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RAPID ALLOPATRIC SPECIATION IN LOGPERCH DARTERS (PERCIDAE: *PERCINA*)

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Abstract.—Theory predicts that clades diversifying via sympatric speciation will exhibit high diversification rates. However, the expected rate of diversification in clades characterized by allopatric speciation is less clear. Previous studies have documented significantly higher speciation rates in freshwater fish clades diversifying via sympatric versus allopatric modes, leading to suggestions that the geographic pattern of speciation can be inferred solely from knowledge of the diversification rate. We tested this prediction using an example from darters, a clade of approximately 200 species of freshwater fishes endemic to eastern North America. A resolved phylogeny was generated using mitochondrial DNA gene sequences for logperches, a monophyletic group of darters composed of 10 recognized species. Divergence times among logperch species were estimated using a fossil calibrated molecular clock in centrarchid fishes, and diversification rates in logperches were estimated using several methods. Speciation events in logperches are recent, extending from 4.20 ± 1.06 million years ago (mya) to 0.42 ± 0.22 mya, with most speciation events occurring in the Pleistocene. Diversification rates are high in logperches, at some nodes exceeding rates reported for well-studied adaptive radiations such as Hawaiian silverswords. The geographic pattern of speciation in logperches was investigated by examining the relationship between degree of sympatry and the absolute age of the contrast, with the result that diversification in logperches appears allopatric. The very high diversification rate observed in the logperch phylogeny is more similar to freshwater fish clades thought to represent examples of sympatric speciation than to clades representing allopatric speciation. These results demonstrate that the geographic mode of speciation for a clade cannot be inferred from the diversification rate. The empirical observation of high diversification rates in logperches demonstrates that allopatric speciation can occur rapidly.

Key words.—Diversification rates, molecular clocks, penalized likelihood, reproductive isolation, sympatric speciation.

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Studies of freshwater fishes have indicated that clades diversifying via sympatric versus allopatric modes of speciation exhibited significantly shorter waiting times between speciation events, hence sympatric speciation clades diversify faster than allopatric clades (McCune and Lovejoy 1998). Despite this interesting result, and the fact that allopatric speciation appears to be the predominate mode of speciation (Mayr 1942, 1963), the diversification rate of very few allopatric clades has been examined. Also, what rates of diversification constitute rapid diversification is not well defined due to a limited number of estimated diversification rates for comparisons among different lineages. To date, there is ample evidence for rapid speciation in sympatric clades, but there are few studies that estimate diversification rates in clades originating by allopatric speciation. Without this empirical data we cannot form a general rule regarding the relationship between the relative diversification rate and the geographic mode of speciation.

Compared to allopatric modes of speciation, sympatric speciation models involve either ecologically mediated reproductive isolation as populations adapt to alternative discrete habitats or resources, or reproductive isolation that results from competition for continuously distributed resources without geographic isolation (Dieckmann and Doebeli 1999; Kondrashov and Mina 1986). Ecological sympatric speciation entails disruptive selection on polymorphic traits and results in nonrandom mating, producing a bimodal distribution of phenotypes (Dieckmann and Doebeli 1999; Turelli et al. 2001). Since selection is acting directly against hybrids during speciation, sympatric speciation may occur more rapidly than allopatric speciation (Bush 1975).

Investigation of the geographic pattern of speciation within clades requires the use of phylogenetic hypotheses, information on relative times of divergence among species, and data documenting the geographic distribution of the constituent species (Barraclough and Vogler 2000; Near et al. 2003). Due to range shifts subsequent to speciation, species diversifying via allopatric modes are expected to display greater sympatry as time since divergence increases, and species resulting from sympatric speciation modes will exhibit reduced geographic overlap as time since common ancestry increases (Lynch 1989; Barraclough and Vogler 2000). These predictions can be used to determine the likely mode of speciation in a clade, and can be combined with studies of diversification rates to examine the relationship between geographic mode of speciation and the tempo of diversification.

An excellent opportunity to study diversification rates in clades characterized by allopatric speciation is found in darters, a clade of approximately 200 freshwater fish species. Darters typically occupy benthic and hyperbenthic habitats in rivers and streams, feed on aquatic macroinvertebrates, and males of many species are characterized by elaborate nuptial coloration (Kuehne and Barbour 1983; Page 1983; Etnier and Starnes 1993; Jenkins and Burkhead 1994). Darters are endemic to North America east of the Rocky Mountains with the majority of species distributed in the regions drained by the Mississippi River (Page 1983). A prevalent pattern that has emerged from comparing geographic distribution and phylogenetic relationships of darter species is that sister species are rarely sympatric (Wiley and Mayden 1985; Near et al. 2000; Near 2002; Page et al. 2003), hinting that allopatry may be the predominant mode of speciation in dart-

ers. However, the geographic mode of speciation in North American freshwater fishes (including darters) has never been investigated using methods that examine the relationship between clade age and degree of observed sympatry (Lynch 1989; Barraclough and Vogler 2000).

In this investigation, we use a molecular clock analysis to investigate diversification rates in logperches, a clade of darters that exhibits little ecological variation among species (Kuehne and Barbour 1983; Page 1983; Jenkins and Burkhead 1994). Unlike many other darter species, logperches exhibit little sexual dimorphism in coloration. However, both males and females of some species have a distinctive red-orange band along the distal margin of the first dorsal fin (Page 1983). The interspecific variation in the presence of the red-orange band has inspired several intensive analyses of meristic and morphometric variation over the past 20 years that has resulted in the doubling of the number of recognized logperch species (Morris and Page 1981; Thompson 1985, 1995, 1997a,b). Logperch species are recognized as morphologically distinct from one another under the same general criteria that have been historically applied to the entire North American freshwater fish fauna. Therefore, logperch species are morphologically diagnosable units hypothesized to be reproductively isolated from other logperch species. Additionally, a lack of naturally occurring hybrids among logperch species corroborates the hypothesis that the species included in our analyses represent ''good'' biological species.

The rate of diversification and the prevalent geographic mode of speciation in logperches were investigated by comparing the degree of geographic range overlap versus estimated divergence times of species in the clade. We attempt to test predictions based on previous analyses of freshwater fish clades that suggest the geographic mode of speciation is correlated with the rate of diversification (McCune 1997; McCune and Lovejoy 1998). The expected pattern is for clades diversifying allopatrically to have significantly lower diversification rates than clades diversifying sympatrically. If logperches exhibit rapid allopatric speciation, this would caution against the generality that diversification rates can be used to infer the geographic mode of speciation, and that rapid allopatric speciation would result only from processes such as adaptive radiation.

Materials and Methods

Species Recognition, Specimen Collection, and DNA Sequencing

Material was gathered for molecular phylogenetic analysis from all 10 recognized logperch species. Specimens were collected from native populations using seine nets and electrofishing techniques. Collection localities, museum voucher information, and GenBank accession numbers for all specimens are given in the Appendix and the approximate collection localities are plotted in Figure 1. *Percina nebulosa* (Haldeman) has long been considered a junior synonym of *P. caprodes* (Rafinesque) (Collette and Knapp 1966). However, we consider *P. nebulosa* to be a valid species, a conclusion supported by mitochondrial DNA (mtDNA) gene trees presented in this study and unpublished morphological analyses (A. George and D. Neely, pers. comm.).

Total genomic DNA was isolated from muscle tissues using standard phenol-chloroform extraction and ethanol precipitation procedures. Complete gene sequences of two mtDNA protein coding genes, cytochrome *b* (cyt*b*) and NADH subunit 2 (ND2), were collected using previously published polymerase chain reaction (PCR) and sequencing protocols and conditions (Near et al. 2003). Individual sequence files were edited using EditView version 1.0.1 (available via http://www.appliedbiosystems.com/support/software/dnaseq/installs.cfm) and complete gene sequences were constructed using the program Sequencher 3.0 (available via http://www.genecodes.com); all gene sequences were verified by completely sequencing both DNA strands.

