Chapter 1

Species diversity, phylogeny and phylogeography of Centrarchidae

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1.1 Introduction

Centrarchidae is a clade of freshwater fishes endemic to North America, a part of the world that harbors more species of freshwater fishes than any other nontropical region on Earth (Briggs 1986; Lundberg *et al*. 2000). Centrarchid fishes have been of interest to biologists for a long period of time because they are commonly the dominant top-level predators in the diverse communities of freshwater fishes in eastern North America, and as such, they are among the world's most popular freshwater sport fishes (Henshall 1881; Etnier and Starnes 1993; Philipp and Ridgway 2002). Interestingly, it is only in the last 10 years or so that comparative morphological and molecular data have been used in conjunction with objective character-based methods to investigate the phylogenetic relationships of Centrarchidae.

The goal of this chapter is to review and assess previous ideas regarding the diversity and relationships of centrarchid species. We hope to provide biologists from all disciplines with a clear picture of the current and best-supported hypotheses of centrarchid phylogeny, and we intend to illustrate how many recent, cutting-edge efforts have agreed remarkably with studies published as far back as the nineteenth century. Although we realize our esoteric interests in centrarchid diversity and phylogeny, as well as our desire to understand the results of modern phylogenetic analyses in the context of the rich past of centrarchid taxonomy and systematics may be confusing to the average fish biologist or ichthyologist, we will attempt to clarify what seems like a morass of trees and classifications for biologists in need of phylogenetic hypotheses. It is our desire that both comparative biologists and conservation agencies exploit the current state of knowledge regarding centrarchid diversity and phylogenetic relationships.

In this chapter we provide a discussion of the currently recognized diversity of both extant and fossil species in Centrarchidae, and we attempt to illuminate some unresolved issues in this area that need attention in future research efforts. We present an overview of previous investigations and hypotheses concerning the evolutionary relationships of Centrarchidae, including a discussion of recent efforts using morphological and molecular data in addition to those that pre-date the development of phylogenetic systematics, or cladistics (Hennig 1966). Many of the pre-cladistic ideas of centrarchid relationships discussed in this review were presented as purely taxonomic hypotheses, where the hypothesized relationships were implied from the composition and ranking of taxa.

Evolutionary biologists often investigate genetic variation within a geographic context, as intraspecific gene trees often show a strong geographic pattern. Such is the science of phylogeography (Avise 2000). We provide a review and discussion of phylogeography in centrarchids, highlighting some of the problems that have made such analyses in Centrarchidae less straightforward than in species from other groups of North American freshwater fishes.

1.2 Species diversity

1.2.1 Extant species and the status of subspecies

Currently, 34 extant species are recognized in Centrarchidae (Table 1.1), with the most recently described species being *Ambloplites constellatus* and *Micropterus cataractae* (Cashner and Suttkus 1977; Williams and Burgess 1999). As in

Table 1.1 Currently recognized centrarchid species and proposed classification. Fossil genera and species are indicated with a dagger.

Centrarchidae (44 species: 33 extant, 11 extinct)
Centrarchinae
Acantharchus pomotis (Baird 1855) Mud sunfish
Ambloplites ariommus (Viosca 1936) Shadow bass
Ambloplites cavifrons (Cope 1868) Roanoke bass
Ambloplites constellatus (Cashner and Suttkus 1977) Ozark bass
Ambloplites ruprestris (Rafinesque 1817) Rockbass
Archoplites †clarki (Smith and Miller 1985) Clarkia perch
Archoplites interruptus (Girard 1854) Sacramento perch
Archoplites †molarus (Smith *et al*. 2000) Ringold sunfish
Archoplites †taylori (Miller and Smith 1967) Lake Idaho sunfish
†Boreocentrarchus smithi (Schlaikjer 1937) Healy Creek sunfish
Centrarchus macropterus (Lacepède 1801) Flier
Enneacanthus chaetodon (Baird 1855) Blackbanded sunfish
Enneacanthus gloriosus (Holbrook 1855) Bluespotted sunfish
Enneacanthus obesus (Girard 1854) Banded sunfish
†Plioplarchus septemspinosus (Cope 1889) John Day sunfish
†Plioplarchus sexspinosus (Cope 1883) Sentinel Butte sunfish
†Plioplarchus whitei (Cope 1883) Laramie sunfish
Pomoxis annularis (Rafinesque 1818) White crappie
Pomoxis †lanei (Hibbard 1936) Ogallala crappie
Pomoxis nigromaculatus (Lesueur 1829) Black crappie
Lepominae
Lepomis auritus (L 1758) Redbreast sunfish
Lepomis cyanellus (Rafinesque 1819) Green sunfish
Lepomis gibbosus (L 1758) Pumpkinseed
Lepomis gulosus (Cuvier 1829) Warmouth
Lepomis humilis (Girard 1858) Orangespotted sunfish
Lepomis †kansasensis (Hibbard 1936) Rhino Hill sunfish
Lepomis macrochirus (Rafinesque 1819) Bluegill
Lepomis marginatus (Holbrook 1855) Dollar sunfish
Lepomis megalotis (Rafinesque 1820) Longear sunfish
Lepomis microlophus (Günther 1859) Redear sunfish
Lepomis miniatus (Jordan 1877) Redspotted sunfish
Lepomis peltastes (Cope 1870) Northern longear sunfish
Lepomis punctatus (Valenciennes 1831) Spotted sunfish
Lepomis †serratus (Smith and Lundberg 1972) Keigh sunfish
Lepomis symmetricus (Forbes 1883) Bantam sunfish

(continued)

Table 1.1 *(continued)*.

Centrarchidae (44 species: 33 extant, 11 extinct)
Micropterinae
Micropterus cataractae (Williams and Burgess 1999) shoal bass
Micropterus coosae (Hubbs and Bailey 1940) Redeye bass
Micropterus dolomieu (Lacepède 1802) smallmouth bass
Micropterus floridanus (LeSueur 1822) Florida bass
Micropterus henshalli (Hubbs and Bailey 1940)
Micropterus notius (Bailey and Hubbs 1949), Suwannee bass
Micropterus punctulatus (Rafinesque 1819) spotted bass
Micropterus †relictus (Cavender and Smith 1975) Chapala bass
Micropterus salmoides (Lacepède 1802) largemouth bass
Micropterus treculi (Vaillant and Bocourt 1874) Guadalupe bass

many groups of animals, there are many more scientific names available than there are recognized species. Not including the names of valid extant species (Table 1.1), there are 118 nominal names that are considered synonyms for species in Centrarchidae. Of these, 11 were either new names for subspecies or were introduced as species names and have been used at some point to designate subspecies (Gilbert 1998). Of the 118 nominal names, 9 are based on hybrid centrarchids; all but 1 of these are the hybrid combinations of *Lepomis cyanellus * L. macrochirus* and *L. cyanellus * L. gibbosus* (Hubbs 1920; Hubbs and Hubbs 1932; Gilbert 1998).

The contemporary view of species diversity in Centrarchidae was fairly well settled by the turn of the nineteenth and twentieth centuries, as the vast majority of valid centrarchid species were described between 1800 and 1883 (Table 1.1; Figure 1.1). This period was also when most of the synonymous names were introduced (Bailey 1938; Gilbert 1998). Through both the nineteenth and twentieth centuries centrarchid species have been described using very similar types of data from external morphology, including meristics (scale row and fin element counts), morphometrics (body proportions), pigmentation patterns, and coloration (Cope 1868, 1870; Hubbs and Bailey 1940; Cashner and Suttkus 1977; Williams and Burgess 1999). To date, comparative phylogenetic methods, using either morphological or molecular data, have not been used in describing new centrarchid species.

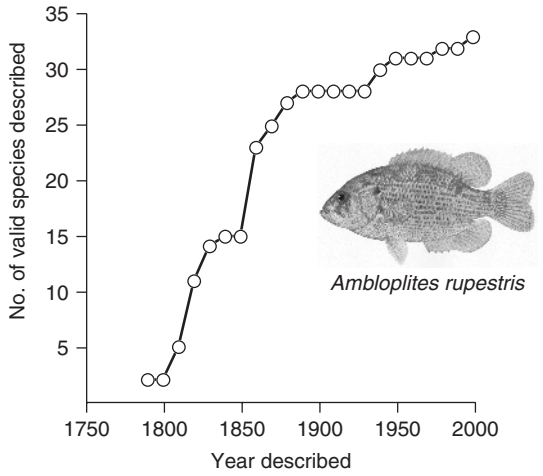

Figure 1.1 Plot illustrating the growth of valid extant centrarchid species descriptions from the nineteenth through twentieth centuries. *Ambloplites rupestris* redrawn from Forbes and Richardson (1920).

The status of subspecies in Centrarchidae is much less resolved when compared to the 33 recognized valid extant species (Table 1.1). The use of subspecies in North American fish taxonomy has a relatively inconsistent history, and since the initial critique of subspecies, most modern workers in systematics have been moving away from using this rank (Wilson and Brown 1953; Burbrink *et al*. 2000). However, there remain 11 names that have been historically designated as centrarchid subspecies. We are able to categorize each of these names into three classes: (i) subspecies that do not exhibit significant variation from the nominal subspecies, (ii) subspecies that are based on hybrid specimens, and (iii) subspecies that merit elevation to species.

Three centrarchid subspecies have been invalidated as it was demonstrated that they did not differ appreciably from other populations of the nominal species. *Acantharchus pomotis mizelli* Fowler and *Enneacanthus chaetodon elizabethae* were both described as subspecies in the 1940s based on six or seven specimens (Bailey 1941; Fowler 1945). In both cases, subsequent analyses that included many more specimens failed to reveal geographic variation consistent with the recognition of the subspecies proposed for each of these species (Sweeney 1972; Cashner *et al*. 1989). A similar situation exists for the Neosho Smallmouth Bass, *Micropterus dolomieu velox* Hubbs and Bailey. This subspecies was described based primarily on slight differences in counts of the second dorsal fin rays, pigmentation patterns, and dentition on the tongue (Hubbs and Bailey 1940). The validity of *M. d. velox* was subsequently dismissed on the basis of slight morphological differences and clinal gradation into the nominal *M. dolomieu* (Bailey 1956; Gilbert 1998), a conclusion supported by more recent analyses of nuclear gene encoded allozymes and mitochondrial DNA (mtDNA) sequence data (Stark and Echelle 1998; Kassler *et al*. 2002).

At least one centrarchid subspecies has turned out to be based on hybrid specimens. *Micropterus punctulatus wichitae* Hubbs and Bailey was described as a subspecies from the Wichita Mountains of southwestern Oklahoma based on differences in scale row counts (Hubbs and Bailey 1940). However, this population was initially described as hybrids of *M. punctulatus* and *M. dolomieu* (Hubbs and Ortenburger 1929). Morphological data from *M. p. punctulatus, M. p. wichitae*, and *M. dolomieu* and historical records of nonnative *M. dolomieu* introductions near the type locality of *M. p. wichitae* support the hypothesis that this subspecies is based on hybrid *M. punctulatus* * *M. dolomieu* specimens (Cofer 1995). Genetic analysis of both nuclear and mtDNA in *M. punctulatus* populations from the Red and Arkansas River Basins did not reveal genetic divergence of the Wichita Mountain populations of *M. punctulatus* (Coughlin *et al*. 2003).

Lepomis megalotis and *L. macrochirus* are two centrarchid species that are thought to be polytypic and contain described subspecies (Mayden *et al*. 1992; Gilbert 1998). Future research documenting morphological and genetic variation in these two complexes has the strong possibility to result in the recognition of additional valid centrarchid species. *L. megalotis* has four, and possibly seven, valid subspecies, *L. m. megalotis* (Rafinesque), *L. m. aquilensis* (Baird and Girard), *L. m. breviceps* (Baird and Girard), and *L. m. occidentalis* Meek (Bailey 1938). In addition, *L. m. convexifrons* (Baird and Girard), *L. m. fallax* (Baird and Girard), and *L. m. popeii* (Girard) are three additional forms from Texas that may represent other unrecognized species related to *L. megalotis* (Gilbert 1998). Unfortunately, there is no published analysis of morphological variation among these subspecies, but a Ph.D. dissertation had detected substantial morphometric variation among four of the described subspecies (Barlow 1980). An analysis of allozyme variation detected appreciable genetic divergence of *L. m. breviceps* and *L. m. aquilensis* relative to the other subspecies (Jennings and Philipp 1992). Based on morphometric and body size differences, *L. peltastes* Cope was elevated as a species from a subspecies of *L. megalotis* (Bailey *et al*. 2004). We suspect that several additional centrarchid species will be recognized as a result of analyses of geographic variation and phylogeny of the *L. megalotis* complex using comparative morphological and molecular data.

There is a degree of uncertainty as to how many subspecies of *Lepomis macrochirus* are recognized. The problem centers on *Pomotis speciosus* described from Brownsville, Texas by Baird and Girard (1854). This species was subsequently synonymized with *L. macrochirus* by Hubbs (1935). At a later date, Hubbs and Lagler (1958) treated *P. speciosus* as a subspecies of *L. macrochirus*, concluding that the geographic range is throughout Texas and northeastern Mexico. Allozyme analyses did not detect genetic differentiation between *L. m. macrochirus* and *L. m. speciosus* (Kulzer and Greenbaum 1986), and subsequent treatments of centrarchid species diversity have not recognized *L. m. speciosus* (Gilbert 1998).

The two valid subspecies of *L. macrochirus* present an interesting problem of nomenclature confusion, morphological and genetic divergence, an area of presumed secondary contact and introgression, and a biogeographic pattern and a timing of divergence seen in another centrarchid sister species pair. The nominal subspecies *L. m. macrochirus* Rafinesque is distributed across eastern North America except for the northern Atlantic Coast (Lee *et al*. 1980), while the other subspecies is endemic to the Florida Peninsula (Felley 1980). Initially, the subspecies found in Florida was designated as *Lepomis*

macrochirus purpurescens Cope under the premise that this subspecies extended from the Atlantic Coast of the Carolinas to the Florida Peninsula (Hubbs and Allen 1943; Hubbs and Lagler 1958). The type locality for *Lepomis purpurescens* is in the Yadkin River Drainage in North Carolina (Cope 1870). Subsequent morphological and molecular analyses demonstrate that this is far north of the range of the Florida subspecies (Avise and Smith 1974a; Felley 1980; Avise *et al*. 1984), and as Gilbert (1998) has pointed out, Cope described a Bluegill from Florida, *Lepomis mystacalis* (Cope 1877). Therefore, the appropriate name for the Florida Bluegill is *L. macrochirus mystacalis*.

Lepomis m. macrochirus and *L. m. mystacalis* are morphologically and genetically distinct, but there is a presumed area of introgression through secondary contact along most of southern Georgia and South Carolina (Felley 1980; Avise *et al*. 1984). Another sister species pair in Centrarchidae, *Micropterus salmoides* and *Micropterus floridanus*, exhibit a very similar distribution and area of secondary contact and introgression (Bailey and Hubbs 1949; Philipp *et al*. 1983). Based on a fossil calibrated molecular phylogeny of Centrarchidae, the divergence time between *M. salmoides* and *M. floridanus* is approximately 2.8 million years ago (mya) (Near *et al*. 2003, 2005b). *Lepomis m. macrochirus* and *L. m. mystacalis* exhibit a very similar divergence time. We found mtDNA cytochrome *b* gene sequences in Genbank for five individuals of *L. m. macrochirus* and a single *L. m. mystacalis* (accession numbers: AY115975, AY115976, AY225667, AY828966, AY828967, and AY828968). The average genetic distance between these two subspecies was 4.5%, which translates to a divergence time of roughly 2.3 mya (Near *et al*. 2003). Future work should aim toward gathering sufficient morphological and molecular data to more precisely determine the geographic distribution of these two forms and assess if *L. mystacalis* is a valid species.

