San Joaquin River Dr., 2 mi. SW of Piedra at Alta Weir. Fresno Co., CA. UAIC 11547.05, AY116004; *Hippocampus zosterae*, AF192706.

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LITERATURE CITED

- AVISE, J. C., AND M. H. SMITH. 1977. Gene frequency comparisons between sunfish (Centrarchidae) populations at various stages of evolutionary divergence. *Syst. Zool.* 26:319-335.
- , D. O. STRANEY, AND M. H. SMITH. 1977. Biochemical genetics of sunfish. IV. Relationships of centrarchid genera. *Copeia* 1977:250-258.
- BAILEY, R. M. 1938. A systematic revision of the centrarchid fishes with a discussion of their distribution, variations, and probable interrelationships. Unpubl. Ph.D. diss., Univ. of Michigan, Ann Arbor.
- BERMINGHAM, E., AND J. C. AVISE. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics* 113:939-965.
- BOLLMAN, C. H. 1891. A review of the Centrarchidae, or freshwater sunfishes of North America. *Rept. U.S. Comm. Fish Fish.* (1888):557-589.
- BOULENGER, G. 1895. Catalog of the Perciform fish of the British Museum. 2d ed. 1-394.
- BRANSON, B., AND G. MOORE. 1962. The lateralis components of the acoustico-lateralis system in the sunfish family Centrarchidae. *Copeia* 1962:1-108.
- BREMER, B., R. K. JANSEN, B. OXELMAN, M. BACKLUND, H. LANTZ, AND K.-J. KIM. 1999. More characters or more taxa for a robust phylogeny-case study from the coffee family (Rubiaceae). *Syst. Biol.* 48:413-435.
- BREMER, K. 1988. The limits of amino acid sequence data in Angiosperm phylogenetic reconstruction. *Evolution* 42:795-803.
- BURR, B. M., AND R. L. MAYDEN. 1992. Phylogenetics and North American freshwater fishes, p. 18-75. *In: Systematics, historical ecology, and North American freshwater fishes.* R. L. Mayden (ed.). Stanford Univ. Press, Stanford, CA.
- CABOT, E. L., AND A. T. BECKENBACH. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comp. Appl. Biosci.* 5:233-234.
- CHANG, C.-H. M. 1988. Systematics of the Centrarchidae (Perciformes: Percoidei) with notes on the haemal-anal-axial character complex. Unpubl. Ph.D. diss., City Univ. of New York, New York.
- COPE, E. D. 1865. Partial catalogue of the cold-blooded vertebrates of Michigan. *Proc. Acad. Nat. Sci. Phila.* 17:78-88.
- . 1868. On the distribution of fresh-water fishes in the Allegheny Region of southwestern Virginia. *J. Acad. Nat. Sci. Phila. N.S.* 6:207-247.
- . 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. *Proc. Am. Philo. Soc.* 11:448-495.
- DOMINEY, W. J. 1981. Maintenance of female mimicry as a reproductive strategy in bluegill sunfish (*Lepomis macrochirus*). *Environ. Biol. Fish.* 6:59-64.
- DOUGLAS, M. E. 1987. An ecomorphological analysis of niche packing and niche dispersion in stream-fish clades, p. 144-149. *In: Community and evolutionary ecology of North American stream fishes.* W. J. Matthews and D. C. Heins (eds.). Univ. of Oklahoma Press, Norman.
- , AND J. C. AVISE. 1982. Speciation rates and morphological divergence in fishes: tests of gradual versus rectangular modes of evolutionary change. *Evolution* 36:224-232.
- GILL, T. N. 1861. Notes on some genera of fishes of the western coast of North America. *Proc. Acad. Nat. Sci. Phila.* 13:164-168.
- GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem. *Syst. Biol.* 47:9-17.
- GREENWOOD, P. H., D. E. ROSEN, S. H. WEITZMAN, AND G. S. MYERS. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. *Bull. Am. Mus. Nat. Hist.* 131:341-455.
- GROSS, M. R., AND E. L. CHARNOV. 1980. Alternative male life histories in bluegill sunfish. *Proc. Natl. Acad. Sci. USA* 77:6937-6940.
- HASSANIN, A., G. LECOINTRE, AND S. TILLIER. 1998. The "evolutionary signal" of homoplasy in protein-coding gene sequences and its consequences for a priori weighting in phylogeny. *C. R. Acad. Sci. Paris* 321:611-620.
- HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise and reliability in phylogenetic analyses. *J. Hered.* 83:189-195.
- JOHNSON, G. D. 1984. Percoidei: development and relationships, p. 464-498. *In: Ontogeny and systematics of fishes.* American Society of Ichthyologists and Herpetologists, Spec. Publ. 1, Lawrence, KS.
- . 1993. Percomorph phylogeny: progress and problems. *Bull. Mar. Sci.* 52:3-28.
- , AND C. PATTERSON. 1993. Percomorph phylogeny: a survey of acanthomorphs and a new proposal. *Ibid.* 52:554-626.
- JONES, W. J., AND J. M. QUATTRO. 1999. Phylogenetic affinities of pygmy sunfishes (*Elassoma*) inferred from mitochondrial DNA sequences. *Copeia* 1999:470-474.
- JORDAN, D. S. 1877. Contributions to North American ichthyology. II, notes on Cottidae, Etheostomatidae, Percidae, Centrarchidae, Aphrododeridae, Umbriidae, Escocidae, Dorysomatidae and Cyprinidae with revisions of the genera and descriptions of new or little known species. *Bull. U.S. Nat. Mus.* 9:1-53.
- , AND B. W. EVERMAN. 1896. The fishes of North and Middle America. *Ibid.* 47:1-1240.
- KLASSEN, G. J., AND M. BEVERLY-BURTON. 1988. North American freshwater ancyrocephalids (Monogea) with articulating haptoral bars: host-parasite coevolution. *Syst. Zool.* 37:179-189.
- KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. F. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mt DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86:6196-6200.
- LAUDER, G. V. 1983. Functional and morphological bases of trophic specialization in sunfishes (Teleostei, Centrarchidae). *J. Morph.* 178:1-21.
- . 1986. Homology, analogy and the evolution of behavior, p. 9-40. *In: Evolution of animal behavior.* M. H. Nitecki and J. A. Kitchell (eds.). Oxford Univ. Press, New York.
- LEVITON, A. E., R. H. GIBBS JR., E. HEAL, AND C. E. DAWSON. 1985. Standards in herpetology and ich-

- thyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia* 1985:802–832.
- LYDEARD, C., AND K. J. ROE. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among actinopterygian fishes, p. 285–303. *In*: Molecular systematics of fishes. T. D. Kocher and C. A. Stepien (eds.). Academic Press, San Diego, CA.
- MABEE, P. M. 1988. Supraneural and predorsal bones in fishes: development and homologies. *Copeia* 1988:827–838.
- . 1993. Phylogenetic interpretation of ontogenetic change: sorting out the actual and artifactual in an empirical case study of centrarchid fishes. *Zool. J. Linn. Soc.* 107:175–291.
- MAYDEN, R. L. 1986. Speciation and depauperate phylads and tests of punctuated and gradual evolution: fact or artifact? *Syst. Biol.* 35:591–602.
- . 1992. Explorations into the past and the dawn of systematics and historical ecology, p. 3–17. *In*: Systematics, historical ecology, and North American freshwater fishes. R. L. Mayden (ed.). Stanford Univ. Press, Stanford, CA.
- MCCULLY, H. H. 1962. The relationship of the Percidae and the Centrarchidae to the Serranidae as shown by the anatomy of their scales. *Am. Zool.* 2: 430.
- MITTELBACH, G. G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). *Ecology* 65:499–513.
- MOK, H.-K. 1981. The phylogenetic implications of the centrarchid kidneys. *Bull. Inst. Zool. Acad. Sin.* 20:59–67.
- MORITZ, C., C. J. SCHNEIDER, AND D. B. WAKE. 1992. Evolutionary relationships within the *Ensatina escholtzii* complex confirm the ring species interpretation. *Syst. Biol.* 41:273–291.
- POE, S., AND D. L. SWOFFORD. 1999. Taxon sampling revisited. *Nature* 398:299–300.
- POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- ROBERTS, F. L. 1964. A chromosome study of 20 species of Centrarchidae. *J. Morph.* 115:401–418.
- ROBINS, R. C., R. M. BAILEY, C. E. BOND, J. R. BROOKER, E. A. LACHNER, R. N. LEA, AND W. B. SCOTT. 1991. Common and scientific names of fishes from the United States and Canada. 5th ed. American Fisheries Society, Spec. Publ. 20, Lawrence, KS.
- SONG, C. B., T. J. NEAR, AND L. M. PAGE. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. *Mol. Phylogenet. Evol.* 10:343–353.
- SHIMODAIRA, H., AND M. HASEGAWA. 1999. Multiple comparisons of log likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16:1114–1116.
- WAINWRIGHT, P. C., AND G. V. LAUDER. 1986. Feeding biology of sunfishes: patterns of variation in the feeding mechanism. *Zool. J. Linn. Soc.* 88:217–228.
- , AND ——. 1992. The evolution of feeding biology in sunfishes (Centrarchidae), p. 472–491. *In*: Systematics, historical ecology and North American freshwater fishes. R. L. Mayden (ed.). Stanford Univ. Press, Stanford, CA.
- WATROUS, L. E., AND Q. D. WHEELER. 1981. The outgroup comparison method of character analysis. *Syst. Zool.* 30:1–11.
- WERNER, E. E. 1977. Species packing and niche complementarity in three sunfishes. *Am. Nat.* 111:553–578.
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