Phylogenetic Analyses

Complete cyt*b* (1140 bp) and ND2 gene sequences (1047 bp) were aligned using the computer program ClustalX (Thompson et al. 1997) and verified by comparing to translated amino acid sequences. There is substantial morphological and molecular evidence that logperches are a monophyletic group (*Percina caprodes* species clade) within the monophyletic genus *Percina* (Page 1981; Near 2002), a fact we exploited in selecting outgroup species for our analyses (Appendix). Darters are classified in Percidae, and along with Moronidae and Centrarchidae are grouped in Perciformes (Nelson 1994). We included nine other darter species, a nondarter percid (*Perca flavescens*), a moronid species, and several species of Centrarchidae as outgroup taxa (Appendix).

Phylogenetic relationships were estimated from the combined cyt*b* and ND2 datasets using both a partitioned mixed model Bayesian (pMM Bayes) method (Ronquist and Huelsenbeck 2003) with posterior probabilities estimated using metropolis-coupled Markov chain Monte Carlo (MC3; Larget and Simon 1999; Huelsenbeck et al. 2001) and maximum parsimony (MP). A total of six data partitions were identified in the cyt*b* and ND2 dataset, corresponding to each of the three codon positions for the two separate genes. Optimal models of DNA sequence evolution were determined for each data partition using hierarchical likelihood ratio tests (LRT) as executed in the computer program Modeltest 3.0 (Posada and Crandall 1998). The models tested for the separate data partitions differed by no more than three parameters, which were a unique substitution model (one, two, or six parameter) corresponding to F81, HKY, or K80; and GTR models of DNA sequence evolution, distribution of among-site rate variation (equal versus gamma distributed rates), and the presence of invariant sites (Swofford et al. 1996).

The pMM Bayes analyses were executed using the computer program MrBayes 3.0 (Ronquist and Huelsenbeck 2003). The optimal models of sequence evolution determined for each of the six data partitions using LRTs were assigned in MrBayes using the APPLYTO command, and appropriate model parameter values were estimated for each partition using UNLINK commands (Ronquist and Huelsenbeck 2003). Each MrBayes search was run for 4×10^6 generations using four chains, and the MC3 was run four separate times. The burn-in period of the MC3 analysis was determined by plotting the marginal likelihood versus generation number to determine the generation number at which likelihood values

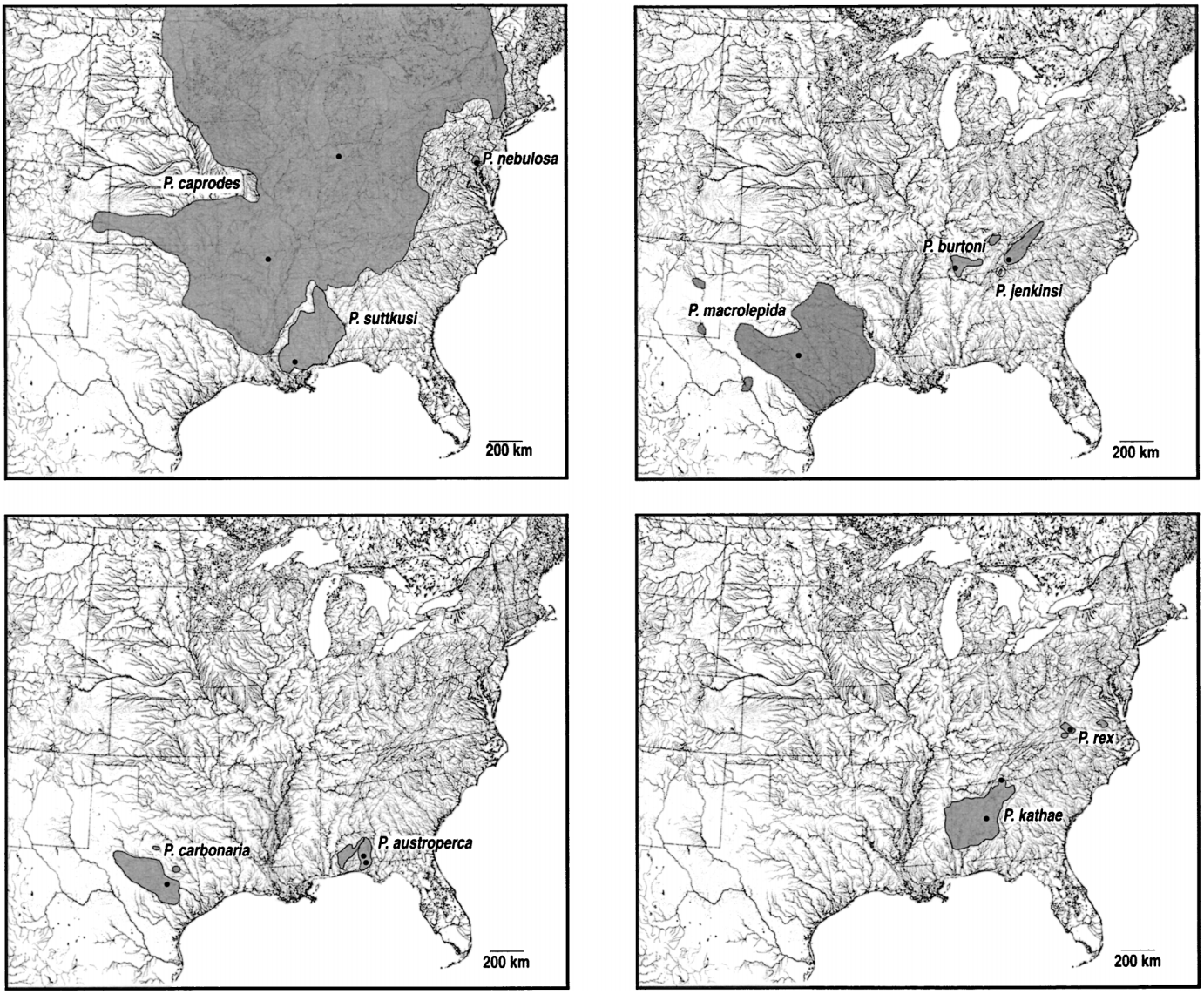

FIG. 1. Geographic distribution of logperch species. Approximate sampling localities are marked with black dots, except *Percina jenkinsi* because a dot would cover the whole portion of its shaded range map.

reach a plateau. Trees and parameter values sampled prior to the burn-in were discarded. The posterior clade probabilities of node support were determined by the frequency of their occurrence in the post-burn-in distribution of trees (Ronquist and Huelsenbeck 2003). Maximum parsimony analyses were executed in PAUP* (Swofford 2000) with heuristic tree searches that completed 100 random sequence addition replicates. Node support in MP trees was assessed using a nonparametric bootstrap analysis with 2000 pseudoreplicates.

Tests of Nucleotide Substitution Rate Heterogeneity, Calibration of the Molecular Clock, and Estimating Divergence Times

We used maximum likelihood tree-based methods to assess heterogeneity of nucleotide substitution rates across darter lineages, and estimate divergence times among logperch species. Unfortunately, maximum likelihood branch lengths cannot be estimated on a fixed user-supplied tree in MrBayes 3.0, limiting the estimation of confidence intervals for divergence times using bootstrap resampling. Therefore, we had to determine the best model of sequence evolution for the concatenated cyt*b* and ND2 dataset using LRTs as executed in Modeltest 3.0, and used the optimal model in PAUP* to calculate model parameter values and estimate maximum likelihood branch lengths on the post-burn-in pMM Bayes tree. Differing from previous molecular clock analyses of cyt*b* and ND2 in centrarchids (Near et al. 2003), we did not use codon based site-specific models of sequence evolution, because these models have been shown to underestimate maximum likelihood branch lengths relative to models using a gamma distribution of among-site rate variation (Buckley and Cunningham 2002).