Recently *Micropterus henshalli* (Hubbs and Bailey) was elevated as a valid species (Baker *et al*. 2008), but was long recognized as a subspecies of *M. punctulatus* (Hubbs and Bailey 1940). *Micropterus henshalli* is endemic to the Mobile Basin and there are slight morphological differences between populations above and below the Fall Line (Gilbert 1973; Baker *et al*. 2008). However, there are substantial differences in several meristic characters between *M. henshalli* and *M. punctulatus* (Gilbert 1973), and there are marked differences in body proportions and surprising life history and dietary differences between these two species (Gilbert 1973). Perhaps the most compelling evidence for the recognition of *M. henshalli* includes measures of genetic divergence and the results of phylogenetic analyses. Among 19 polymorphic allozyme loci surveyed for all *Micropterus* species, not a single allele was shared exclusively between *M. henshalli* and *M. punctulatus*, and a fixed unique allele was found in *M. p. henshalli* (Kassler, *et al*., 2002). In a phylogenetic analysis of *Micropterus* species using gap coded continuous morphological characters *M. henshalli* and *M. punctulatus* did not form a clade (Harbaugh 1994), and these two species were sister lineages in frequency parsimony of allozyme alleles (Kassler *et al*. 2002). In addition, molecular phylogenetic analyses of mtDNA sequences from cyt*b* and ND2 resulted in tree topologies where *M. henshalli* was nested within *M. coosae* and distantly related to *M. punctulatus* (Kassler *et al*. 2002). Given the evidence presented above, the classification of *M. henshalli* as a subspecies of *M. punctulatus* was not compelling and the recognition of this species is supported by the substantial comparative data.

1.3 Centrarchid fossils

The fossil record of Centrarchidae is fairly rich and extends in geologic time from the Late Eocene to Early Oligocene of approximately 35 mya to the very early Holocene of approximately 10 years ago. Both extant centrarchid species and centrarchid fossils are found only in North America, indicating that origin and diversification of this clade did not involve other continental regions. There are 11 valid and extinct centrarchid species known only from fossil material (Table 1.1; Figures 1.2–1.17), and there are fossils of seven extant species.

Despite an excitingly abundant centrarchid fossil record, at least four of the oldest fossil centrarchid species are generally unknown to science. These fossil species are undescribed and have been under study for at least three decades. Unfortunately, they have not been made available to other researchers for study, which has significantly hindered progress in understanding the evolutionary origin of Centrarchidae and its patterns of diversification. The meager information available for these four undescribed fossil species that we present here is from general synopses of the fossil record of North American freshwater fishes (Cavender 1986, 1998). The first of these four we call the High Plains Sunfish, from the northwestern part of Montana near the foothills of the Rocky Mountains. Cavender (1986, 1998) indicates that they are found in Late Eocene to Early Oligocene deposits, but more precise age estimates are unavailable. The High Plains Sunfish has three anal spines and an emarginate caudal fin. The second of these undescribed fossils is the Chadron Sunfish

† *Plioplarchus sexspinosus*

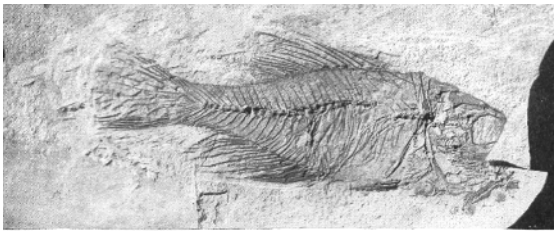

Figure 1.2 Photos and drawings of fossil Centrarchidae species: *†Plioplarchus sexspinosus* Sentinel Butte Sunfish, photo redrawn from Eastman (1917).

†Plioplarchus whitei

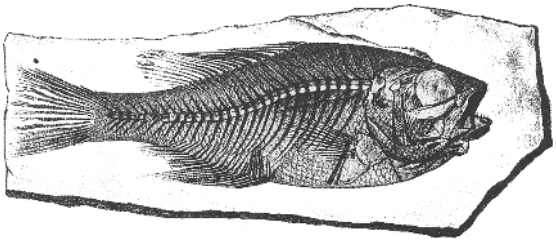

Figure 1.3 Photos and drawings of fossil Centrarchidae species: *†Plioplarchus whitei* Laramie Sunfish, redrawn from Cope (1884).

†Plioplarchus septemspinosus

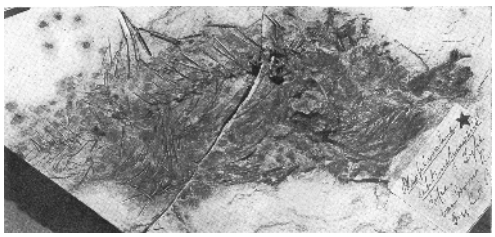

Figure 1.4 Photos and drawings of fossil Centrarchidae species: *†Plioplarchus septemspinosus* John Day Sunfish, photo redrawn from Eastman (1917).

†Boreocentrarchus smithi

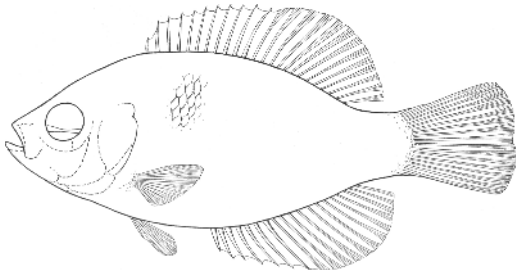

Figure 1.5 Photos and drawings of fossil Centrarchidae species: *†Boreocentrarchus smithi* Healy Creek Sunfish, redrawn from Schlaikjer (1937).

Figure 1.6 Photos and drawings of fossil Centrarchidae species: *Pomoxis †lanei* Ogallala Crappie, photo redrawn from Hibbard (1936).

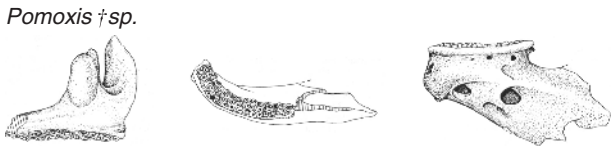

Figure 1.7 Photos and drawings of fossil Centrarchidae species: Pomoxis *†sp.* Wakeeney Crappie, redrawn from Wilson (1968).

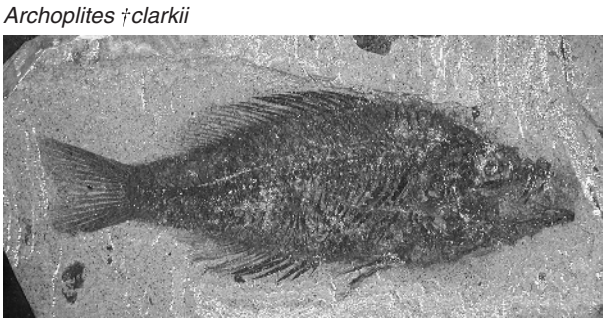

Figure 1.8 Photos and drawings of fossil Centrarchidae species: *Archoplites †clarki* Clarkia Perch, photo provided by Smith (1963).

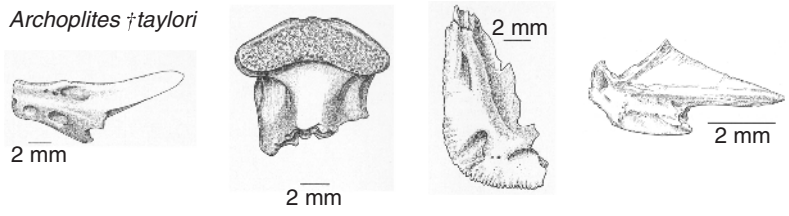

Figure 1.9 Photos and drawings of fossil Centrarchidae species: *Archoplites †taylori* Lake Idaho Sunfish, redrawn from Miller and Smith (1967).

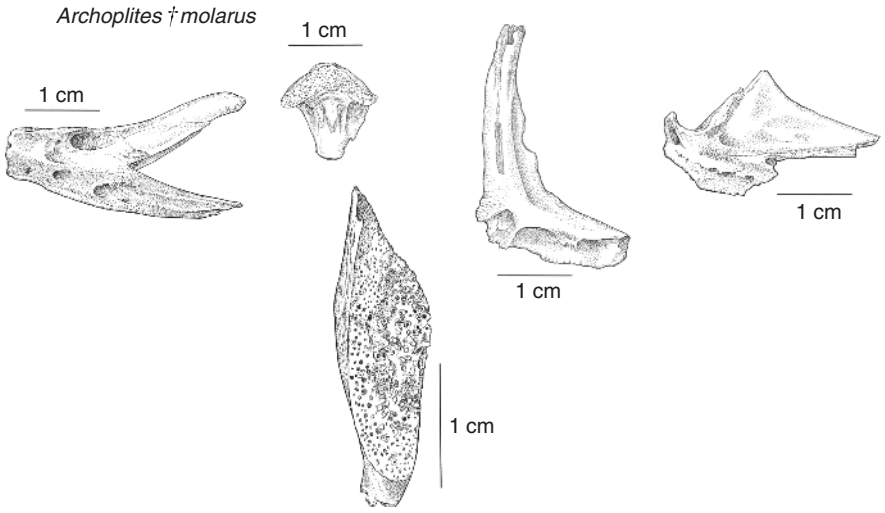

Figure 1.10 Photos and drawings of fossil Centrarchidae species: *Archoplites †molarus* Ringold Sunfish, redrawn from Smith *et al*. (2000).

Figure 1.11 Photos and drawings of fossil Centrarchidae species: *Lepomis †kansasensis* Rhino Hill Sunfish, photo redrawn from Hibbard (1936).

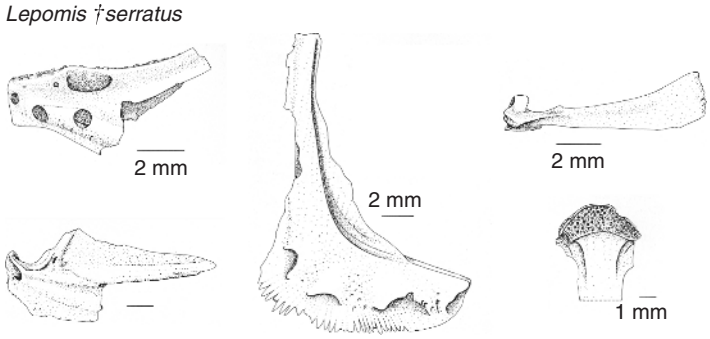

Figure 1.12 Photos and drawings of fossil Centrarchidae species: *Lepomis †serratus* Keigh Sunfish, redrawn from Smith and Lundberg (1972).

Lepomis †sp. A

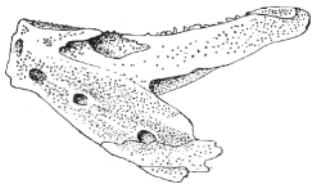

Figure 1.13 Photos and drawings of fossil Centrarchidae species: *Lepomis †sp.* A Valentine Sunfish, redrawn from Smith (1962).

Lepomis †sp. B

Figure 1.14 Photos and drawings of fossil Centrarchidae species: *Lepomis †sp.* B Wakeeney Sunfish, redrawn from Wilson (1968).

Micropterus †relictus

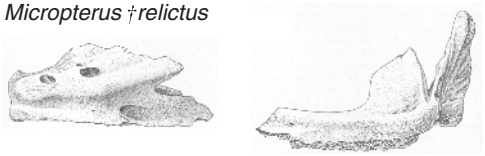

Figure 1.15 Photos and drawings of fossil Centrarchidae species: *Micropterus †relictus* 1975 Chapala Bass, redrawn from Smith *et al*. (1975).

Micropterus † sp. B

Figure 1.16 Photos and drawings of fossil Centrarchidae species: *Micropterus †sp.* B Wakeeney Bass, redrawn from Wilson (1968).

Micropterus †sp. C

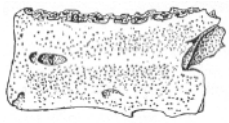

Figure 1.17 Photos and drawings of fossil Centrarchidae species: *Micropterus †sp.* C Laverne Bass, redrawn from Smith (1962).

from Lower Oligocene limestone deposits in the South Dakota Badlands, dating this fossil to the White River group of approximately 28 to 35 mya (Tedford *et al*. 1987). The Chadron Sunfish has three anal spines and 27 to 28 vertebrae (Cavender 1986). The third fossil sunfish in this group of undescribed forms is from Lower Miocene deposits in South Dakota, and Cavender (1986) provides an age of approximately 25 mya. These are very similar in morphology to the Chadron Sunfish, but have 29 vertebrae (Cavender 1986). The last of the four undescribed fossils in Cavender (1986) is from Middle Miocene deposits, but no location is given. This fossil species has six or seven anal fin spines and is similar to fossils that were assigned to *†Plioplarchus* (Cope 1884).

There are two extinct genera of Centrarchidae known from the fossil record, *†Plioplarchus* and *†Boreocentrarchus. †Plioplarchus* contains three species (Table 1.1), and is the oldest of the described centrarchid fossils (Figures 1.2–1.4). *†Plioplarchus sexspinosus* and *†P. whitei* were described from Oligocene age freshwater limestone deposits from the Sentinel Butte of North Dakota (Cope 1883) that date to approximately 30 mya (Feldman 1962) (Figures 1.2 and 1.3). *†Plioplarchus sexspinosus* and *†P. whitei* are also found in the Badlands of South Dakota in the White River Group. Specimens that are either *†P. sexspinosus or †P. whitei* are found at the contact between the Chadron and Brule Formations (Welzenbach 1992), and this is dated to approximately 31 mya (Tedford *et al*. 1987). *†Plioplarchus septemspinosus* was described from the John Day River in Oregon (Cope 1889) in the geological deposits that make up the John Day Fauna (Figure 1.4), and is dated between 18 and 31 mya (Tedford *et al*. 2004). Fossils currently assigned to *†P. septemspinosus* are also found in the Trout Creek Flora in Oregon and this is dated at 13 mya (Graham 1999). Morphological analyses indicate that *†P. septemspinosus* from the John Day and Trout Creek locations in Oregon are different from each other and both of these are quite divergent from *†P. sexspinosus* and *†P. whitei* (Schlaikjer 1937; Bailey 1938; Smith and Miller 1985). These differences were substantial enough for Bailey (1938) in his unpublished Ph.D. dissertation to describe a new genus for *†P. septemspinosus*.

†Boreocentrarchus smithi was described from Healy Creek, Alaska in deposits that were thought to age from the Oligocene to the Early Miocene (Figure 1.5) (Schlaikjer 1937; Uyeno and Miller 1963), and a more precise estimate of this formation at 24 to 18 mya agrees with these earlier estimates (Merritt 1987). Schlaikjer (1937) argues that *†B. smithi* is closely related to *†P. septemspinosus*, but others have questioned whether *†B smithi* is a centrarchid (Uyeno and Miller 1963). Both *†Plioplarchus* and *†Boreocentrarchus* are classified in the Centrarchinae (Table 1.1), because these species possess more than three anal fin spines. Undescribed fossil species in this clade include one from the Horse Creek Fish Quarry in Laramie Co., Wyoming, that dates to approximately 19 mya (Cassiliano 1980), another from the Bear Valley, California (Smith and Miller 1985), and a third from the Humboldt Formation, Nevada, that dates to 9 mya (Smith and Miller 1985; Smith *et al*. 2002).