Using PAUP*, maximum likelihood scores with and without a molecular clock constraint were compared using LRTs

with a chi-squared distribution. The degrees of freedom were equal to $s - 2$, where s is equal to the number of taxa in the analysis. Upon the discovery of rate heterogeneity of DNA sequence evolution among lineages, we estimated divergence times using the penalized likelihood method, which includes a roughness penalty for the autocorrelated rate transformation between ancestor and descendent branches in the phylogeny. This permits an examination of many solutions to smooth rate variation in the tree, with the penalty level determined by cross-validation using r8s (Sanderson 2003).

We used a fossil calibration of the molecular clock in centrarchid fishes as an external calibration for logperches. We did not use darter fossils because the group is poorly represented in the fossil record, with the earliest fossils dated to the Late Pleistocene (Smith 1981; Cavender 1986). Also, we did not use age information from geologic events that may have led to vicariant speciation as calibration points, because of pseudocongruence (Donoghue and Moore 2003) between darter lineages with similar geographic distributions (Strange and Burr 1997). We assume that using the centrarchid external fossil calibration will not to lead to grossly inaccurate estimates of divergence times among logperch species due to the relatively close phylogenetic affinity between centrarchids and darters, as well as our use of methods to smooth rate variation across the phylogeny.

Two centrarchid fossil calibration points were used in penalized likelihood analyses. These calibration points were the most recent common ancestor (MRCA) of *Micropterus* and its sister taxon dated at 23.0 millions of years ago (mya), and the MRCA of *Archoplites interruptus* and *Ambloplites* dated at 15.5 mya. These calibration points and methods of assigning fossil dates to nodes in the phylogeny are discussed in Near et al. (2003), and cross-validation analysis has demonstrated that both of these calibration points provide robust estimates of divergence times in centrarchid fishes. Fossil-dated calibration points were fixed in r8s analyses using the FIXAGE command.

Error contribution from data sampling was estimated using a nonparametric bootstrap procedure outlined in Baldwin and Sanderson (1998). One hundred bootstrap replicate datasets were generated using the seqboot program in Phylip (Felsenstein 1993), and branch lengths from each dataset were calculated for the pMM Bayes tree using the optimal model of sequence evolution for the concatenated cyt*b* and ND2 dataset. The distribution of divergence times across the 100 bootstrap datasets was determined using the PROFILE command in r8s. The central 95% of the distribution of age estimates provided confidence intervals for age estimates at each node (Baldwin and Sanderson 1998; Sanderson 1998).

Estimation and Analysis of Diversification Rates in Logperches

The diversification rate among species in the logperch clade was investigated with a stochastic model of lineage growth, the Yule pure birth process (Yule 1924; Baldwin and Sanderson 1998; Magallon and Sanderson 2001; Nee 2001). The Kendall-Moran estimator (eq. 1 in Near et al. 2003; eq. 7 in Nee 2001) was used to calculate the diversification rate (S). The 95% confidence interval for S was calculated using the exact estimator derived in Nee (2001, eq. 15).

To compare diversification rates in logperches with other clades of fishes reported in the literature, we calculated the time for speciation (TFS) index that was previously used to compare clades of freshwater fishes diversifying via sympatric versus allopatric modes (McCune 1997; McCune and Lovejoy 1998). McCune and Lovejoy (1998) did not calculate S for the clades in their study, and because many of these clades are not closely related to either darters or centrarchids, estimation of S for these clades is complicated by the lack of lineage-specific molecular clock calibrations. When comparing the diversification rate in logperches with other clades of freshwater fishes, we relied on TFS calculations that require only the age of the MRCA of the lineage and the number of species in the clade. In a symmetrical model of branching, TFS_{ln} was used as a measure of the mean doubling time for the species in a clade. $TFS_{ln} = t(\ln 2)(\ln n)^{-1}$, where t is the age of the MRCA of the clade and n is the number of species (McCune 1997). A confidence interval for the logperch TFS_{ln} value was calculated using the confidence intervals of the divergence time estimate for the MRCA of all logperches. The TFS_{ln} value calculated for logperches was compared to published TFS_{ln} values for lineages with hypothesized sympatric and allopatric origins (McCune and Lovejoy 1998).

We identified lineages within the logperches with higher-than-expected diversification rates using the relative cladogenesis statistic (Nee et al. 1994) as executed in the computer program End-Epi version 1.0 (Rambaut et al. 1997). The statistic Pk is the probability in a constant rates birth-death model of lineage diversification that a specific lineage, extant at time t, will have k descendent tip taxa, relative to the total number of tip taxa at the present time (Nee et al. 1995).

Geographic Pattern of Speciation in Logperches

Geographic distribution data for logperch species were analyzed using recently developed comparative methods to investigate geographic patterns of speciation (Chesser and Zink 1994; Barraclough and Vogler 2000). The likelihood that sympatric speciation has been a mechanism of diversification in darters is limited since substantial evidence from previous phylogenetic analyses indicates that allopatric modes are the predominant mechanism of speciation in North American freshwater fishes (Wiley and Mayden 1985; Near et al. 2000). We were interested in determining whether true sister species of logperches were ever sympatric, and the relative age of logperch species that exhibit sympatry. Distributional data for each logperch species were collected from several publications that are based on documented collections in museums (Lee et al. 1980; Thompson 1985, 1995, 1997a,b; Mettee et al. 1996), and transferred to equal area maps (Fig. 1). A transparent grid sheet with squares measuring 2 mm^2 was overlaid onto shaded range maps, and the occurrence of a species in a particular grid was recorded in Microsoft Excel (Microsoft Corp., Redmond, WA). The range size of a species was recorded as the number of grids occupied on the equal area maps. Distributions for ancestral species at internal nodes in the phylogeny were ''reconstructed'' by summing

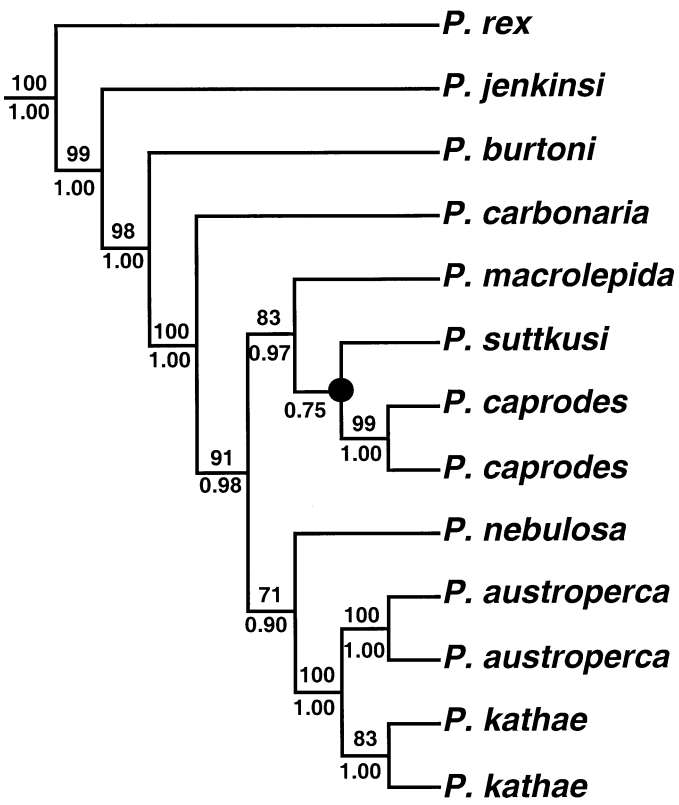

FIG. 2. One of two trees resulting from maximum parsimony analysis and the single tree from partitioned mixed model Bayesian analysis showing relationships among logperch species. The node collapsed between the two parsimony trees is indicated with a black dot. Maximum parsimony bootstrap scores are listed above nodes, and Bayesian posterior probabilities are listed below the nodes.

the ranges of the species subtending the node (Lynch 1989; Barraclough and Vogler 2000). The degree of sympatry between any two species or lineages was calculated using the equation presented in Chesser and Zink (1994) and Barraclough and Vogler (2000), where the area of overlap is divided by the range size of the species or clade with the smaller range. For visual inspection, the degree of range overlap at a given node was plotted against the estimated age of the MRCA for the node. Following Barraclough and Vogler (2000), we also regressed arcsine-transformed sympatry index on node age.