The remaining centrarchid fossil species are classified in genera that also contain extant species (Table 1.1). *Pomoxis* is known from the fossil record with one described species, *P. †lanei*, and one undescribed fossil species. *Pomoxis †lanei* was found in the Rhino Hill Quarry in Logan Co., Kansas (Hibbard 1936), and age of this fossil formation is correlated with Coffee Ranch mammals that date to 6.6 mya (Wallace 1997; Passey *et al*. 2002). The holotype of *P. †lanei* is a complete and crushed skeleton (Figure 1.6). The specimen is a remarkable impression and many morphological features can be scored, counted, or measured (Hibbard 1936). The phylogenetic position of *P. †lanei* in *Pomoxis* is unresolved due to conflicting characters. The presence of seven dorsal fin spines and a long dorsal fin base supports the hypothesis that *P. †lanei* and *P. nigromaculatus* are sister species (Smith 1962). However, the hypothesis that *P. nigromaculatus* and *P. annularis* are sister species is supported by the presence of 17 to 20 anal fin rays in these species versus 12 anal fin rays in *P. †lanei* (Uyeno and Miller 1963). There is a second fossil species of *Pomoxis* that is undescribed. These fossils were found in the Wakeeney local fauna that is a part of the Ogallala Formation in Kansas (Wilson 1968). The age of this formation was placed in the lower portion of the Ash Hollow or upper Valentine Formation (Wilson 1968), and this dates to approximately 12 mya (Tedford *et al*. 2004). These are the oldest *Pomoxis* fossils and they are fragmentary, consisting of a dentary and premaxillary fragments (Figure 1.7).

Archoplites contains three fossil species and only one extant species (Table 1.1). The oldest of the *Archoplites* fossil species is *A. †clarki* from the Clarkia Lake Beds in Idaho (Figure 1.8) (Smith and Miller 1985). This fossil formation has been dated at 15.5 mya (Golenberg *et al*. 1990; Wing 1998). *Archoplites †taylori* is found in seven different fossil locations in southwestern Idaho and these sites are characterized as lacustrine deposits (Figure 1.9). The oldest of the fossil sites containing *A. †taylori* is the Poison Creek formation and is dated at 9 mya (Smith and Cossel 2001). The youngest formation containing *A. †taylori* fossils is Jackass Butte, a part of the Grandview local fauna dated at 2.2 mya (Smith 1975; Lundelius *et al*. 1987). *Archoplites †molarus* was recently described from the Ringold Formation

in Washington (Figure 1.10). Fossils of *A. †molarus* are found at three different locations in the Ringold Formation and the ages of these deposits extend through the Pliocene. Fossils from the White Bluffs local fauna are the oldest at 4.5 mya, the Blufftop Locality and local fauna dates to 3.7 mya, and Tauton Locality dated at 2.9 mya contains the youngest *A. †molarus* fossils (Smith *et al*. 2000; Van Tassell *et al*. 2001). The oldest fossils assigned to the extant species *Archoplites interruptus* are from the Cache Formation in Lake Co., California and date to the Early Pleistocene, approximately 2.5 mya (Casteel and Rymer 1975). The youngest *A. interruptus* fossils are from Sacramento Co., California and date to the Pleistocene of approximately 100,000 years ago (Hansen and Begg 1970). There is the possibility of undescribed fossil species of *Archoplites*. Van Tassell *et al*. (2001) mention specimens from Grande Ronde Valley in Union Co., Oregon that date to 3.7 mya, and there are other *Archoplites* fossil specimens dated to the Early or Middle Pleistocene from Moses Lake in Washington (Miller 1965).

There are four fossil species of *Lepomis*, and two of these are closely related to *L. gulosus*. *Lepomis †kansasensis* was found in the same fossil formation as *P. †lanei*, so it is dated at 6.6 mya (Hibbard 1936; Wallace 1997; Passey *et al*. 2002). The holotype is a nearly complete skeletal impression with a badly crushed head, but morphological features such as dentition can be distinguished (Figure 1.11). The presence of pterygoid teeth in *L. †kansasensis* led to the original classification of this species in *Chaenobryttus* that also contained *L. gulosus* (Hibbard 1936; Bailey 1938). *Lepomis †serratus* was described from fossils collected at the Keim Formation in the Sand Draw local fauna in Brown Co., Nebraska (Smith and Lundberg 1972). The age of this formation is dated at 3.4 mya (Repenning 1987). The Sand Draw *L. †serratus* fossils are dentaries, articulars, maxillae, prevomers and preopercles (Figure 1.12). *Lepomis †serratus* maxillae, prevomers, articulars, and preopercles, premaxillae, and dentaries are also reported from the Seneca local fauna in Hooker Co., Nebraska (Bennett 1979), and this is a younger fossil formation dated between 2.5 and 2.0 mya (Bell *et al*. 2004). *Lepomis †serratus* was classified in *Chaenobryttus* on the basis of morphological similarity of the preopercle with *L. gulosus* (Smith and Lundberg 1972). The initial classifications proposed for *L. †kansasensis* and *L. †serratus* indicate a fairly close phylogenetic affinity with *L. gulosus*, illustrated by the fact that at one point *L. †kansasensis* was synonymized with *L. gulosus* (Branson and Moore 1962). However, Smith and Lundberg (1972) argue that *L. †kansasensis* is more closely related to other *Lepomis* species than to *L. gulosus*, but concur that a definitive conclusion on this issue would result only from a more thorough analysis of the fossil material.

There are at least two undescribed *Lepomis* fossil species. Both of these fossils are quite old and are represented by fragmentary material of the lower jaw. The first of these is referred to as *L. †sp.* A, and was initially identified as *L.* cf. *microlophus* (Smith 1962). The fossil comes from the Lower Valentine Formation in Brown Co., Nebraska (Figure 1.13) (Smith 1962). This fossil location was later identified as the Norden Bridge local fauna (Estes and Tihen 1964), and is dated at 13.5 mya (Tedford *et al*. 2004). The second undescribed *Lepomis* fossil species is referred to as *L. †sp.* B (Figure 1.14), and is found in the same fossil location as the undescribed *Pomoxis* fossil species discussed earlier (Wilson 1968), so this fossil also dates to approximately 12 mya.

There are four fossil *Micropterus* species, but only one is described. *Micropterus †relictus* was described from and its jaw element fossils found in Late Pliocene–Early Pleistocene deposits in the Lake Chapala Basin, Mexico (Figure 1.15) (Smith *et al*. 1975). *Micropterus †sp.* A consists of fragmentary skull pieces and vertebrae from the Lower Snake Creek fauna in Sioux Co., Nebraska (Matthew 1924), and dates between 16 and 15 mya (Tedford *et al*. 2004). *Micropterus †sp.* B was originally identified as *M*. cf. *punctulatus*, and the fossil material is a lower pharyngeal jaw and a dentary fragment (Figure 1.16). The fossils were collected in the same formation as *Pomoxis †sp*. and *Lepomis †sp*. B and date to 12 mya (Wilson 1968). *Micropterus †sp.* C. is a dentary from the Laverne Formation in Beaver Co., Oklahoma (Figure 1.17) with an estimated age between 10.5 and 9.5 mya (Smith 1962; Tedford *et al*. 2004).

In addition to *A. interruptus* discussed earlier, there are at least six extant centrarchid species present in the fossil record. The oldest formations that contain extant species are the Rexroad local fauna in Meade Co., Kansas and the Sand Draw local fauna in Brown Co., Nebraska (Smith 1962; Smith and Lundberg 1972), both dated at 3.4 mya (Bell *et al*. 2004). These two formations combined contain fossil specimens of *Ambloplites rupestris*, *L. cyanellus*, and *L. humilis*. Both *L. cyanellus* and *L. humilis* occur in numerous Pleistocene fossil deposits. *Lepomis cyanellus* is found in Pleistocene formations ranging in age from 2.3 mya to 10,000 years ago and these sites are spread across Kansas, Michigan, Nebraska, Oklahoma, and Texas (Smith 1954, 1958, 1963; Schultz 1965; Hibbard and Dalquest 1966; Lundberg 1967; Eshelman 1975; Neff 1975; Shoshani and Smith 1996). Pleistocene formations containing *L. humilis* fossils range in age from 2.5 mya to 250,000 years ago and are restricted to Kansas, Nebraska, and South Dakota (Smith 1963; Ossian 1973; Neff 1975; Bennett 1979; Cross *et al*. 1986). *L. megalotis* fossils are found at two Pleistocene fossil formations. The oldest of

these two locations is the Rita Blanca Lake Deposit in Hartley Co., Texas and this formation is dated at 2.4 mya (Anderson and Kirkland 1969; Koster 1969; Lindsay *et al*. 1975; Lundelius *et al*. 1987; Repenning 1987), whereas the Kanopolis local fauna in Ellsworth Co., Kansas has yielded much younger *L. megalotis* fossils dated at 300,000 years ago (Neff 1975; Repenning 1987). *Micropterus salmoides* fossils have been reported from Pleistocene deposits in Kansas, Michigan, and South Dakota that are dated from 300,000 to 14,000 years (Smith 1963; Wilson 1967; Ossian 1973; Neff 1975). Fossil *L. gibbosus* specimens are known only from a single Pleistocene locality in South Dakota (Ossian 1973).

1.4 Phylogeny

1.4.1 Pre-Cladistic concepts of centrarchid evolutionary relationships

The nineteenth century was the time when most of the valid centrarchid species were described (Figure 1.1). Associated with this period of activity was the initial development of hypotheses of centrarchid relationships. In these early studies evolutionary relationships were reflected by the composition of species in particular taxonomic groups that were arranged in nested hierarchical ranks. For example, at the taxonomic rank of family, centrarchids were initially classified with Percidae (Günther 1859), implying a close relationship with pikeperches (*Sander*), perches (*Perca*), and darters (e.g., *Etheostoma* and *Percina*). The name Ichthelidae was applied to the centrarchids when they were first grouped apart from Percidae as a distinct family (Holbrook 1860). This family name was not used by later authors as *Icthelis* was regarded as a synonym (Bailey 1938). The first use of the name Centrarchidae came at a time when most of the valid species were described (Cope 1868).

Subsequent studies adopted Centrarchidae as the family rank name and presented nested classifications that were meant to imply evolutionary relationships (Jordan 1877; McKay 1881; Bollman 1891). Jordan's (1877) classification had two subfamilies with one containing *Micropterus* and all other genera were classified in the second subfamily, Lepominae. Within the Lepominae, the genera *Ambloplites, Archoplites, Acantharchus*, and *Chaenobryttus* (*Lepomis gulosus*) were grouped together. The remaining *Lepomis* species were classified into five genera that are no longer recognized, and *Enneacanthus, Centrarchus*, and *Pomoxis* were placed in the same grouping.

The classification presented by McKay (1881) did not include taxonomic ranks above genus. Previous to this classification all *Lepomis* species were classified into eight genera, *Chaenobryttus, Apomotis, Xenotis, Bryttus, Helioperca, Xystroplites, Eupomotis*, and *Lepomis*. McKay (1881) placed all of these species, except for *L. gulosus*, into *Lepomis*. The classification presented by Bollman (1891) is important in that it recognized three subfamilies, Centrarchinae, Lepominae, and Micropterinae that are still in use (Table 1.1). Bollman's (1891) proposed Centrarchinae contained *Centrarchus* and *Pomoxis*, whereas the Lepominae contained *Archoplites, Ambloplites, Chaenobryttus, Acantharchus, Enneacanthus, Mesogonistius*, and *Lepomis. Lepomis gulosus* was retained in *Chaenobryttus* as it was considered distantly related to other *Lepomis* species. The Micropterinae contained two recognized *Micropterus* species.

After the studies of McKay (1881) and Bollman (1891), pharyngeal jaw morphology provided important information for hypotheses of relationships among *Lepomis* species. Hypertrophied lower pharyngeal arches were presented as evidence to remove *L. gibbosus* from *Lepomis* and into the genus *Eupomotis* (Richardson 1904). A later review of the pharyngeal arches resulted in an amplification of Bollman's (1891) proposal that all species previously classified in *Apomotis, Xenotis, Bryttus, Helioperca, Xystroplites*, and *Eupomotis* were closely related and all species from these genera were placed in *Lepomis* (Bean and Weed 1911). However, as pointed out by Bailey (1938), some of the discussion in Richardson (1904) and Bean and Weed (1911) was based on hybrid individuals.

The first representation of centrarchid relationships presented as a branching dendrogram was in Schlaikjer's (1937) "suggestions on the phylogeny of the recent Centrarchidae." This schematic of centrarchid relationships did not result from the current concept of a phylogenetic analysis, but was inferred from body depth, relative mouth size, and numbers of rays and spines on the dorsal and anal fins (Figure 1.18). In this "phylogeny" *Centrarchus macropterus* was depicted as the ancestral centrarchid species with *Pomoxis* and *Archoplites* being represented as early splits from this ancestral lineage. Elongated body shape was an important characteristic that motivated the grouping of *Acantharchus, Ambloplites*, and *Micropterus* (including the invalid *Huro*) (Figure 1.18). In contrast to the classifications of McKay (1881) and Bollman (1891), Schlaikjer (1937) classifies *Lepomis* species (except *L. gulosus*) into three genera (*Lepomis, Apomotis*, and *Eupomotis*) that were depicted as a group in the branching diagram (Figure 1.18). The *Enneacanthus* species, including

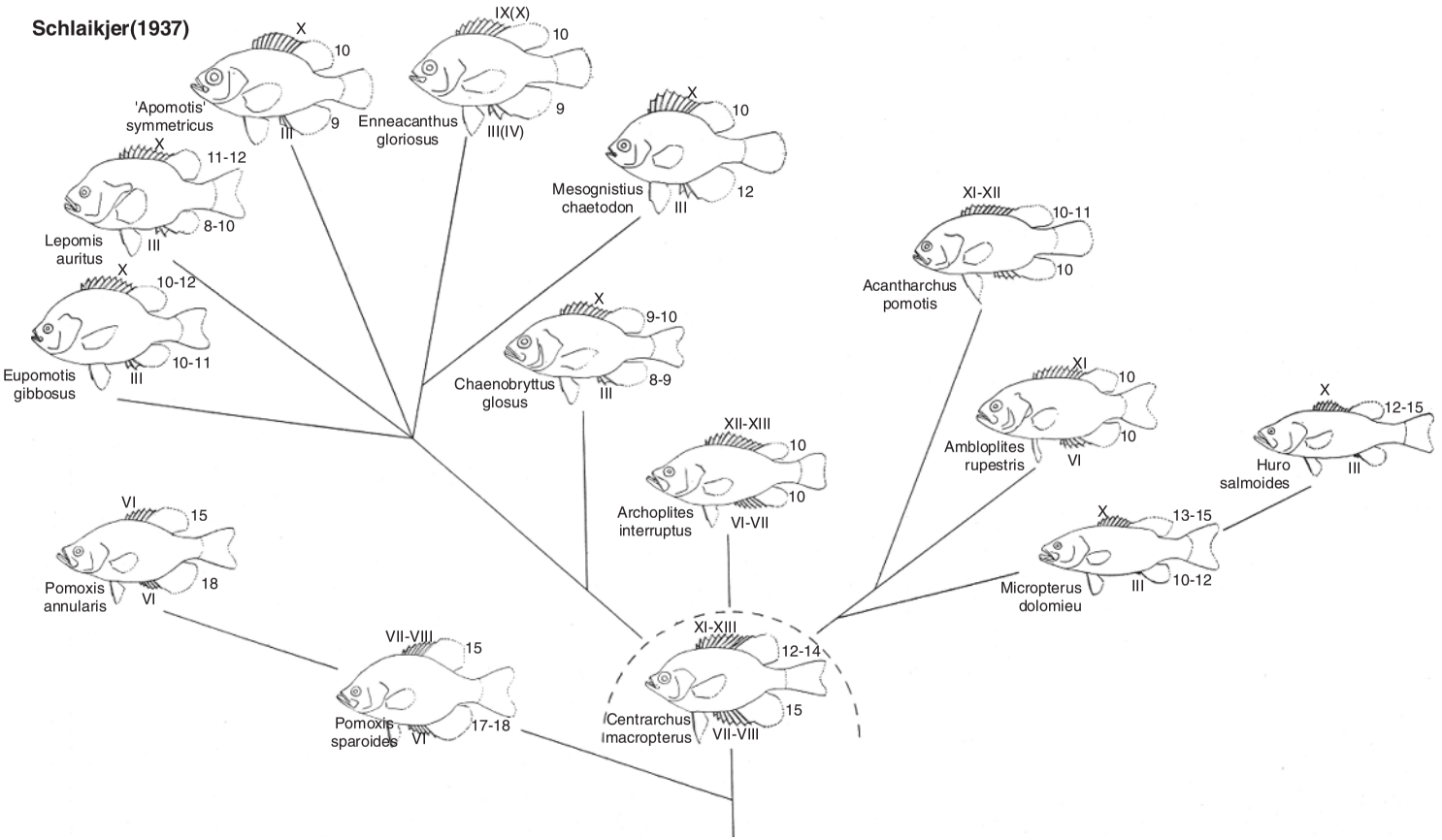

Figure 1.18 Phylogeny of Centrarchidae presented in Schlaikjer (1937).