RESULTS

Phylogenetic Relationships of Logperches

The combined cyt*b* and ND2 gene sequences totaled 2187 aligned nucleotide sites, with only 103 parsimony-informative nucleotide sites present in logperches. Previous analyses of logperch relationships using only the cyt*b* gene were poorly resolved, especially with regard to apical nodes in the phylogeny (figs. 3 and 6 in Near 2002). The MP analysis of combined cyt*b* and ND2 sequences resulted in the resolution of all but one node, and all resolved interspecific nodes were supported in greater than 70% of the bootstrap pseudoreplicates (Fig. 2).

Likelihood ratio tests selected four different models of sequence evolution for the six data partitions, with the third codon positions from cyt*b* and ND2 each sharing the same model (Table 1). Running the pMM Bayes analysis four times resulted in the same tree topology after the burn-in, and essentially the same Bayesian posterior probabilities. The pMM Bayes tree resulting from the last of the four MC3 runs was chosen and is nearly identical to the MP analysis, with the exception that all nodes were resolved among the collection of trees after the burn-in. One of the two interspecific nodes not supported with Bayesian posterior probabilities greater than 0.95 was the node not resolved in the MP analysis (Fig. 2).

TABLE 1. Summary of models of DNA substitution selected for codon-based data partitions using maximum likelihood ratio tests. GTR, general time reversible model; HKY85, Hasegawa-Kishino-Yano 1985 model.

Data partition	DNA substitution model	No substitution types	Invariant sites?	Substitution rates[1]
cyt*b* 1st	GTR[2]	6	no	gamma distributed
cyt*b* 2nd	HKY85[3]	2	no	gamma distributed
cyt*b* 3rd	GTR[3]	6	yes	gamma distributed
ND2 1st	HKY85[2]	2	no	gamma distributed
ND2 2nd	HKY85[2]	2	yes	gamma distributed
ND2 3rd	GTR[3]	6	yes	gamma distributed

[1] Among-site rate variation.

Nucleotide Substitution Rate Heterogeneity and Divergence Time Estimates

Using the pMM Bayes tree, LRTs identified the GTR + I + G model as optimal for the concatenated cyt*b* and ND2 dataset. The log-likelihood scores for the rate-constant molecular clock enforced model (GTR + I + Gc; −20,794.49) and the rate-variable model where the molecular clock was relaxed (GTR + I + G; −20,754.11) were used in a LRT that resulted in the rejection of the molecular clock model ($\chi^2 = 80.76$, df = 30, $P > 0.001$).

The divergence time estimates from penalized likelihood analysis using the external centrarchid fossil calibration points resulted in a divergence time estimate of 4.20 ± 1.06 mya for the MRCA of the logperch clade (Fig. 3, node A). The estimated ages of interspecific nodes in the logperch phylogeny ranged between 4.20 ± 1.06 mya and 0.42 ± 0.22 mya (Table 2), and are graphically represented in a chronogram (Fig. 3). The rate of nucleotide substitution in the concatenated cyt*b* and ND2 dataset among the logperch species was estimated as 0.010 ± 0.001 substitutions per site per million years per lineage, or roughly a pairwise rate of 2.0 ± 0.2% per million years.

Diversification Rates in Logperches

The net diversification rate of the entire logperch clade using the Kendall-Moran estimate of a pure birth process (*S*) was 0.46 species per million years (s/my) (95% CI: 0.20 s/my, 0.82 s/my). The relative cladogenesis statistic (*Pk*) was significant for node D (*Pk* = 0.05) in the logperch phylogeny (Fig. 3), indicating a faster diversification rate at this node relative to all other nodes in the phylogeny. The Kendall-

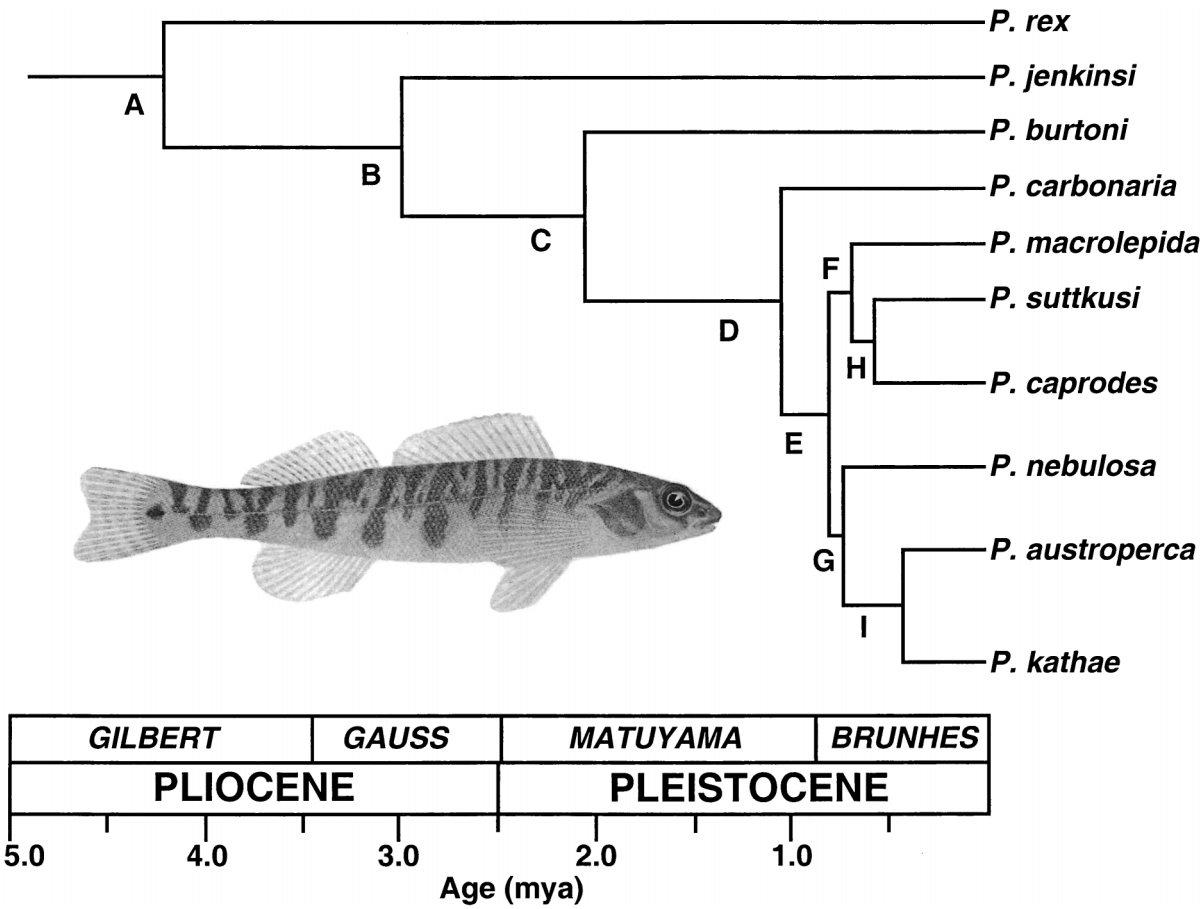

FIG. 3. Time-calibrated phylogram (chronogram) of the partitioned mixed-model Bayesian tree. Exact age estimates for labeled nodes (A through I) are given in Table 2. Geologic time scale is given for paleomagnetic chrons and epochs (Repenning 1987). *Percina caprodes* redrawn from Forbes and Richardson (1920). mya, millions of years ago.

Moran estimate for node D was 0.96 species per million years (95% CI: 0.31 s/my, 1.97 s/my).