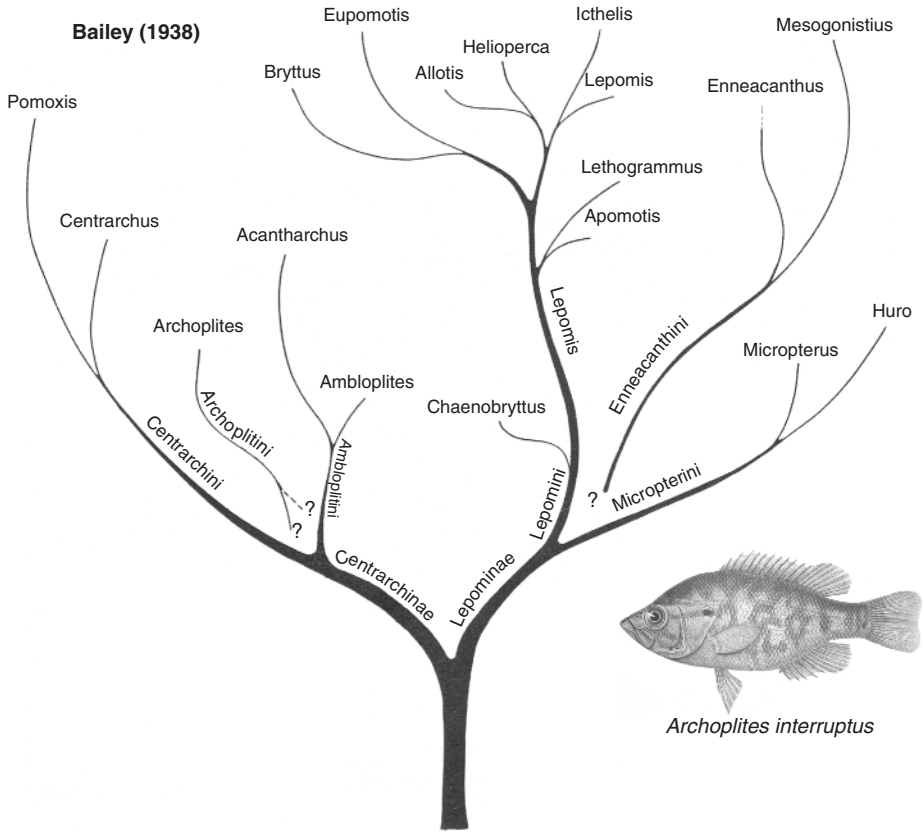

Figure 1.19 Phylogeny of Centrarchidae presented in Bailey (1938). *Archoplites interruptus* redrawn from Girard (1858).

the invalid *Mesogonistius*, were placed as closely related to *Lepomis*, with *L. gulosus* (in *Chaenobryttus*) outside of this group (Figure 1.18).

Bailey (1938) in an unpublished Ph.D. dissertation presented a classification of Centrarchidae and a "hypothetical phylogeny" for the group that was depicted as a branching diagram (Figure 1.19). The characters used by Bailey (1938) were primarily anal fin spines, branchiostegal rays, dentition, body shape, opercle serration, scale morphology, and gill raker morphology. By classifying *Centrarchus, Pomoxis, Archoplites, Acantharchus*, and *Ambloplites* in the subfamily Centrarchinae, Bailey (1938) was the first author to propose that species in these genera are closely related (Figure 1.19). The genera *Chaenobryttus* (*L. gulosus*), *Lepomis, Enneacanthus, Mesogonistius* (*Enneacanthus chaetodon*), *Micropterus*, and *Huro* (*M. salmoides*) were classified together in the subfamily Lepominae (Figure 1.19). Given the uncertainty of Bailey's (1938) placement of *Enneacanthus* and *Mesogonistius* in the phylogenetic tree (Figure 1.19), *Lepomis* and *Chaenobryttus* were depicted as the sister lineages and most closely related to *Micropterus*. Eight subgenera were proposed for ten recognized *Lepomis* species. Sister species pairs proposed within *Lepomis* were *L. cyanellus–L. symmetricus, L. macrochirus–L. humilis, L. gibbosus–L. microlophus*, and *L. megalotis–L. marginatus*. A detailed "phylogeny" of *Lepomis* species, as proposed by Bailey (1938), is given in Figure 1.20.

A "theoretical phylogeny" was presented in a taxonomic revision of *Micropterus* that described four new species and subspecies (Figure 1.21; Hubbs and Bailey 1940). This *Micropterus* "phylogeny" was intuitively derived and based on character variation in scale row and fin counts, and pigmentation patterns (Figure 1.21). In this phylogeny *Huro* was still used as a monotypic genus to contain *M. salmoides*. Also, the subspecies of *M. punctulatus* were not presented as a group that is most closely related to one another (Figure 1.21). This is explained by the fact that Hubbs and Bailey (1940, p. 41)

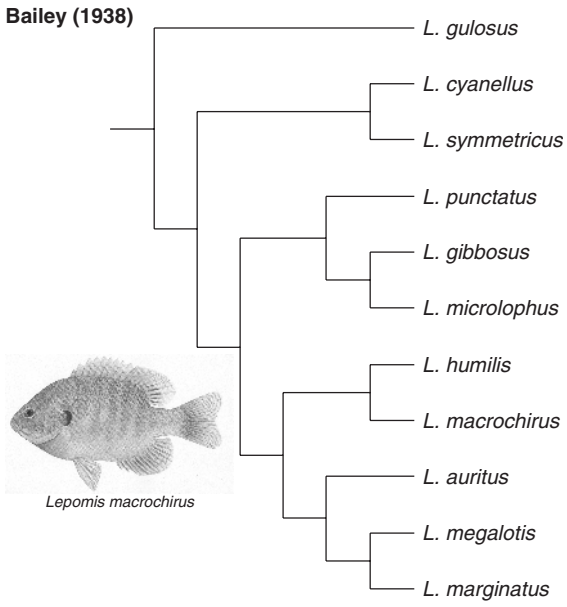

Figure 1.20 Detailed phylogeny of *Lepomis* presented in Bailey (1938). Subgenera of *Lepomis* in Figure 1.19 were translated to species names using tables presented in Bailey (1938). *Lepomis macrochirus* redrawn from Forbes and Richardson (1920).

did not rule out a scenario where *M. coosae* originated through hybridization and introgression between *M. dolomieu* and *M. punctulatus*. This explains the "paraphyletic" depiction of *M. punctulatus* in their branching diagram (Figure 1.21).

The phylogeny presented in Bailey (1938) was slightly modified and used to study the evolution of dorsal fin supports in percoid fishes, and particularly in Centrarchidae (Figure 1.22) (Smith and Bailey 1961). In this branching diagram *Archoplites* is resolved as the sister species of a group containing *Pomoxis* and *Centrarchus* (Figure 1.22). The phylogenetic position of *Enneacanthus* (*Mesogonistius* was no longer recognized) was still unresolved, but was hypothesized as closely related to *Lepomis* and *Micropterus* and not to genera in Centrarchinae (Smith and Bailey 1961).

The lateralis system and osteology provided characters for inferences regarding centrarchid phylogeny (Branson and Moore 1962). In this study, relationships were proposed separately for centrarchid genera, species in *Lepomis*, and species in *Micropterus*. The "hypothetical dendography" presented as a phylogeny among centrarchid genera is quite different from the hypotheses presented in Bailey (1938) (Figure 1.19) and Smith and Bailey (1961) (Figure 1.22). In Branson and Moore's (1962) hypothesis, Centrarchinae, as proposed by Bailey (1938), is paraphyletic relative to the Lepominae, and *Chaenobryttus* is nested outside of a sister group containing *Lepomis* and *Micropterus* (Figure 1.23a). Within *Lepomis*, Branson and Moore (1962) converge on a hypothesis of relationships that is less resolved than Bailey's (1938) (Figure 1.20), but agree with Bailey (1938) in presenting the *L. cyanellus–L. symmetricus* and *L. macrochirus–L. humilis* species pairs (Figure 1.23b). The proposal of relationships among *Micropterus* species presented by Branson and Moore (1962) agrees with that of Hubbs and Bailey (1940) (Figure 1.21) in depicting *M. salmoides* as the sister species to all other *Micropterus* species. Also, Branson and Moore (1962) provide a phylogenetic hypothesis for *M. treculi* and *M. notius* (Figure 1.23c), two species that were either not recognized or not described when Hubbs and Bailey (1940) revised *Micropterus*.

The last of the pre-cladistic hypotheses of centrarchid relationships discussed in this review was published after the development of cladistic methods, but is a verbal hypothesis of relationships among *Micropterus* species based primarily on pigmentation and ecological characteristics (Ramsey 1975). Three lineages in *Micropterus* were identified and Ramsey's (1975) hypothesis was converted into a generally unresolved phylogeny. In this tree *M. salmoides* is grouped by itself, *M. coosae* and *M. dolomieu* are sister species, and *M. punctulatus, M. treculi, M. notius*, and *M. cataractae* are placed in an unresolved grouping (Figure 1.23d).

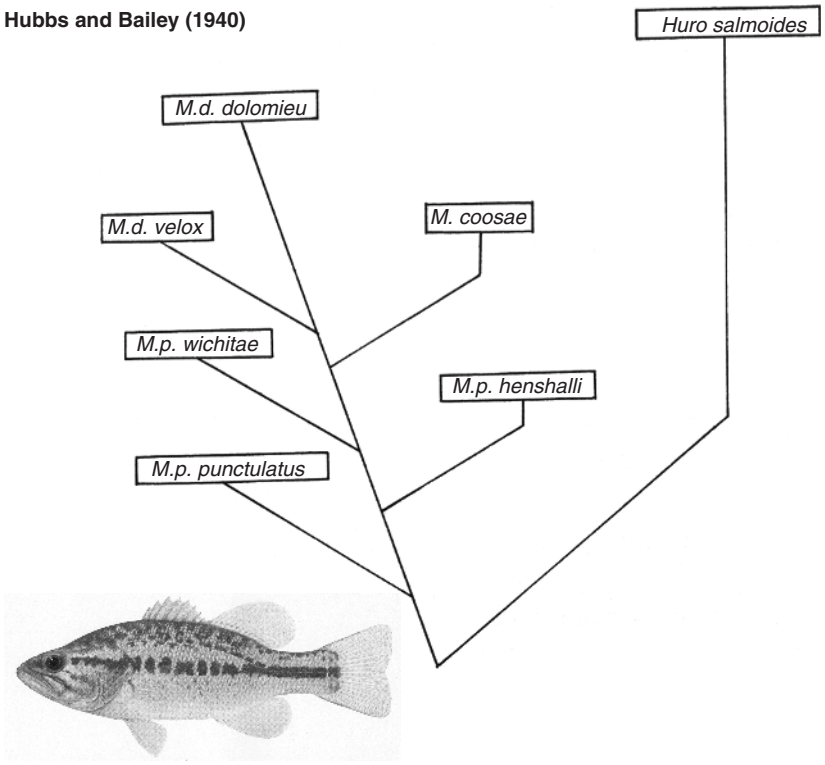

Figure 1.21 Phylogeny of *Micropterus* presented in Hubbs and Bailey (1940). *Micropterus salmoides* redrawn from Forbes and Richardson (1920).

1.4.2 Phylogenetic hypotheses derived from analysis of character data

The preceding section reviewed ideas about centrarchid evolutionary relationships that were intuitive, and did not utilize forms of character optimization seen in the current practice of phylogenetic systematics (Swofford *et al*. 1996). This section reviews more recent hypotheses of centrarchid relationships, and includes those that use a particular optimality criterion to analyze a coded character dataset. As a result of the publication of several studies and datasets over the past 30 years, Centrarchidae has come to provide an exciting system to investigate very relevant issues in systematics such as character congruence among independent molecular datasets (Near *et al*. 2004), the use of fossil data for calibrating molecular phylogenies (Near *et al*. 2005b), and the optimal use of phylogenies and divergence time estimates in comparative studies (Bolnick and Near 2005; Collar *et al*. 2005).

The first studies of centrarchid relationships that used a defined optimality criterion to analyze a comparative data matrix were also the first studies to use genetic data in reconstructing centrarchid phylogeny. Allozymes, which are alternative forms of an enzyme produced by different alleles of a given locus that are usually detected by protein electrophoresis, were used to investigate relationships among centrarchid genera and among *Lepomis* species (Avise and Smith 1974b, 1977; Avise *et al*. 1977). In these studies allozyme variation was converted to pair-wise genetic distances, and the unweighted pair-group method (UPGMA) was used for cluster analyses that resulted in branching dendrograms. The phylogeny resulting from the UPGMA analysis that included the greatest taxon sampling among these studies is presented in Figure 1.24a (Avise and Smith 1977). These analyses agreed with earlier, pre-cladistic hypotheses by presenting *Micropterus* and *Lepomis* as sister lineages (Bailey 1938; Branson and Moore 1962), but *Pomoxis* grouped with this clade instead of with other

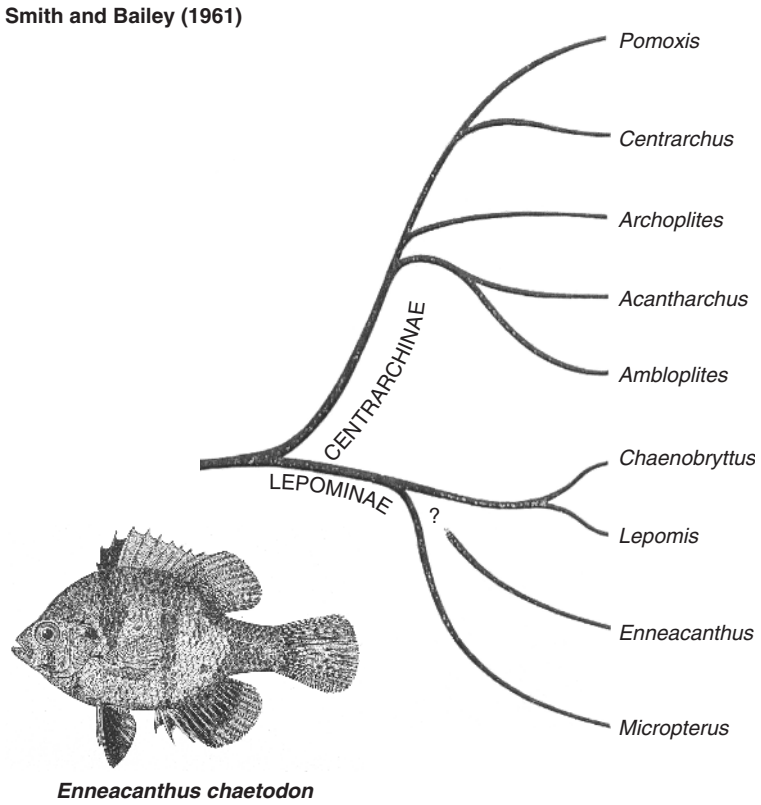

Figure 1.22 Phylogeny of Centrarchidae presented by Smith and Bailey (1961). *Enneacanthus chaetodon* redrawn from Smith (1907).