Calculated TFS values from McCune and Lovejoy (1998) for 11 clades of freshwater fishes, and the hypothesized geographic mode of speciation are given in Table 3. The mean TFS values between sympatric and allopatric clades were significantly different ($t_9 = 4.185$, $P < 0.0024$) in a two-sample *t*-test (Fig. 4). The TFS value for the entire logperch clade (Fig. 3, node A) was 1.26 million years (my; bootstrap confidence interval: 0.95 my, 1.58 my), and TFS for node D (Fig. 3) was 0.37 my (bootstrap confidence interval: 0.16 my, 0.57 my). The TFS value for the MRCA of the 10 extant logperch species (Fig. 3, node A) is similar to TFS values for other allopatric fish clades, whereas the TFS value for node D is more similar to TFS values calculated in fish lineages that are examples of sympatric speciation (Fig. 4).

TABLE 2. Estimated ages and bootstrap estimates of standard error in nodes in the logperch chronogram (see Fig. 3). mya, millions of years ago.

Node	Age estimate (mya)	Bootstrap estimate of standard error (mya)
A	4.20	±1.06
B	2.97	±0.75
C	2.04	±0.57
D	1.03	±0.58
E	0.78	±0.38
F	0.67	±0.35
G	0.71	±0.39
H	0.55	±0.24
I	0.42	±0.22

Geographic Pattern of Speciation in Logperches

Plotting the degree of sympatry versus the MRCA age of each node in the logperch phylogeny illustrates a pattern of increasing range overlap with age (Fig. 5). This is the expected pattern in clades diversifying via allopatric modes with range shifts after speciation (Barraclough and Vogler 2000; Losos and Glor 2003). The regression of arcsine-transformed sympatry index onto node age was not significant ($n = 9$; df = 1,8; $F = 1.42$; $P = 0.272$). The low intercept was consistent with Barraclough and Vogler's (2000); simulations for allopatric speciation (intercept 0.10, SE = 0.34). Two true sister species pairs are present in the logperch phylogeny (*P. caprodes–P. suttkusi*, and *P. austroperca–P. kathae*), and each of these species pairs is completely allopatric. The largest three instances of observed sympatry among logperches involve *P. carbonaria* and *P. macrolepida* (MRCA node D), *P. caprodes* and *P. burtoni* (MRCA node C), and *P. kathae*

TABLE 3. Time-for-speciation (TFS) values reported in McCune and Lovejoy (1998) for examples of sympatric and allopatric speciation in freshwater fishes. my, million years.

Taxa	No. of species	TFS (my/species)	Speciation mode
Lake Bermin cichlids	9	0.07	sympatric
Lake Malawi cichlids	400	0.10	sympatric
Barombi Mbo, cichlids	11	0.32	sympatric
Lamprologini cichlids	65	0.53	sympatric
Ectodini cichlids	30	0.77	sympatric
Tropheus cichlids	6	0.79	sympatric
Xiphophorus	13	1.00	allopatric
Gambusia	45	1.10	allopatric
Melanotaenia	32	1.43	allopatric
Rivulus	70	1.54	allopatric
Osmerus	3	2.27	allopatric

and *P. jenkinsi* (MRCA node B; Figs. 1 and 5). The basal split in the logperch phylogeny (Figs. 2 and 3) is characterized by no sympatry (Fig. 5).

DISCUSSION

Speciation in logperches has been rapid. The Kendall-Moran (*S*) estimate of the diversification rate for the entire logperch clade, 0.46 s/my, is similar to the rate estimated for the adaptive radiation of Hawaiian silverswords (0.56 s/my; Baldwin and Sanderson 1998), higher than North American tiger beetles (0.29 s/my; Barraclough and Vogler 2002), and more than four times the rate observed in the North American endemic *Micropterus* black basses (0.11 s/my; Near et al. 2003).

The clade of logperches subtended by node D (Fig. 3) was identified as having a higher diversification rate relative to all other nodes in the logperch tree using the relative cladogenesis statistic (Nee et al. 1996). The diversification rate (*S*) at this node was 0.96 species per my, which is among the highest Kendall-Moran estimates of diversification ever calculated from molecular phylogenies (Klak et al. 2004). Because the development of the methods using the Kendall-Moran estimator for analyzing chronograms has been rather recent (Baldwin and Sanderson 1998; Sanderson 1998; Nee 2001), too few comparisons among clades have been published to make broad generalizations about the differences and similarities in diversification rates between logperches and other lineages. Regardless, the value of *S* calculated for node D in the logperch phylogeny is exceptionally high, and such a result could inspire a search for biological correlates that drive such high rates of speciation. However, logperch species are very similar in morphology, habitat, and resource use, making it unlikely that mechanisms such as adaptive radiation (Simpson 1953; Schluter 2000) or the evolution of key innovations (Heard and Hauser 1995; Bond and Opell 1998) are responsible for the high diversification rate observed in logperches.

Speciation in logperches has also been allopatric, a conclusion derived from examination of the relationship between degree of sympatry and estimated ages of the MRCA of nodes in the logperch phylogeny (Fig. 5). In particular, the two true sister species pairs in the logperch tree exhibited complete allopatry, and instances of extensive sympatry were observed only between distantly related logperch species (Figs. 3 and 5).

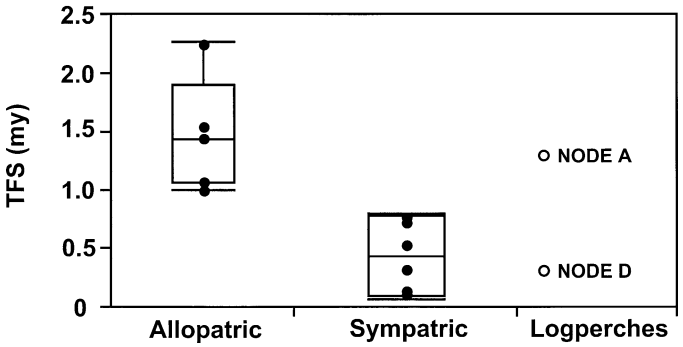

FIG. 4. Box plot of time-for-speciation (TFS) values estimated for hypothesized allopatric and sympatric diversifying freshwater fish clades (Table 3), and two nodes (A, D) in the logperch phylogeny (Fig. 3). The box represents the central 50% of values, with the median marked with the central horizontal line. Values within 1.5 times the interquartile range are shown with vertical bars. my, million years.

One major critique of this approach to studying the geographic pattern of speciation is the fact that the geographic distribution of ancestral taxa (internal nodes) must be inferred by adding the ranges of the constituent species. This method is confounded when species distributions change subsequent to speciation, because sympatry results as a consequence of range shifts (Losos and Glor 2003). Despite this caveat, it is important to point out that the greatest degrees of sympatry involve contrasts between logperch species that are distantly related (Figs. 1 and 5), with one species usually phylogenetically basal and distributed in highland areas characterized by extensive endemism in other lineages of freshwater fishes and aquatic organisms (Lydeard and Mayden 1995). In ad-

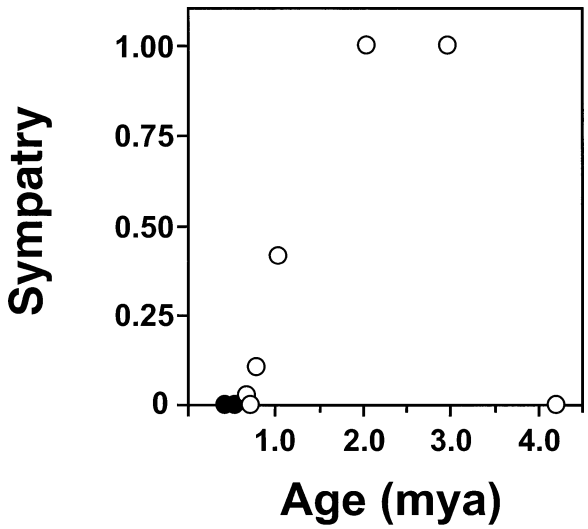

FIG. 5. Degree of sympatry versus age of nodes in the logperch phylogeny (Figs. 1 and 3). Open circles mark nodes where geographic ranges were reconstructed by summing geographic ranges for at least one of the two lineages contrasted. Filled circles mark contrasts between the two true sister species pairs (*P. kathae–P. austroperca* and *P. caprodes–P. suttkusi*). mya, millions of years ago.

dition, the logperch species with the largest ranges are among the youngest (Figs. 1 and 3), indicating that range expansion subsequent to speciation is common in this clade. Despite the potential complication of inferring the geographic mode of speciation introduced by postspeciation range shifts, the pattern of speciation in logperches appears allopatric, since the youngest nodes in the phylogeny exhibit little or no sympatry (Fig. 5).