Centrarchinae (*Acantharchus, Archoplites, Centrarchus*, and *Ambloplites)*. However, it is important to note that the branch length for this node in the allozyme genetic distance dendrogram was very short (Avise and Smith 1977, Figure 5). The allozyme phylogenies differ from several of the earlier morphological hypotheses in having *Enneacanthus* closely related to genera comprising Bailey's (1938) concept of Centrarchinae (*Acantharchus, Archoplites, Centrarchus*, and *Ambloplites*), and not *Lepomis* and *Micropterus*. Also, relationships within *Lepomis* were different from the hypotheses presented in Bailey (1938) and Branson and Moore (1962) (Figures 1.20 and 1.23a, b), perhaps most notable is that *L. gulosus* was nested well within *Lepomis*, and not in a separate clade that would warrant recognition of *Chaenobryttus*. A later allozyme study that used a distance Wagner method to construct a centrarchid phylogeny (Parker *et al*. 1985) resulted in a fairly similar tree (Figure 1.24b). One noticeable difference was the nonmonophyly of *Lepomis*, a result that may have been an artifact of the genetic distance calculations or the distance clustering method used in this study (Figure 1.24b).

Characters from kidney morphology, anal fin spine counts, and olfactory organ morphology were used in the first explicit cladistic analysis of centrarchid phylogeny (Mok 1981). Two separate trees were presented, as Mok (1981) did not combine all the morphological characters for one cladistic analysis. The first phylogeny lacked resolution and was based on five characters from kidney morphology (Figure 1.25a). The presence of an extreme posterior kidney was interpreted as a shared derived character (synapomorphy) for all Centrarchidae except *Micropterus*. The phylogeny has a basal polytomy with the outgroup taxon (*Elassoma*), *Micropterus*, and all other centrarchid genera (Figure 1.25a). Despite the lack of phylogenetic resolution Mok's (1981) analysis of kidney morphology resulted in a clade containing *Centrarchus* and *Pomoxis* that agreed with earlier pre-cladistic hypotheses (Bailey 1938; Smith and Bailey 1961; Branson and Moore 1962). Mok (1981) stressed that the kidney morphology does not support the previous hypotheses that presented *Lepomis* and *Micropterus*

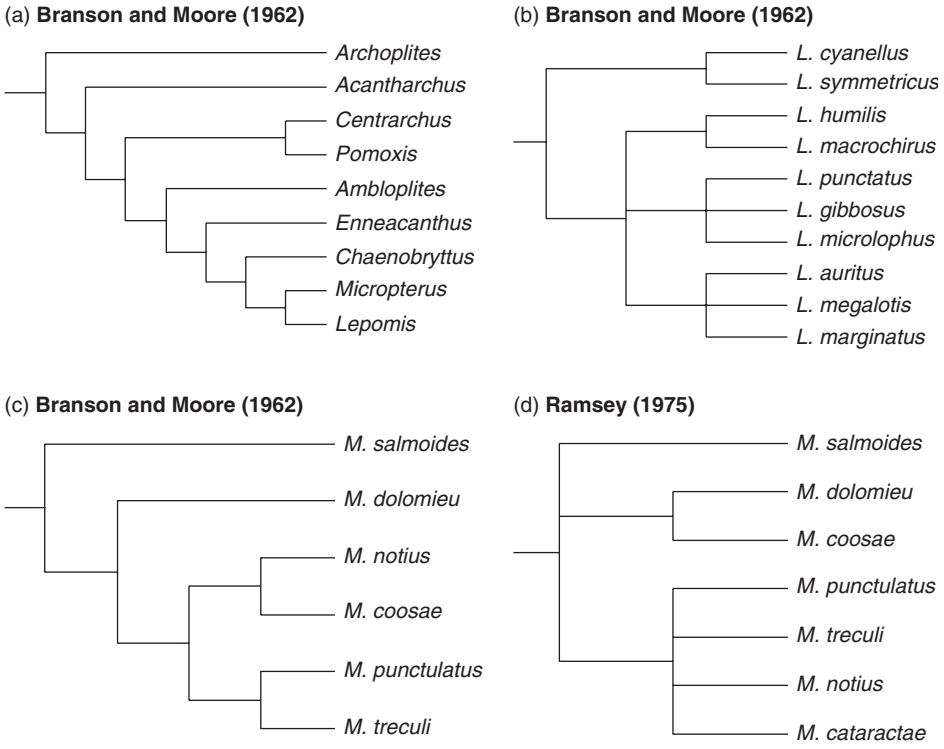

Figure 1.23 (a) Phylogeny of centrarchid genera presented by Branson and Moore (1962). (b) Phylogeny of *Lepomis* presented by Branson and Moore (1962). (c) Phylogeny of *Micropterus* presented by Branson and Moore (1962). (d) Phylogeny of *Micropterus* converted from a verbal hypotheses presented by Ramsey (1975).

as sister taxa (Bailey 1938; Smith and Bailey 1961; Branson and Moore 1962). The second phylogeny in Mok (1981) was based on two characters, the number of anal spines and folding of the olfactory sac, as presented in Eaton (1956). This tree was also unresolved, but it did argue that more than three anal fin spines was a synapomorphy for *Ambloplites, Acantharchus, Archoplites, Centrarchus*, and *Pomoxis* (Figure 1.25b), a result that agreed closely with Bailey's (1938) concept of Centrarchinae (Figures 1.19 and 1.25b).

An undefined set of morphological characters was used for a cladistic analysis of centrarchids, and the resulting tree served as the basis for a comparative study of diet, functional feeding morphology, and behavior (Lauder 1986). The phylogeny had a basal polytomy with *Micropterus, Lepomis*, and clade containing *Pomoxis, Centrarchus, Acantharchus, Archoplites*, and *Ambloplites* (Figure 1.25c). *Lepomis* was monophyletic and *L. gulosus* was not closely related to *Micropterus*. In agreement with Bailey (1938), *L. gibbosus* and *L. microlophus* were sister species (Figures 1.20 and 1.25c)

The next morphological phylogeny of Centrarchidae was presented in an unpublished Ph.D. dissertation and was based on cladistic analyses of 27 morphological characters (Chang 1988). This phylogeny was pectinate, or completely imbalanced, with *Micropterus* as the basal sister taxon to all other centrarchids (Figure 1.25d). One interesting aspect of this phylogeny was the placement of *Enneacanthus* as the sister taxon of the genera that comprise Bailey's (1938) concept of Centrarchinae, and not closely related to *Micropterus* or *Lepomis*. Also, in agreement with several previous studies (Bailey 1938; Smith and Bailey 1961; Mok 1981; Parker *et al*. 1985), *Centrarchus* and *Pomoxis* were sister taxa (Figure 1.25d). Chang's (1988) study is particularly important because it identified four morphological synapomorphies for Centrarchidae (exclusive of *Elassoma*), a posterior bifurcation of the swim bladder, the first hemal spine of the same length as the second, a deep groove on the first hemal spine, and contact between the first and second hemal spines.

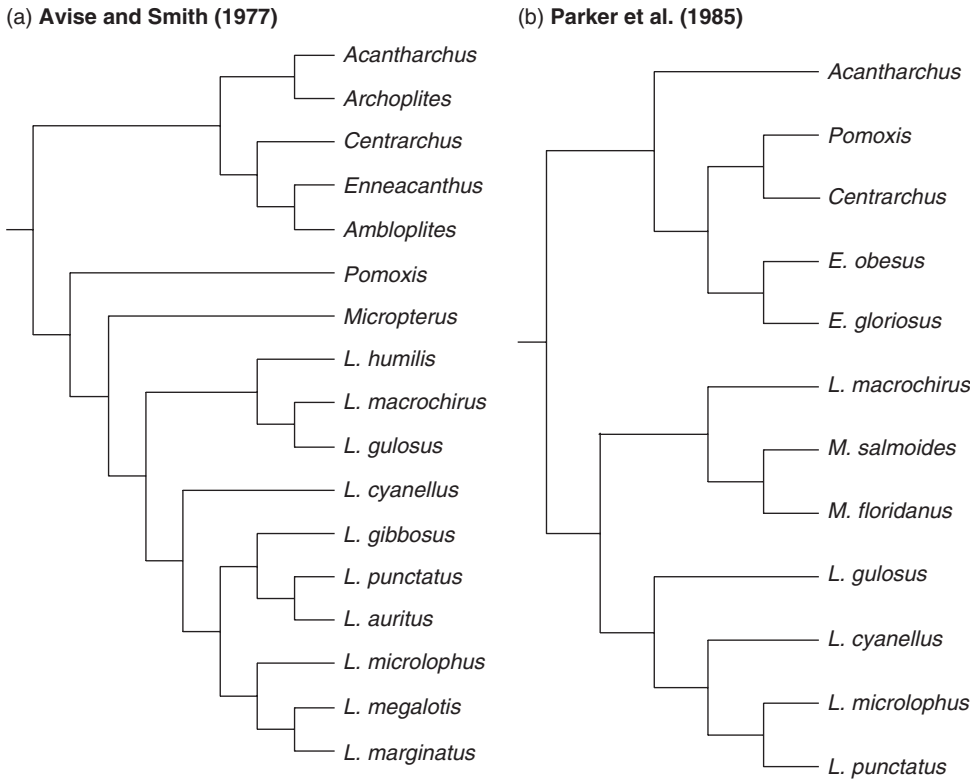

Figure 1.24 (a) Allozyme inferred phylogeny of Centrarchidae presented by Avise and Smith (1977). (b) Allozyme inferred phylogeny of Centrarchidae presented by Parker *et al*. (1985).

In a study examining the evolutionary patterns in functional morphological aspects of feeding in centrarchids, Wainwright and Lauder (1992) used a centrarchid phylogeny that resulted from a cladistic analysis of 53 undefined morphological characters. The tree was similar to that of Chang (1988) in that *Micropterus* is the basal sister taxon to all other Centrarchidae (Figure 1.26a). Also, in agreement with Bailey's (1938) concept of Centrarchinae, this phylogeny presented *Acantharchus, Ambloplites, Pomoxis, Centrarchus*, and *Archoplites* as a monophyletic group (Figure 1.26a). *Archoplites interruptus* and *C. macropterus* were recovered as sister species, a relationship that had not been proposed in any of the previous hypotheses; however, Mok (1981) presented a tree based on olfactory organ folding that had a clade containing *Pomoxis, Centrarchus*, and *Archoplites* (Figure 1.25b). Interestingly, in Wainwright and Lauder's (1992) phylogeny, *Enneacanthus* was nested within *Lepomis* and *L. gulosus* was the phylogenetically basal species in this clade. Some details of the relationships in *Lepomis* proposed by Wainwright and Lauder (1992) are consistent with previous hypotheses (Bailey 1938; Branson and Moore 1962), and others are unique to this analysis (Figure 1.26a).

Mabee (1989, 1993) presented a phylogenetic analysis of Centrarchidae using 61 morphological characters. The trees were used to study the ontogenetic criterion in phylogenetics, asking if an ontogenetic series for a particular character provided a reasonable method to polarize the character in a phylogenetic analysis (Mabee 1989, 1993). From our own reanalysis of the data matrix and other published analyses of this dataset (Mabee 1993; Patterson 1996), it is clear that parsimony analysis using outgroup rooting results in hundreds (if not thousands) of most parsimonious trees. However, a single tree from the set of most parsimonious trees was selected for purposes of Mabee's (1989, 1993) analyses of ontogenetic character evolution (Figure 1.26b). Despite the seemingly arbitrary nature of the selection of this tree, the strict consensus of the most parsimonious trees is quite well resolved (see Patterson 1996, Figure 1a), and is completely resolute with regard to the details of the phylogenetic relationships discussed in this review.

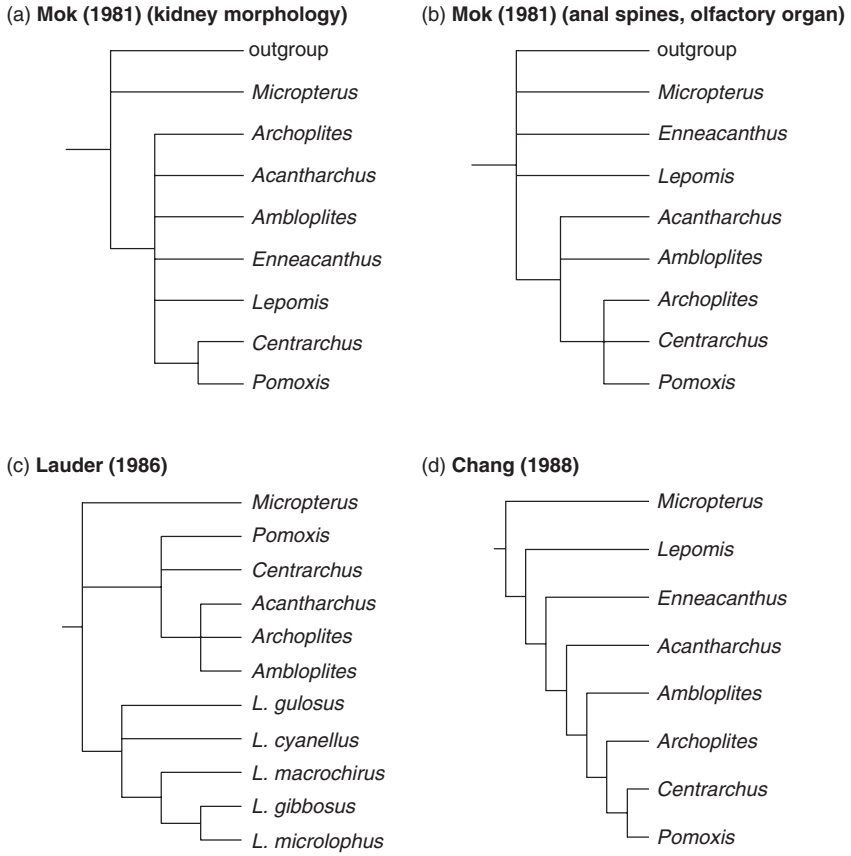

Figure 1.25 (a) Phylogeny of Centrarchidae based on a cladistic analysis of kidney morphology presented by Mok (1981). (b) Phylogeny of Centrarchidae based on a cladistic analysis of anal spine counts and scale morphology presented by Mok (1981). (c) Phylogeny of Centrarchidae based on a cladistic analysis of morphological characters presented by Lauder (1986). (d) Phylogeny of Centrarchidae based on a cladistic analysis of 27 morphological characters presented by Chang (1988).

The phylogeny presented by Mabee (1989, 1993) is interesting in many respects (Figure 1.26b). In agreement with two of the other morphological cladistic analyses (Chang 1988; Wainwright and Lauder 1992), *Micropterus* is the sister lineage of all other Centrarchidae. The relationships within *Lepomis* were very similar to that presented by Wainwright and Lauder (1992)—*L. gulosus* was the sister species to all others in the clade, and *Enneacanthus* was nested in *Lepomis*. Within *Lepomis*, Mabee's (1989, 1993) phylogeny has two sister species pairs, *L. megalotis–L. marginatus* and *L. microlophus–L. gibbosus* seen in other phylogenies (Bailey 1938; Avise and Smith 1977; Lauder 1986; Wainwright and Lauder 1992); however, the sister species pairs *L. macrochirus–L. humilis* and *L. cyanellus–L. symmetricus* proposed by Bailey (1938) and Branson and Moore (1962) were not supported by these analyses (Figure 1.26b). In agreement with many of the earlier, pre-cladistic, morphological hypotheses (Bailey 1938; Branson and Moore 1962), a monophyletic Centrarchinae, exclusive of *Enneacanthus*, was present in the selected single tree from the pool of most parsimonious trees (Figure 1.26b). However, *Acantharchus* falls out of this clade in the strict consensus tree (Patterson 1996, Figure 1a).

Over the past 5 years DNA data has increasingly been used in phylogenetic analyses of Centrarchidae. Three studies have focused on relationships of *Micropterus* species (Johnson *et al*. 2001; Kassler *et al*. 2002; Near *et al*. 2003) and have produced fairly congruent results; however, there are some unresolved issues with regard to species recognition in the clade that are illuminated by these molecular studies. Johnson *et al*. (2001) analyzed the phylogeny of *Micropterus* species using a maximum parsimony analysis of restriction enzyme digests of whole mtDNA genomes. The monophyly

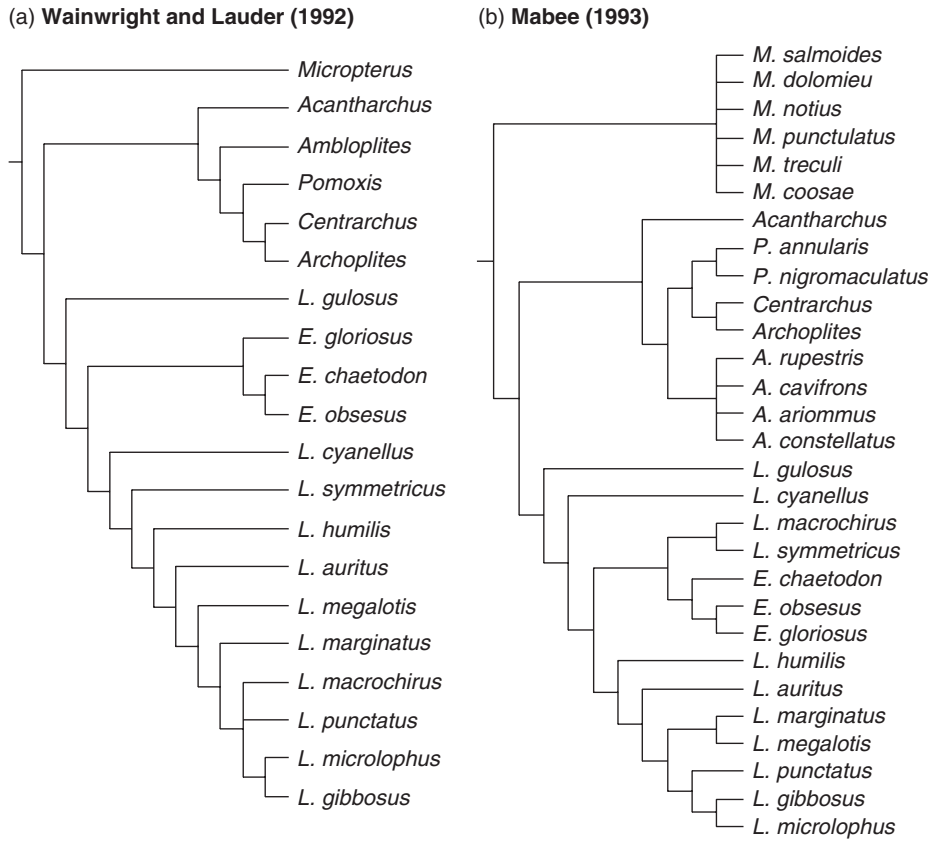

Figure 1.26 (a) Phylogeny of Centrarchidae based on a cladistic analysis of morphological characters presented by Wainwright and Lauder (1992). (b) Phylogeny of Centrarchidae based on a cladistic analysis of 61 morphological characters presented by Mabee (1989, 1993).

of *Micropterus* was not tested as only a single outgroup species was used, but the phylogeny depicts *M. salmoides* as the sister species to all other *Micropterus* (Figure 1.27a). Near *et al*. (2003) presented a maximum likelihood analysis of DNA sequences from two mtDNA genes, cyt*b* and ND2 that were collected from 50 individuals sampled from 8 *Micropterus* species. This maximum likelihood phylogeny was similar to the tree presented by Johnson *et al*. (2001), but differs primarily where the root was placed. This difference was most likely a consequence of the use of a single outgroup taxon. Also, Johnson *et al*. (2001) did not provide support values for nodes in the phylogeny, and Near *et al*. (2003) presented a phylogeny that had most of the interspecific nodes supported with high bootstrap pseudoreplicate scores (Figure 1.27b). In Near *et al*.'s (2003) tree *M. dolomieu* and *M. punctulatus* were sister species, and this clade was sister to the remaining *Micropterus* species (Figure 1.27b). Differing from Johnson *et al*. (2001), Near *et al*. (2003) found *M. treculi* as the sister species of a clade containing *M. salmoides* and *M. floridanus*. There are two aspects of the *Micropterus* phylogeny presented by Near *et al*. (2003) that support the recognition of *M. floridanus* as a species distinct from *M. salmoides*: (i) the two species exhibit reciprocally monophyletic mtDNA haplotypes, and (ii) the intraspecific branch lengths are shorter than those subtending the interspecific node (Figure 1.27b).

Kassler *et al*. (2002) utilized the sampling of cyt*b* and ND2 mtDNA sequences from Near *et al*. (2003), but added more *M. treculi* specimens and included *M. henshalli* in the phylogenetic analyses. Also, Kassler *et al*. (2002) presented phylogenies that are derived from the analysis of 19 polymorphic allozyme loci. The mtDNA maximum likelihood phylogeny yielded two very surprising results. First, two distinct *M. treculi* mtDNA haplotypes were discovered. One of

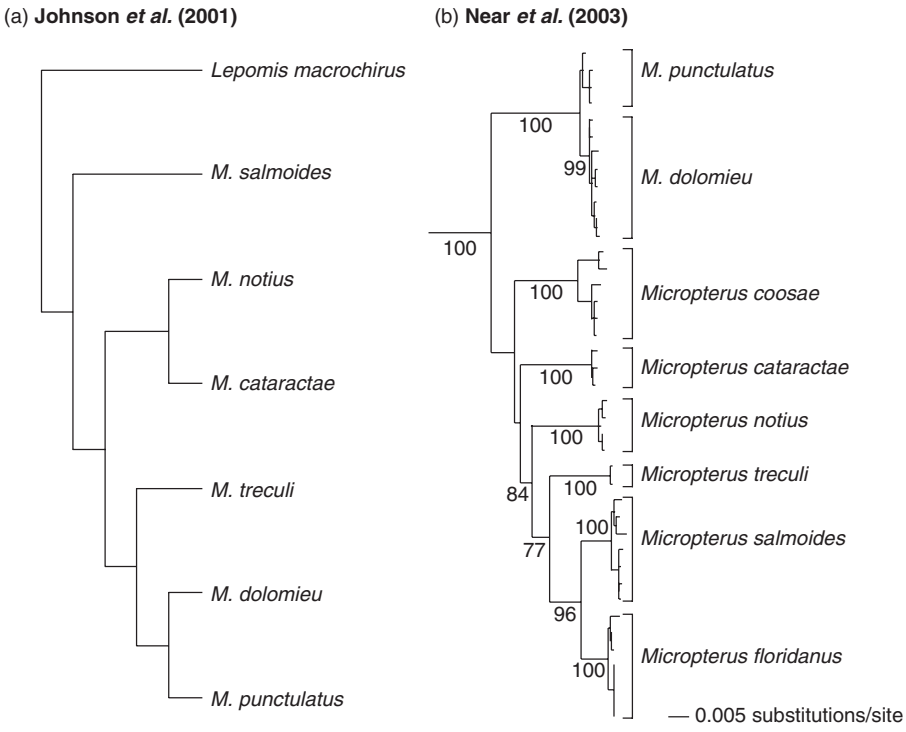

Figure 1.27 (a) Phylogeny of *Micropterus* based on a cladistic analysis of restriction digests of whole mtDNA genomes presented by Johnson *et al*. (2001). (b) Phylogeny of *Micropterus* based on a maximum likelihood analysis of mtDNA gene sequences presented in Near *et al*. (2003). Outgroup species are not shown. A scale bar for the expected number of substitutions is given in the lower right, and numbers at nodes are percent recovery in a bootstrap analysis.

these was resolved as the sister taxon of the clade containing *M. floridanus* and *M. salmoides*, and the other was closely related to *M. punctulatus*. Second, *M. henshalli* was nested within the haplotypes sampled for *M. coosae* (Figure 1.28a). These two patterns could be attributed to mtDNA introgression, a process known to occur in fishes (Avise 2001), but the allozyme inferred phylogeny offers some important clues to the unexpected results in the mtDNA phylogeny (Figure 1.28b).

In an unpublished study, we have screened 100 *M. treculi* from three locations within the species' limited native range and found 49 individuals with the haplotype that is sister to the *M. floridanus–M. salmoides* clade, and 51 individuals with the haplotype that is closely related to *M. punctulatus* (Figure 1.28a). There is no geographic pattern within *M. treculi* as the two haplotypes were found in equal frequency within the three populations sampled. However, regardless of which of the two divergent mtDNA haplotypes are found in a given *M. treculi* specimen, there is virtually no intraspecific variation among allozyme alleles or DNA sequences from nuclear genes. In the allozyme phylogeny constructed using a frequency parsimony method (Swofford and Berlocher 1987), *M. treculi* is closely related to *M. punctulatus*, a result that is expected from the previous classification of *M. treculi* as a subspecies of *M. punctulatus* (Hubbs and Bailey 1942). The presence of a divergent mtDNA haplotype that is closely related to the *M. floridanus–M. salmoides* clade that has no counterpart in the nuclear gene phylogeny (Figure 1.28b) cannot be explained by human introductions of *M. punctulatus* into the native range of *M. treculi*. As it stands, the mystery of the two divergent mtDNA haplotypes in the background of what appears to be a homogenous nuclear genome of *M. treculi* will have to be solved in future studies.

The case of *M. henshalli*, or the Alabama Spotted Bass, is equally puzzling as the pattern revealed in *M. treculi*. The mtDNA maximum likelihood phylogeny resolves *M. p. henshalli* as distantly related to *M. punctulatus* and the haplotypes are nested within *M. coosae* (Figure 1.28a). *Micropterus henshalli* and *M. coosae* are sympatric throughout the Mobile Basin (Mettee *et al*. 1996; Boschung and Mayden 2004) and the similarity of the mtDNA haplotypes would indicate a

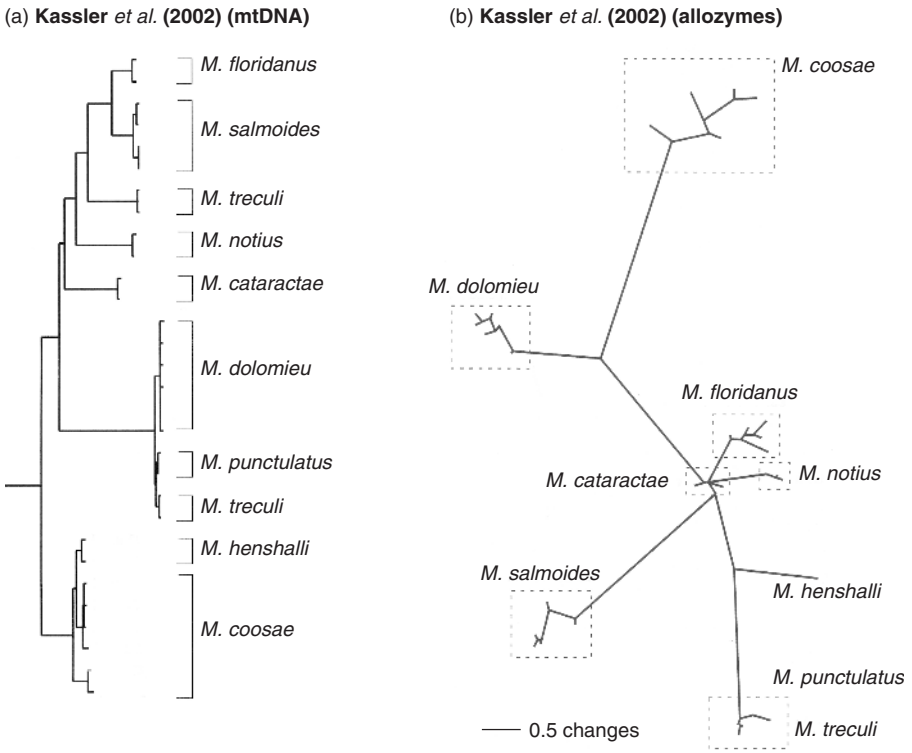

Figure 1.28 (a) Phylogeny of *Micropterus* based on a maximum likelihood analysis of mtDNA gene sequences presented by Kassler *et al*. (2002). Outgroup species are not shown. (b) Phylogeny of *Micropterus* based on a frequency parsimony analysis of allozyme alleles at 19 loci presented by Kassler *et al*. (2002).

recent introgression of mtDNA from *M. coosae* to *M. henshalli*. The allozyme phylogeny resolves *M. henshalli* as the sister taxon of a clade containing *M. punctulatus* and *M. treculi* with a very long branch separating *M. henshalli* from this clade (Figure 1.28b). In our own work we have collected *M. coosae* and *M. henshalli* in sympatry in the upper Coosa River system. These individuals have very similar mtDNA haplotypes (1.3% uncorrected genetic distance), but despite the sympatry of these species the *M. p. henshalli* haplotypes cluster together exclusive of the paraphyletic *M. coosae* mtDNA haplotypes (Figure 1.28b). Even if mtDNA introgression is obscuring the true phylogeny of *M. henshalli*, it is apparent that it is quite distinct from *M. punctulatus* at nuclear encoded loci (Figure 1.28b) and exhibits substantial morphological divergence (Gilbert 1973).

Complete coding sequences from the mtDNA cyt*b* gene were used to examine intraspecific and interspecific relationships of *Lepomis* species (Harris *et al*. 2005). All species in the clade, except the very recently elevated *L. peltastes*, were sampled and multiple individuals were included from each sampled species. As reported by Harris *et al*. (2005), mtDNA haplotypes from five *Lepomis* species were not reciprocally monophyletic (Figure 1.29). However, for reasons outlined in the following text, we have found it necessary to reanalyze the cyt*b* data from Harris *et al*. (2005). The phylogeny presented in this review was obtained using a Bayesian analysis similar to that used by Near *et al*. (2005b), and we present the phylogeny as a phylogram (Figure 1.29).