A potential explanation for the rapid allopatric speciation in logperches is that the species have been ''over-split'' taxonomically, and the clade actually contains fewer than 10 species. Three lines of evidence support the premise that logperch species diversity has not been overestimated. First, with respect to the criteria used by ichthyologists in recognizing species that is applied across the diverse North American freshwater fish fauna, all logperch species are morphologically diagnosable and patterns of morphological variation used to identify species have been determined after examination of large numbers of specimens sampled throughout the ranges of the species (Stevenson 1971; Morris and Page 1981; Thompson 1985, 1995, 1997a,b). Second, despite the occurrence of five sympatric pairs of logperch species, there is a conspicuous lack of hybrids reported between logperch species. However, hybrids are reported between logperches and more distantly related darter species in both *Percina* and *Etheostoma* (Schwartz 1972; Page 1976; Hubbs et al. 1988). Third, the interspecific variation in the presence of a conspicuous red-orange band along the distal margin of the first dorsal fin in both males and females exhibits an interesting pattern among sympatric logperch species. In four of the five sympatric pairs of logperch species the red-orange band is present in one species and absent in the other, indicating that the presence and absence of this coloration may serve as a mechanism of inter- and/or intraspecific species recognition.

Some authors conclude that sympatric speciation should always be faster than allopatric speciation (Bush 1975; McCune and Lovejoy 1998). This is based on the premise that allopatric speciation is a passive process, merely the byproduct of drift and local adaptation that will eventually lead to reproductive isolation. However, the variance of the time to allopatric speciation may be much higher than for sympatric speciation since mutations that cause postzygotic reproductive incompatibilities are stochastic (Orr and Turelli 2001), and processes such as divergent natural selection and sexual conflict can also contribute to the acceleration of allopatric speciation (Rice and Hostert 1993; Funk 1998; Gavrilets 2000, 2003; Orr and Turelli 2001; Turelli et al. 2001; Fitzpatrick 2002).

The theoretical models that show that allopatric speciation can occur rapidly require testing by comparing diversification rates in sympatric and allopatric speciation clades. McCune and Lovejoy (1998) presented a novel approach in comparing the rate of speciation between clades of freshwater fishes that were hypothesized to represent examples of sympatric and allopatric speciation. We reanalyzed their data (Fig. 4) and found a significant difference in the mean TFS value between allopatric and sympatric freshwater fish clades. Notably, the TFS value for the MRCA node of the entire logperch clade (1.26 my) falls within the distribution of McCune and Lovejoy's (1998) allopatric clades. However, the TFS value for node D in the logperch phylogeny (0.37 my) is less than the median value of the sympatric clades (Fig. 4). This very low TFS value, and a high Kendall-Moran estimate of the diversification rate ($S = 0.96$ s/my), involves a portion of the logperch phylogeny that exhibits minimal sympatry among the constituent species (Figs. 3 and 5). Thus our data indicate that allopatric processes are driving diversification, and allopatric speciation has occurred at a very rapid rate in this clade.

The Gulf Coast Allopatric Speciation Model

If adaptive diversification among species of logperches can be ruled out, what possible mechanisms could be responsible for the rapid allopatric speciation observed in this clade? One possibility is the Gulf Coast allopatric speciation model that has been formulated to explain the repeated pattern of endemism in this region across many clades of North American freshwater fishes (Wiley and Mayden 1985; Swift et al. 1986; Near et al. 2003). In particular, the model proposes that speciation events in freshwater fishes endemic to this region were driven by sea level fluctuations acting as vicariant isolating mechanisms with speciation events dating between 8.5 and 3.5 mya, with peak diversification rates occurring between 8.0 and 6.0 mya (Near et al. 2003). These predictions were based on the geographic distribution and timing of diversification in the freshwater fish clade *Micropterus* (Near et al. 2003). The distribution of logperch species is similar to that of *Micropterus* and consistent with this model, as six of the 10 species are endemic to river systems that drain into the Gulf of Mexico (Fig. 1). However, the timing of speciation in logperches does not fit the Gulf Coast allopatric speciation model as presented by Near et al. (2003), because diversification is much more recent, as evidenced by seven of the nine logperch diversification events being dated to the Pleistocene (Fig. 3).

Sea level fluctuations have occurred repeatedly since the Oligocene, with the highest rate in the Pleistocene (Riggs 1984; Haq et al. 1987). This high rate of change in sea level may have produced a rapid series of geographic isolating barriers among rivers of the Gulf of Mexico. The mechanism of geographic isolation through sea level fluctuations is the same as proposed for *Micropterus* in the Gulf Coast allopatric speciation model (Near et al. 2003), but the timing of geographic isolation in logperches and *Micropterus* is very different. Rapid speciation in logperches could have resulted from the combination of phases of rapid geographic isolation driven by sea level fluctuations coupled with a possible rapid accumulation of post- and/or prezygotic isolating barriers.

The comparison of the timing of speciation in logperches and *Micropterus* leads to a caution for future studies using cladistic biogeographic methods to investigate patterns of speciation in freshwater fishes distributed in Gulf of Mexico river drainages. Previous cladistic biogeographic investigations of North American freshwater fishes have ignored temporal information, hypothesizing that similar area relationships imply a common cause responsible for speciation across multiple clades (Wiley and Mayden 1985; Mayden 1987a,b, 1988). The demonstration of pseudocongruence between Gulf Coast speciation in logperches and *Micropterus*, in which

similar area relationships are exhibited across multiple clades with vastly different ages (Cunningham and Collins 1994; Donoghue and Moore 2003), should encourage future historical biogeographic studies of North American freshwater fishes to incorporate information on the absolute timing of diversification within lineages.

In this study we have documented a very high rate of allopatric diversification in a clade of freshwater fishes. This result demonstrates that the mode of speciation cannot be reliably predicted from the rate of speciation. The methods and strategies employed in this investigation can easily be extended and applied to future macroevolutionary studies of North American freshwater fishes. In addition, a promising avenue for research is the experimental determination of postzygotic reproductive isolation in laboratory hybrid crosses. Previous work in darters has proven fruitful in demonstrating that hybrid viability decreases with taxonomic and genetic divergence (Hubbs and Strawn 1957; Hubbs 1959; Mendelson 2003). We predict that the ''speciation clock'' in darters will vary among clades as a result of the stochastic nature of accumulating postzygotic reproductive incompatibilities. Also, rates of diversification will correlate with the rate of postzygotic reproductive isolation as determined by the relationship between hybrid viability and/or fitness and the age since common ancestry among interspecific experimental crosses. Finally, we anticipate the discovery of additional clades of organisms that exhibit rapid allopatric diversification, which will enhance our conclusion that the geographic mode of speciation cannot be reliably inferred from the diversification rate.

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Literature Cited

Baldwin, B. G., and M. J. Sanderson. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. USA 95:9402–9406.

Barraclough, T. G., and A. P. Vogler. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. Am. Nat. 155:419–434.

———. 2002. Recent diversification rates in North American tiger beetles estimated from a dated mtDNA phylogenetic tree. Mol. Biol. Evol. 19:1706–1716.

Bond, J. E., and B. D. Opell. 1998. Testing adaptive radiation and key innovation hypotheses in spiders. Evolution 52:403–414.

Buckley, T. R., and C. W. Cunningham. 2002. The effects of nucleotide substitution model assumptions on estimates of nonparametric bootstrap support. Mol. Biol. Evol. 19:394–405.

Bush, G. L. 1975. Modes of animal speciation. Annu. Rev. Ecol. Syst. 6:339–364.

Cavender, T. M. 1986. Review of the fossil history of North American freshwater fishes. Pp. 699–724 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Chesser, R. T., and R. M. Zink. 1994. Modes of speciation in birds: a test of Lynch's method. Evolution 48:490–497.

Collette, B. B., and L. W. Knapp. 1966. Catalog of type specimens of the darters (Pisces, Percidae, Etheostomatini). Proc. US Natl. Mus. 119:1–88.

Cunningham, C. W., and T. M. Collins. 1994. Developing model systems for molecular biogeography: vicariance and interchange in marine invertebrates. Pp. 405–433 *in* B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle, eds. Molecular ecology and evolution: approaches and applications. Birkhauser Verlag, Basel, Switzerland.

Dieckmann, U., and M. Doebeli. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

Donoghue, M. J., and B. R. Moore. 2003. Toward an integrative historical biogeography. Integr. Comp. Biol. 43:261–270.

Etnier, D. A., and W. C. Starnes. 1993. The fishes of Tennessee. Univ. of Tennessee Press, Knoxville, TN.

Felsenstein, J. 1993. Phylip: Phylogenetic inference package. University of Washington, Seattle, WA.

Fitzpatrick, B. M. 2002. Molecular correlates of reproductive isolation. Evolution 56:191–198.

Forbes, S. A., and R. E. Richardson. 1920. The fishes of Illinois. Illinois Natural History Survey, Springfield, IL.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. Evolution 52:1744–1759.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. Nature 403:886–889.

Gavrilets, S. 2003. Models of speciation: What have we learned in 40 years? Evolution 57:2197–2215.

Haq, B. U., J. Hardenbol, and P. R. Vail. 1987. Fluctuating sea levels since the Triassic. Science 235:1156–1167.

Heard, S. B., and D. L. Hauser. 1995. Key evolutionary innovations and their ecological mechanisms. Hist. Biol. 10:151–173.

Hubbs, C. 1959. Laboratory hybrid combinations among etheostomatine fishes. Tex. J Sci. 11:49–56.

Hubbs, C., F. B. Cross, and F. Stevens. 1988. Occurrence of natural hybrids between *Etheostoma* and *Percina* (Pisces: Percidae). Southwest. Nat. 33:97–126.

Hubbs, C., and K. Strawn. 1957. Survival of F_1 hybrids between fishes of the subfamily Etheostomatinae. J. Exp. Zool. 134: 31–60.

Huelsenbeck, J. P., F. Ronquist, R. Nielsen, and J. P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310–2314.

Jenkins, R. E., and N. M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD.

Klak, C., G. Reeves, and T. Hedderson. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. Nature 427:63–65.

Kondrashov, A. S., and M. V. Mina. 1986. Sympatric speciation: when is it possible? Biol. J. Linn. Soc. 27:201–223.

Kuehne, R. A., and R. W. Barbour. 1983. The American darters. Univ. Press of Kentucky, Lexington, KY.

Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16:750–759.

Lee, D. S., C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. J. R. Stauffer. 1980. Atlas of North American

freshwater fishes. North Carolina State Museum of Natural History, Raleigh, NC.
Losos, J. B., and R. E. Glor. 2003. Phylogenetic comparative methods and the geography of speciation. Trends Ecol. Evol. 18: 220–227.
Lydeard, C., and R. L. Mayden. 1995. A diverse and endangered aquatic ecosystem of the southeast United States. Conserv. Biol. 9:800–805.
Lynch, J. D. 1989. The gauge of speciation. Pp. 527–553 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.
Magallon, S., and M. J. Sanderson. 2001. Absolute diversification rates in angiosperm clades. Evolution 55:1762–1780.
Mayden, R. L. 1987a. Historical ecology and North American highland fishes: A research program in community ecology. Pp. 210–222 *in* W. J. Matthews and D. C. Heins, eds. Community and evolutionary ecology of North American stream fishes. Univ. of Oklahoma Press, Norman, OK.
———. 1987b. Pleistocene glaciation and historical biogeography of North American central-highland fishes. Pp. 141–152 *in* W. C. Johnson, ed. Quaternary environments of Kansas. Kansas Geological Survey, Lawrence, KS.
———. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Syst. Zool. 37:329–355.
Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.
———. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, MA.
McCune, A. R. 1997. How fast is speciation? Molecular, geological, and phylogenetic evidence from adaptive radiations of fishes. Pp. 585–610 *in* T. J. Givnish and K. J. Sytsma, eds. Molecular evolution and adaptive radiation. Cambridge Univ. Press, Cambridge, U.K.
McCune, A. R., and N. R. Lovejoy. 1998. The relative rate of sympatric and allopatric speciation in fishes. Pp. 172–185 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, New York.
Mendelson, T. C. 2003. Sexual isolation evolves faster than hybrid inviability in a diverse and sexually dimorphic genus of fish (Percidae: *Etheostoma*). Evolution 57:317–327.
Mettee, M. F., P. E. O'Neil, and J. M. Pierson. 1996. Fishes of Alabama and the Mobile Basin. Oxmoor House, Birmingham, AL.
Morris, M. A., and L. M. Page. 1981. Variation in western logperches (Pisces: Percidae) with description of a new subspecies from the Ozarks. Copeia 1981:95–108.
Near, T. J. 2002. Phylogenetic relationships of *Percina* (Percidae: Etheostomatinae). Copeia 2002:1–14.
Near, T. J., T. W. Kassler, J. B. Koppelman, C. B. Dillman, and D. P. Philipp. 2003. Speciation in North American black basses, *Micropterus* (Actinopterygii: Centrarchidae). Evolution 57: 1610–1621.
Near, T. J., J. C. Porterfield, and L. M. Page. 2000. Evolution of cytochrome *b* and the molecular systematics of *Ammocrypta*. Copeia 2000:701–711.
Nee, S. 2001. Inferring speciation rates from phylogenies. Evolution 55:661–668.
Nee, S., T. G. Barraclough, and P. H. Harvey. 1996. Temporal changes in biodiversity: detecting patterns and identifying causes. Pp. 230–252 *in* K. J. Gaston, ed. Biodiversity: a biology of numbers and difference. Blackwell Science, Oxford, U.K.
Nee, S., E. C. Holmes, R. M. May, and P. H. Harvey. 1995. Estimating extinction from molecular phylogenies. Pp. 164–182 *in* J. H. Lawton and R. M. May, eds. Extinction rates. Univ. of Oxford Press, Oxford, U.K.
Nee, S., R. M. May, and P. H. Harvey. 1994. The reconstructed evolutionary process. Philos. Trans. R. Soc. Lond. B 344: 305–311.
Nelson, J. S. 1994. Fishes of the world. Wiley, New York.
Orr, H. A., and M. Turelli. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution 55:1085–1094.
Page, L. M. 1976. Natural darter hybrids: *Etheostoma gracile* × *Percina maculata*, *Percina caprodes* × *Percina maculata*, and *Percina phoxocephala* × *Percina maculata*. Southwest. Nat. 21: 145–149.
———. 1981. The genera and subgenera of darters (Percidae, Etheostomatini). Occ. Pap. Mus. Nat. Hist. Univ. Kans. 90:1–69.
———. 1983. Handbook of darters. TFH Publications, Neptune City, NJ.
Page, L. M., M. Hardman, and T. J. Near. 2003. Phylogenetic relationships of barcheek darters (Percidae: *Etheostoma*, subgenus *catonotus*) with descriptions of two new species. Copeia 2003: 512–530.
Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
Rambaut, A., P. H. Harvey, and S. Nee. 1997. End-Epi: an application for reconstructing phylogenetic and population processes from molecular sequences. Comput. Appl. Biosci. 13:303–306.
Repenning, C. A. 1987. Biochronology of the microtine rodents of the United States. Pp. 236–268 *in* M. O. Woodburne, ed. Cenozoic mammals of North America. Univ. of California Press, Berkeley, CA.
Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution 47: 1637–1653.
Riggs, S. R. 1984. Paleoceanographic model of Neogene phosphorite deposition, U.S. Atlantic Continental Margin. Science 223: 123–131.
Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
Sanderson, M. J. 1998. Estimating rate and time in molecular phylogenies: beyond the molecular clock? Pp. 242–264 *in* D. E. Soltis, P. S. Soltis, and J. J. Doyle, eds. Molecular systematics of plants, II: DNA sequencing. Kluwer Academic Publishers, Amsterdam.
———. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.
Schluter, D. 2000. The ecology of adaptive radiation. Oxford Univ. Press, Oxford. U.K.
Schwartz, F. J. 1972. World literature to fish hybrids with an analysis by family, species, and hybrid. Publ. Gulf Coast Res. Lab. 3:1–328.
Simpson, G. G. 1953. The major features of evolution. Columbia Univ. Press, New York.
Smith, G. R. 1981. Late Cenozoic freshwater fishes of North America. Annu. Rev. Ecol. Syst. 12:163–193.
Stevenson, M. M. 1971. *Percina macrolepida* (Pisces, Percidae, Etheotomatinae), a new percid fish of the subgenus *Percina* from Texas. Southwest. Nat. 16:65–83.
Strange, R. M., and B. M. Burr. 1997. Intraspecific phylogeography of North American highland fishes: a test of the Pleistocene vicariance hypothesis. Evolution 51:885–897.
Swift, C. C., C. R. Gilbert, S. A. Bortone, G. H. Burgess, and R. W. Yerger. 1986. Zoogeography of the freshwater fishes of the southeastern United States: Savannah River to Lake Pontchartrain. Pp. 213–265 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.
Swofford, D. L. 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.
Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. Sinauer Associates, Sunderland, MA.
Thompson, B. A. 1985. *Percina jenkinsi*, a new species of logperch (Pisces, Percidae) from the Conasauga River, Tennessee and Georgia. Occ. Pap. Mus. Zool. La. State Univ. 61:1–23.
———. 1995. *Percina austroperca*: a new species of logperch (Percidae, subgenus *Percina*) from the Choctawhatchee and Escambia Rivers in Alabama and Florida. Occ. Pap. Mus. Nat. Sci. La. State Univ. 69:1–19.
———. 1997a. *Percina suttkusi*, a new species of logperch (sub-