Harris *et al*. (2005) state that introgression and the presence of cryptic species best explain the pattern of extensive nonmonophyly observed in *Lepomis* species. Despite a reasonable probability for this scenario, hybridization cannot be detected without genetic data from nuclear genes or morphological analyses (Neff and Smith 1979; Shaw 2002), and identification of cryptic species would minimally require some degree of assessment of morphological divergence, but such data were not presented (Harris *et al*. 2005). One possible explanation for the nonmonophyly of *Lepomis* species not

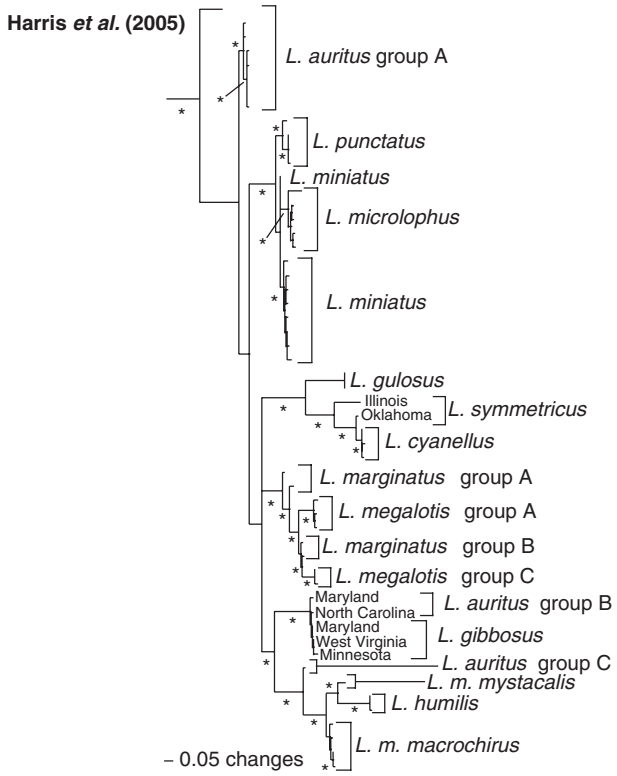

Figure 1.29 Phylogeny of *Lepomis* based on a reanalysis of mtDNA gene sequence data presented by Harris *et al*. (2005). The phylogram resulted from a Bayesian mixed model analysis. Outgroup species are not shown. A scale bar for the expected number of substitutions is given in the lower left, and asterisks at nodes indicate support with significant ($\leqslant$0.95) Bayesian posterior probabilities.

explored by Harris *et al*. (2005) is misidentification of specimens. For example, the haplotype of *L. symmetricus* sampled from McCurtain Co., Oklahoma is very similar (low genetic divergence) to the haplotypes sampled from *L. cyanellus* (Figure 1.29). Given that *L. cyanellus* is sympatric with *L. symmetricus* in this region of Oklahoma (Miller and Robison 2004), specimens of *L. cyanellus* from the same location as this divergent *L. symmetricus* haplotype were not sampled, and Harris *et al*. (2005) do not state that they verified the identification of these specimens together, which means that specimen misidentification cannot be ruled out. The same circumstance can possibly be applied to the phylogenetic resolution of haplotypes from *L. auritus* group B that nests in the same clade as the sampled *L. gibbosus* haplotypes (Figure 1.29). The striking similarity of the haplotypes in *L. auritus* group B and *L. gibbosus*, in addition to the fact that the two species are sympatric where the *L. auritus* group B specimens were collected, points to a possible instance of specimen misidentification.

Ancestral polymorphism was not considered as a mechanism that could result in the pattern of extensive species nonmonophyly observed in the *Lepomis* phylogeny (Figure 1.29). Ancestral polymorphism can result in nonmonophyly of a species' alleles when the ancestral species is polymorphic at the locus, and the random sorting of the alleles during the splitting into multiple daughter species results in a gene tree that is incongruent with the species phylogeny (Neigel and Avise 1986; Pamilo and Nei 1988; Wu 1991; Hudson 1992; Hudson and Coyne 2002). The time to reach coalescence, when the species haplotypes are monophyletic, is proportional to the effective population size. Due to maternal inheritance, the coalescent time for mtDNA haplotypes is one quarter that expected for alleles of an autosomal locus (Moore 1995). One heuristic method to assess if ancestral polymorphism is driving a phylogenetic result is to determine if interspecific branches

(genetic distances) are longer than intraspecific branches in the phylogeny, with the assumption that long interspecific branches indicate that sufficient time has elapsed to expect coalescence and reciprocal monophyly (Moore 1995). The paraphyly of *L. miniatus* and *L. marginatus–L. megalotis* are in regions of the *Lepomis* phylogeny that have fairly short interspecific branch lengths relative to the intraspecific branch lengths, so ancestral polymorphism should not be ruled out as a cause for the observed paraphyly of these species.

The first phylogenetic investigation among centrarchid genera using DNA sequences was an analysis of the mitochondrial cyt*b* gene by Roe *et al*. (2002). The importance of this study was limited by the sampling of only one half of all centrarchid species, and by sparse phylogenetic resolution.

Two recent studies have examined relationships of all extant centrarchid species, except the recently elevated *L. peltastes* and *M. henshalli*, using DNA sequences from multiple genes. Near *et al*. (2004) presented phylogenetic trees resulting from maximum parsimony and Bayesian analyses of a three gene data set consisting of the mtDNA, ND2, and two nuclear genes (S7 ribosomal protein intron 1 and the protein coding *Tmo4C4*). Two important conclusions were discussed in Near *et al*. (2004). First, separate analyses of each of the three sampled gene regions resulted in very similar phylogenies that indicated little incongruence between mtDNA and nuclear gene trees. Second, Shimodaira–Hasegawa tree topology tests indicated that 13 of 20 previous hypotheses of centrarchid relationships examined were significantly different from the best tree that resulted from the Bayesian analysis of the mitochondrial and nuclear gene dataset (Table 1.2). This allowed a unique perspective on how these earlier hypotheses compared in the context of a large set of characters that were sampled for most of the species level diversity in Centrarchidae.

The phylogenies inferred from mitochondrial and nuclear gene DNA sequences demonstrated the monophyly of all polytypic genera, and in agreement with earlier studies resolved *Lepomis* and *Micropterus* as sister lineages (Bailey 1938; Smith and Bailey 1961; Branson and Moore 1962; Avise and Smith 1977), and provided strong support for a clade containing *Enneacanthus Centrarchus, Archoplites, Ambloplites*, and *Pomoxis* (Figure 1.30a, b). Other interesting relationships resolved in these analyses included *Archoplites* and *Ambloplites* as sister taxa, and the identification of two sister species pairs within *Ambloplites*. Relationships within *Lepomis* were highly resolved and most nodes received strong support in maximum parsimony bootstrap analysis or had significant Bayesian posterior probabilities (Figure 1.30a, b). The sister species pairs *L. cyanellus–L. symmetricus* and *L. humilis–L. macrochirus*, proposed by Bailey (1938) and Branson and Moore (1962) (Figures 1.20 and 1.23b), were strongly supported in the mtDNA and nuclear gene phylogenies (Figures 1.30a, b). Also, *L. megalotis* and *L. marginatus* were resolved as sister species, supporting the results from several earlier studies (Bailey 1938; Avise and Smith 1977; Mabee 1993). Previous investigations of *Lepomis* phylogeny have hypothesized that *L. microlophus* and *L. gibbosus* are sister species. This relationship was not supported in the mtDNA and nuclear gene phylogenies (Figure 1.30a, b). These two species are the only *Lepomis* species that exhibit specialized diets, feeding primarily on snails. Both *L. microlophus* and *L. gibbosus* possess morphological and behavioral specializations that function in crushing snails (Lauder 1983, 1986; Wainwright and Lauder 1992), and many of these characters had been used as evidence of common ancestry for these two species (Bailey 1938; Branson and Moore 1962; Lauder 1986; Wainwright and Lauder 1992; Mabee 1993). These phylogenies indicated that the evolution of these characters involved with molluscivory have a more complex evolutionary history than previously hypothesized.

The dataset used in Near *et al*. (2004) was expanded to include one additional mitochondrial gene (16S ribosomal RNA) and two additional nuclear genes (calmodulin intron 4 and rhodopsin) for a total of 5553 base pairs of aligned DNA sequence data (Near *et al*. 2005b). The purpose of this study was to use fossil information to calibrate the molecular phylogeny to estimate divergence times in Centrarchidae. Ten centrarchid fossils were used to provide minimal age estimates for nodes in the phylogeny. Using a fossil cross-validation method (Near and Sanderson 2004; Near *et al*. 2005a), Near *et al*. (2005b) were able to identify four fossil calibrations that provided inconsistent molecular age estimates, and six consistent centrarchid fossils were used to calibrate the molecular phylogeny. Molecular divergence times of centrarchid species were estimated using penalized likelihood, a method that account for lineage specific molecular evolutionary rate heterogeneity (Sanderson 2002). The centrarchid phylogeny was presented as a chronogram, where the branch lengths are drawn to reflect estimates of absolute evolutionary ages (Figure 1.31). Given the temporal context of centrarchid diversification, Near *et al*. (2005b) point out that the origin of Centrarchidae at approximately 35 mya in the late Eocene–early Oligocene corresponds to a time of major global climate change to cooler conditions, and a signature in the fossil record of both lineage extinction and origination for many disparate clades across the tree of life.

Another important result from the centrarchid chronogram that was exploited by later studies of functional character evolution and patterns of post-zygotic reproductive isolation was the finding that the major centrarchid lineages had

Table 1.2 Shimodaira–Hasegawa tests of alternative phylogenetic hypotheses of centrarchid fishes. Significant results are presented with an asterisk.

Hypothesis	*p*
Three gene Bayesian phylogeny; Figure 1.30b	–
Schlaikjer (1937); Figure 1.18	<0.001*
Bailey (1938); Figure 1.19	<0.001*
Smith and Bailey (1961); Figure 1.22	<0.001*
Branson and Moore (1962); Figure 1.23a	<0.001*
Avise *et al*. (1977); Figure 1.24a	0.452
Mok (1981); Figure 1.25a	<0.001*
Mok (1981); Figure 1.25b	<0.001*
Parker *et al*. (1985); Figure 1.24b	0.002*
Lauder (1986); Figure 1.25c	0.912
Chang 1988; Figure 1.25d	0.472
Wainwright and Lauder (1992); Figure 1.26a	<0.001*
Mabee (1993); Figure 1.26b	<0.001*
Lepomis	
Bailey (1938); Figure 1.20	<0.001*
Branson and Moore (1962); Figure 1.23b	0.089
Micropterus	
Hubbs and Bailey (1940); Figure 1.21	0.014*
Branson and Moore (1962); Figure 1.23c	<0.001*
Ramsey (1975); Figure 1.23d	<0.001*
Johnson *et al*. (2001); Figure 1.27a	0.900
Near *et al*. (2003); Figure 1.27b	0.989
Kassler *et al*. (2002); Figure 1.28a	0.986

different ages (Bolnick and Near 2005; Collar *et al*. 2005; Bolnick *et al*. 2006). For instance, the Centrarchinae (exclusive of *Acantharchus*) was the oldest major centrarchid clade followed by *Lepomis*, then *Micropterus*. Interestingly, despite the fact that *Lepomis* and *Micropterus* were sister lineages, the chronogram revealed that the ages of the most recent common ancestor (MRCA) in each clade was quite different with the *Lepomis* MRCA being substantially older than the MRCA of *Micropterus* (Figure 1.31).

The phylogeny inferred from the expanded mitochondrial and nuclear gene data set presented in Near *et al*. (2005b) is very similar to those estimated from the earlier mitochondrial and nuclear gene DNA study (Near *et al*. 2004) (Figures 1.30a, b and 1.31). Important differences included the resolution of *Acantharchus pomotis* as the sister taxon of all other Centrarchidae (Figure 1.31), where maximum parsimony and Bayesian analyses differed on the placement of this species in the phylogeny in Near *et al*. (2004) (Figure 1.30a, b).

1.5 Phylogeography

Phylogeography investigates the relationships between phylogeny and geography for a species or group of related species. It is an approach to understanding intraspecific geographic subdivision, evolutionary pathways to that subdivision, and

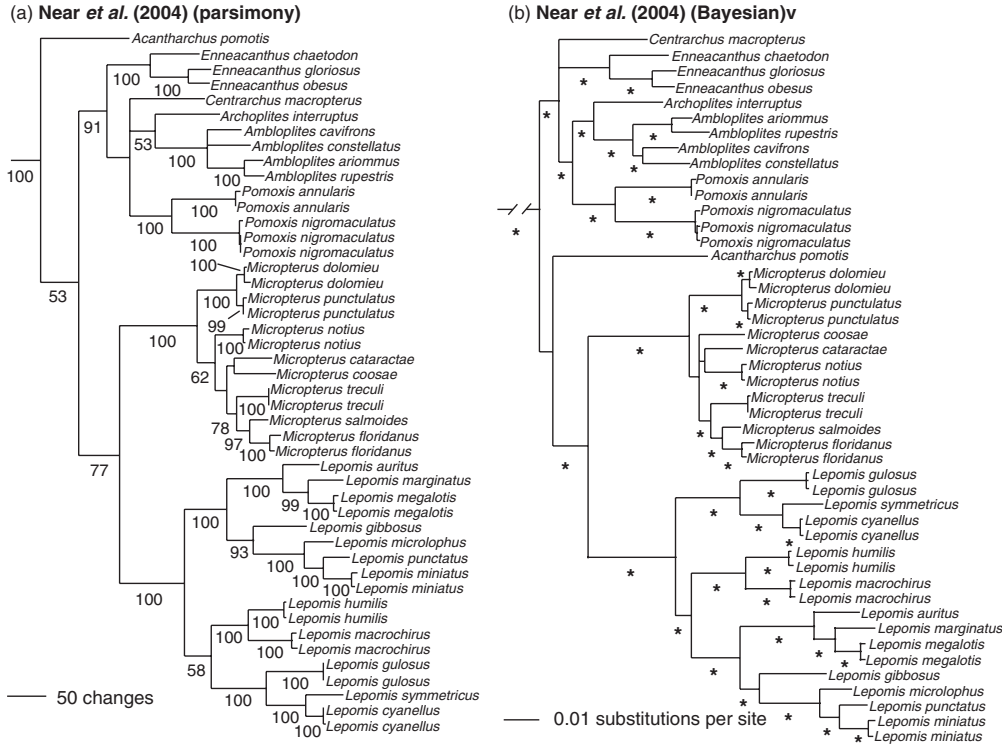

Figure 1.30 (a) Phylogeny of nearly all centrarchid species based on a maximum parsimony analysis of mitochondrial and nuclear gene DNA sequences. The phylogram is a strict consensus of four most parsimonious trees. Outgroup species are not shown. A scale bar for the number of optimized changes is given in the lower left, and numbers at nodes are percent recovery in a bootstrap analysis. (b) Phylogeny of nearly all centrarchid species based on a partitioned mixed-model Bayesian analysis of mitochondrial and nuclear gene DNA sequences. Outgroup species are not shown. A scale bar for the expected number of substitutions is given in the lower left, and asterisks at nodes indicate support with significant ($\leqslant$0.95) Bayesian posterior probabilities.

geographic patterns of speciation. In simple terms, phylogeography involves putting phylogenies on geographic maps (Avise *et al*. 1987), and the term was derived to simplify the description of patterns that emerge from co-analysis of a species' genetic diversity and the geographic framework on which that diversity is distributed. Phylogeography also involves understanding the segregation of genetic diversity under varying conditions of behavioral, physiological, and abiotic separation as they have affected gene flow (Templeton 1998). Both historic and current geographic barriers to dispersal and gene flow have shaped distributional patterns of fish species, and in many instances these patterns are evident across phylogenetically divergent lineages inhabiting the same environments and geographic regions (Avise *et al*. 1987; Mayden 1987; Strange and Burr 1997; Near *et al*. 2001).