genus *Percina*) from Louisiana, Mississippi, and Alabama (Perciformes, Percidae, Etheostomatini). Occ. Pap. Mus. Nat. Sci. La. State Univ. 72:1–27.

———. 1997b. *Percina kathae*, a new logperch endemic to the Mobile Basin in Mississippi, Alabama, Georgia, and Tennessee (Percidae, Etheostomatini). Occ. Pap. Mus. Nat. Sci. La. State Univ. 73:1–34.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 22:4673–4680.

Turelli, M., N. H. Barton, and J. A. Coyne. 2001. Theory and speciation. Trends Ecol. Evol. 16:330–343.

Wiley, E. O., and R. L. Mayden. 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Mio. Bot. Gard. 72:596–635.

Yule, G. U. 1924. A mathematical theory of evolution based on the conclusions of Dr. J. C. Willis. Philos. Trans. R. Soc. Lond. B 213:21–87.

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Appendix

Voucher specimens (if available) are deposited in the University of Tennessee Research Collection of Fishes (UT), Illinois Natural History Survey (INHS), University of Alabama Ichthyology Collection (UAIC), and North Carolina State Museum (NCSM). Collection localities, museum catalog numbers, University of Tennessee Tissue Collection (UTTC) catalog numbers, and GenBank accession numbers (cyt*b* and ND2) are as follows: **Moronidae**: *Morone chrysops*, Mississippi River, Clinton County, Iowa, INHS 40069, UTTC 381, AY770838, AY770844; **Centrarchidae**: *Ambloplites cavifrons*, Tar River, Franklin County, North Carolina, NCSM 30358, UTTC 2099, AY770839, AY517728; *Ambloplites ruprestris*, Lake Andrusia, Beltrami County, Minnesota, UT 90.3358, UTTC 284, AY225663, AY225723; *Archoplites interruptus*, Hume Lake, Fresno County, California, INHS 59069, UTTC 1077, AY225665, AY225725; *Centrarchus macropterus*, Mud Creek, Hardin County, Tennessee, INHS 38384, UTTC 384, AY225666, AY225726; *Lepomis macrochirus*, Blue River, Crawford County, Indiana, INHS 41369, UTTC 424, AY225667, AY225727; *Lepomis miniatus*, Conasauga River, Bradley County, Tennessee, UT 90.3364, UTTC 444, AY225668, AY225728; *Micropterus salmoides*, Lipsett Lake, Burnett County, Wisconsin, no voucher, UTTC MsalA, AY225675, AY225735; *Micropterus dolomieu*, Fox River, Kenosha County, Wisconsin, no voucher, UTTC MdolD, AY225687, AY225747; **Percidae**: *Perca flavescens*, Lake Andrusia, Beltrami County, Minnesota, INHS 39508, UTTC 261, AF386600, AY225721; *Percina roanoka*, Blackwater River, Franklin County, Virginia, INHS 64359, UTTC 76, AF386597, AY225722; *Percina peltata*, South Anna River, Louisa County, Virginia, UAIC 9825.11, UTTC 132, AF386595, AY770845; *Percina austroperca*, Big Escambia Creek, Escambia County, Alabama, UAIC 9993.19, UTTC 129, AF386546, AY770846; *Percina austroperca*, Escambia River, Escambia County, Florida, INHS 38433, UTTC 220, AF386547, AY770847; *Percina burtoni*, Spring Creek, Polk County, Tennessee, UAIC 9819.17, UTTC 164, AY770840, AY770848; *Percina burtoni*, Buffalo River, Wayne County, Tennessee, INHS 38531, UTTC 335, AF386554; *Percina caprodes*, Lake Wawasee, Kosciusko County, Indiana, INHS 68983, UTTC 93, AF386550, AY770849; *Percina caprodes*, Big Piney Fork, Sharp County, Arkansas, INHS 41160, UTTC 396, AY770841, AY770850; *Percina carbonaria*, Colorado River, Travis County, Texas, UAIC 11412.18, UTTC 309, AF386553, AY770851; *Percina jenkinsi*, Conasauga River, Whitfield County, Georgia, UAIC 11680.01; UTTC 160, AF386555, AY770852; *Percina kathae*, Conasauga River, Bradley County, Tennessee, INHS 41653, UTTC 439, AY770842, AY770853; *Percina kathae*, Hilabee Creek, Tallapoosa County, Alabama, INHS 38632, UTTC 166, AF386549, AY770854; *Percina macrolepida*, South Fork San Gabriel River, Williamson County, Texas, UAIC 11680.01, UTTC 158, AF386552, AY770855; *Percina nebulosa*, Conowingo Creek, Maryland, no voucher, UTTC 2311, AY770843, AY770856; *Percina rex*, Roanoke River, Roanoke County, Virginia, UAIC 7932.15, UTTC 147, AF386556, AY770857; *Percina suttkusi*, Bogue Chitto River, Washington Parrish, Louisiana, UAIC 10466.12, UTTC 159, AF386551, AY770858; *Percina maculata*, Dismal Creek, Fayette County, Illinois, no voucher, UTTC 75, AF386557, AY517725; *Percina phoxocephala*, Embarrass River, Cumberland County, Illinois, no voucher, UTTC 77, AF386563, AY770859; *Percina copelandi*, Green River, Green County, Kentucky, no voucher, UTTC 89, AF386568, AY770860; *Percina vigil*, Bayou de Chien, Hickman County, Kentucky, no voucher, UTTC 90, AF386569, AY770861; *Percina sciera*, Strong River, Simpson County, Mississippi, INHS 38604, UTTC 345, AF386573, AY770862; *Etheostoma squamiceps*, Big Creek, Hardin County, Illinois, INHS 48199, UTTC 1081, AF412523, AF412537; *Etheostoma flabellare*, Middle Fork Vermillion River, no voucher, UTTC 1438, AF412526, AF412540.