Intraspecific phylogeographic assessments are not without confounding factors. In the case of centrarchid species of recreational fishing importance, stocking over the last 100+ years has spread fish from often-distant sources across virtually all of the major watersheds in North America. Any phylogeographic analysis would have to potentially sort out the admixture of native and introduced genotypes to correctly assess the distribution of diversity. In extreme cases, the original ancestral type could be lost, leaving an anomaly in the genetic landscape (Epifanio and Philipp 2000). For example, a recent study of the distribution of alleles at two loci fixed for *M. salmoides* and *M. floridanus* concluded that the extent of mixing was so great in Virginia reservoirs that it was uncertain which species was native (Dutton *et al*. 2006).

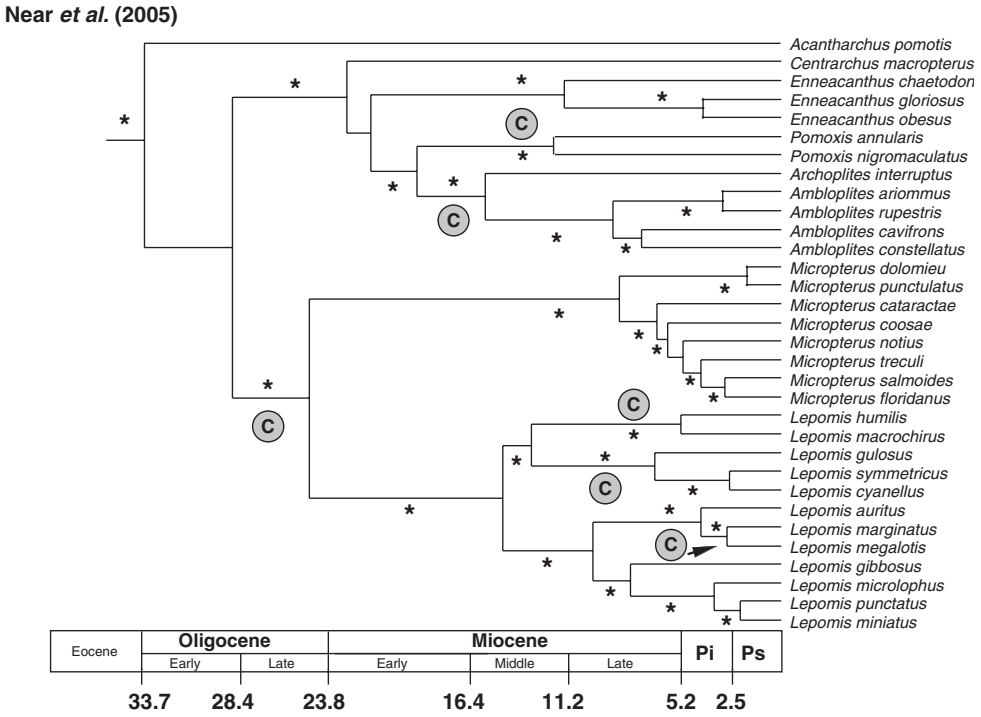

Figure 1.31 Phylogeny of nearly all centrarchid species based on a partitioned mixed-model Bayesian analysis of a seven gene dataset of mitochondrial and nuclear gene DNA sequences. The phylogeny is time-calibrated (chronogram) using six centrarchid fossils to provide minimal age estimates for nodes in the tree. Nodes calibrated with fossils are indicated with a circled "C." The chronogram is calibrated against the geological time scale. Outgroup species are not shown. Asterisks at nodes indicate support with significant ($\leqslant$0.95) Bayesian posterior probabilities.

1.5.1 Phylogeography of Micropterus

Although studies of polymorphic genetic loci had been conducted on *M. salmoides* (Whitt *et al*. 1971), Philipp *et al*. (1983) represented the first phylogeographic analysis of a *Micropterus* species. Based on fixed allelic differences at two allozyme loci, the native range of *M. salmoides* was divided into three areas that corresponded to *M. salmoides, M. floridanus*, and intergrades between the two species. Coupled with allele frequency data at two additional allozyme loci, latitudinal clines were revealed that indicate the distribution of populations follows thermal clines (Philipp *et al*. 1983). Additional allozyme studies on a more-local scale have had mixed results. In South Carolina, variation among sites verified that this region of the Atlantic Slope was an area of intergradation between *M. salmoides* and *M. floridanus*, and observed clinal variation appeared to correspond to mean annual temperature (Bulak *et al*. 1995). However, further north in Virginia, there was no geographic pattern with respect to the fixed alleles that differentiate *M. salmoides* and *M. floridanus*, and the authors concluded that this pattern was indicative of either stocking of nonnative species, or that *M. salmoides* is not native to the region sampled (Dutton *et al*. 2006). In addition to the two fixed allelic differences, there is substantial mtDNA divergence between *M. salmoides* and *M. floridanus* (Nedbal and Philipp 1994; Kassler *et al*. 2002; Near *et al*. 2003).

Allozyme variation was used to investigate the phylogeography of *M. dolomieu* across its geographic range, but with a biased sampling of Interior Highlands (Ozark and Ouachita uplands) populations (Stark and Echelle 1998). Substantial genetic heterogeneity among sampling sites was detected (i.e. high F_{ST} values). Multivariate analysis of genetic variation between populations identified four clusters of populations: southwestern Ozarks, northern Ozarks and upper Mississippi, and Ohio drainages, and two distinct Ouachita Highland clusters. Phylogenetic analysis using frequency

parsimony indicated that *M. dolomieu* populations from northern Ozark rivers were more closely related to populations sampled from the Ohio and Upper Mississippi River Basins than to other Ozark populations (Stark and Echelle 1998).

Phylogeographic analysis of western populations of *M. punctulatus* using mtDNA sequences from the control region and allele frequencies from five microsatellite DNA loci found little genetic variation (Coughlin *et al*. 2003). However, it was determined that populations from the Arkansas River were more similar to Ouachita River populations, relative to Red River populations. Based on the paleogeography of the river drainages (Mayden 1985, 1988), it was expected that the Red and Ouachita River populations would be most similar genetically. Coughlin *et al*. (2003) hypothesized mtDNA introgession between *M. punctulatus* and *M. dolomieu* because they discovered a shared haplotype. Considering the sister species relationship and recent divergence time between *M. punctulatus* and *M. dolomieu* (Kassler *et al*. 2002; Near *et al*. 2003, 2005b), we argue that retention of ancestral polymorphism better explains this instance of mtDNA haplotype sharing.

Analysis of the mtDNA cyt*b* and ND2 has revealed extensive phylogeographic structuring among populations of the Mobile Basin endemic *M. coosae*. A small sample of individuals from three different sites in the eastern Mobile Basin exhibited appreciable variation at the mtDNA genes with an estimated intraspecific divergence time of approximately 1.0 mya (Near *et al*. 2003). Considering that the Mobile Basin is characterized by a substantial number of endemic fish species (Lydeard and Mayden 1995; Mettee *et al*. 1996; Boschung and Mayden 2004), the discovery of genetic differentiation among such a paltry sampling of *M. coosae* populations is not surprising. A more thorough phylogeographic analysis based on a sampling of *M. coosae* throughout its limited geographic range has potential to reveal interesting cryptic diversity.

1.5.2 Phylogeography of Lepomis

Despite the fact that most *Lepomis* species have fairly large geographic ranges, there have been few published studies of intraspecific phylogeography. As discussed in Section 1.2.1 on subspecies, these types of studies have the potential to discover patterns of geographic variation, identify cryptic species, and test species boundaries.

The first centrarchid phylogeographic study was Avise and Smith (1974a) who examined allozyme allelic variation in southern *L. macrochirus* populations. This study revealed an area of intergradation between two described subspecies, *L. m. macrochirus* and *L. m. mystacalis*, and genetic differentiation of Texas populations. Subsequent studies have found similar patterns resulting from analyses of mtDNA (Avise and Smith 1977; Avise *et al*. 1984).

Phylogeography of four *Lepomis* species (*L. miniatus*, *L. punctatus*, *L. microlophus*, and *L. gulosus*) along the southeastern seaboard of the United States was examined with mtDNA haplotype variation (Bermingham and Avise 1986). Intraspecific patterns in two of these species exhibited phylogeographic discontinuities that were concordant with previously defined biogeographic boundaries identified from the distributional limits of other organisms (Wiley and Mayden 1985). The sister species *L. miniatus* and *L. punctatus* exhibited a pattern similar to the intraspecific-level analyses of *L. gulosus* and *L. microlophus* with a phylogeographic break at the Apalachicola River (Figure 1.32). Morphological differentiation between the sister species *L. miniatus* and *L. punctatus* and between eastern and western populations of *L. microlophus* is concordant with the mtDNA inferred phylogeographic breaks (Bailey 1938; Warren 1992).

The phylogeographic discontinuities exhibited in *L. microlophus, L. gulosus*, and between *L. punctatus* and *L. miniatus* were attributed to sea-level fluctuations along the Costal Plain that had the effect of connecting and isolating costal rivers at different times during the Pliocene and Pleistocene (Bermingham and Avise 1986). The effect of water level fluctuation on the extinction and colonization dynamics of Everglades *L. punctatus* populations was investigated with allozyme and microsatellite markers (McElroy *et al*. 2003). As predicted, annual environmental fluctuations in the Everglades of Florida, in the form of water level reductions, led to increases in variation on a local level. Sampling design in this case could have resulted in a very different result if recolonization from areas with higher water levels was not considered (McElroy *et al*. 2003).

In Section 1.2.1 on centrarchid species and subspecies, we discussed that *L. megalotis* contains four to possibly seven recognized subspecies. The distribution of *L. megalotis* includes most of the Ohio, middle and lower Mississippi river drainages, and several Gulf of Mexico drainages in Alabama, Mississippi, and Texas. The recent elevation of *L. peltastes* (Table 1.1; Bailey *et al*. 2004) based on morphological differences has only partially resolved the problem of the status of subspecies and intraspecific relationships within *L. megalotis*. Substantial allozyme allelic frequency differences were observed in *L. megalotis* with differences detected between eastern and western populations (Jennings and Philipp 1992).

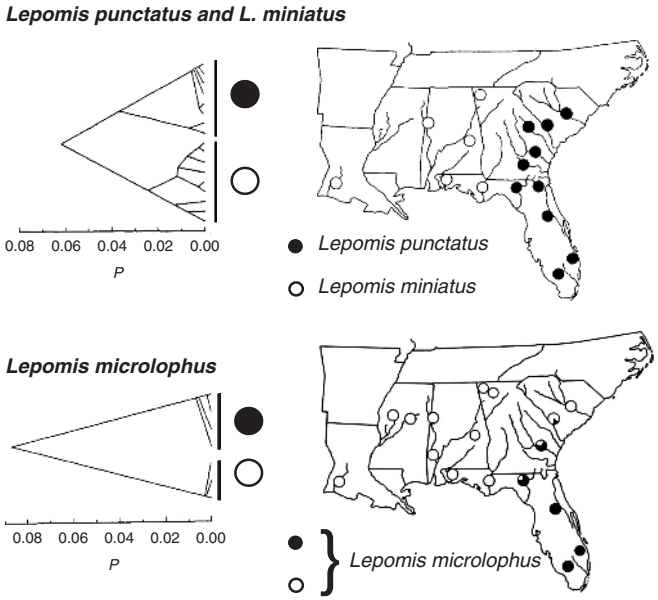

Figure 1.32 Phylogeography of *Lepomis punctatus, L. miniatus*, and *L. microlophus* based on mtDNA haplotype variation (Bermingham and Avise 1986).

There were no fixed allelic differences among the populations and subspecies examined, including comparisons involving *L. megalotis* and *L. peltastes*.

1.5.3 Phylogeography of Centrarchinae

A survey of allozyme variation in *A. constellatus, A. rupestris*, and *A. ariommus* came to three conclusions (Koppelman *et al*. 2000). First, *A. constellatus* exhibited substantial genetic differentiation from *A. rupestris* and *A. ariommus* and is restricted to the White River and sporadic localities in north-flowing tributaries of the upper Osage River. Second, the sister species *A. rupestris* and *A. ariommus* did not exhibit any fixed allelic differences at three polymorphic loci. Third, human introductions might have obscured the phylogeographic patterns between *A. ariommus* and *A. rupestris*, and may have been responsible for the presence of *A. constellatus* in the Osage basin (Koppelman *et al*. 2000).

Recently 23 polymorphic microsatellite markers have been isolated from *A. interruptus* for studies to document population structure. Information on genetic variation is being collected as a prerequisite for efforts to reestablish populations in the species' native range (Schwartz and May 2004). Given the close phylogenetic relationship between *A. interruptus* and the four *Ambloplites* species (Figures 1.30 and 1.31), these microsatellite markers may be helpful in examining the lack of coalescence observed for the allozyme markers between *A. rupestris* and *A. ariommus*.

1.6 Conclusions and future directions

The vast majority of valid centrarchid species were described in the nineteenth century (Figure 1.1). Despite a long and rich history of species descriptions, taxonomic revisions, and studies aiming to resolve phylogenetic relationships of Centrarchidae, there is still much that is unresolved. Centrarchid fishes are among the most economically important group of freshwater fishes in the world, but many species remain unrecognized. In this review, we have tried to illustrate

that many of these species are probably masquerading as subspecies. A resolution to this problem will only come from published studies that examine morphological and genetic variation within polytypic centrarchid species.

With regard to the phylogeny of Centrarchidae, we argue that the analyses using both mitochondrial and nuclear gene sequences provide the best estimates of centrarchid relationships (Figure 1.31). This confidence is based on the near-complete taxon sampling that is not seen in most of the other phylogenetic analyses, a sampling of a large number of characters, congruence between the mitochondrial and nuclear gene phylogenies (Near *et al*. 2004), and the fact that the DNA dataset is able to reject many of the previous centrarchid phylogenetic relationships (Table 1.2).

The most important question facing future phylogenetic studies of Centrarchidae involves the apparent phylogenetic incongruence of the morphological and molecular datasets. For example, the resolution of *Enneacanthus* as nested in *Lepomis* is a result in the cladistic analyses of morphology that is never resolved in the DNA inferred phylogenies (Figures 1.26a, b, 1.30a, b, and 1.31). Specifically, combined morphological and DNA character analyses may allow the identification of particular morphological character states that are convergent among centrarchid lineages, and do not reflect common ancestry. Also, it will be useful to compile the morphological characters used in the separate cladistic analyses that presumably did not share many character states (Lauder 1986; Chang 1988; Wainwright and Lauder 1992; Mabee 1993). The mitochondrial and nuclear gene DNA sequence phylogeny is not completely resolved with strongly supported nodes (Figure 1.31). It has been demonstrated that phylogenetic resolution and node support in molecular phylogenies can be increased by adding more base pairs of DNA to the dataset (de Queiroz *et al*. 2002). In order to increase phylogenetic resolution, we recommend the inclusion of additional mitochondrial and single-copy nuclear gene DNA sequences to the phylogenetic dataset.

Given the scientific and economic importance of centrarchids, it is surprising that such little phylogeographic information is available for centrarchid species. The few published studies have provided a glimpse into the cryptic patterns of variation perhaps not readily apparent in external morphological characters.

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