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Comparative Conservation Genetics of Two Endangered Darters, *Percina rex* and *Percina jenkinsi*

Comparative Conservation Genetics of Two Endangered Darters, *Percina rex* and *Percina jenkinsi*

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ABSTRACT

Within the logperch group, a clade of 10 darter species from North America, two species are afforded federal protection. *Percina rex* is found in four major systems of the Roanoke and Chowan river drainages, and *Percina jenkinsi* is restricted to less than 45 river kilometers of the Conasauga River. Two complete mitochondrial genes, NADH dehydrogenase subunit 2 and cytochrome *b*, were sequenced to assess genetic diversity and conservation status of these two species. Levels of haplotype diversity were higher in *P. rex* ($h = 0.919$) than *P. jenkinsi* ($h = 0.889$), but nucleotide diversity was higher in *P. jenkinsi* ($p = 0.00485$) than *P. rex* ($p = 0.00367$). Four haplogroups were recovered in *P. rex*, and two distinct clades of haplotypes were recovered from phylogenetic analysis of *P. jenkinsi*. These results are interpreted as reflecting differing causes of decline for the two logperch species: recent geographic fragmentation with subsequent bottleneck events in *P. rex* versus a historical bottleneck of the restricted *P. jenkinsi*. Within *P. rex*, a combination of natural isolation of subpopulations in different tributary systems and recent anthropomorphic fragmentation of the drainage is likely responsible for observed patterns of differentiation among the extant populations. *Percina jenkinsi* is at far greater risk of extinction, and both the species and its habitat are in need of immediate conservation actions.

INTRODUCTION

Aquatic vertebrates are imperiled at rates twice as high as terrestrial species (Richter et al., 1997). Drainages of the southeastern U.S. contain a particularly diverse and imperiled ichthyofauna (Master, 1990; Burr and Mayden, 1992; Lydeard and Mayden, 1995; Warren et al., 2000). Etnier (1997) identified the top three causes of imperilment of this fauna to be nonpoint-source pollution, alteration of water flow, and small native range. Fishes with habitat preferences of medium-sized rivers are disproportionately affected. While these habitats support only 20% of southeastern fish species, they contain 40% of the fishes considered imperiled from all habitats (Etnier, 1997). Two families of fishes exhibiting close associations with these habi-

tats, the Ictaluridae and Percidae, have disproportionately higher percentages of jeopardized species than other southeastern fish families (Etnier and Starnes, 1991; Etnier, 1997).

Within the family Percidae, darters are a clade of over 200 species of smaller-bodied fishes endemic to eastern North America (Song et al., 1998; Sloss et al., 2004). While the relationships within this group have been the subject of considerable debate (Near, 2002; Sloss et al., 2004; Lang and Mayden, 2007), one group consistently recovered is a clade of logperches of the genus *Percina*, subgenus *Percina* (Near, 2002). The 10 described species of logperch are ideal for comparative conservation genetics studies due to their rapid diversification rates (Near and Benard, 2004) and a broad disparity in rarity and abundance, from extremely common and broadly distributed taxa to critically rare and localized endemics. The clade is characterized by an elongate and conical snout, a pigmentation pattern consisting of tiger-stripe bars, and the behavioral synapomorphy of rock-flipping. Individuals use their conical snout to turn over rocks and gravel to prey upon hiding macroinvertebrates (Jenkins and Burkhead, 1994), a behavior unique among darters. Because this feeding strategy requires nonembedded gravel or cobble substrates, logperch are negatively affected by siltation (Rosenberger and Angermeier, 2003). The two basal most species in this group, *Percina rex* (Jordan and Evermann) and *Percina jenkinsi* Thompson, are the only two in the subgenus listed as Federally Endangered (Near, 2002; Near and Benard, 2004; George et al., 2006).

Percina rex, the Roanoke Logperch, was described from the upper Roanoke River in 1889 (Jordan, 1889), but not widely considered a valid species until the late 1960s (Jenkins and Burkhead, 1994). Until 2006, *P. rex* was only known from four river systems in Virginia (Fig. 1; Jenkins and Burkhead, 1994). Three of these systems, the Roanoke, Pigg, and Smith rivers (Roanoke River drainage), are separated from the fourth, Nottoway River (Chowan River drainage), by Albemarle Bay. This disjunct distribution has been invoked as evidence for the loss of many populations across the Piedmont physiographic province over the past 150 years (Jenkins and Burkhead, 1994). This hypothesis was supported through the recent

discovery of *P. rex* in several other systems of the Roanoke River drainage: Goose Creek and Big Otter River in the middle Roanoke River and Mayo River and downstream Smith River in North Carolina (Rosenberger, 2007; Roberts et al., 2009). The most recent management plan for *P. rex* recognizes six populations: upper Roanoke River, middle Roanoke River, Pigg River, Smith River upstream of Philpott Reservoir, Smith River downstream of Philpott Reservoir, and Chowan Reservoir (Rosenberger, 2007). The populations in the Mayo and Smith rivers in North Carolina had not been discovered yet (Roberts et al., 2009).

Of the four populations of *P. rex*, the one in the upper Roanoke River has consistently been considered the healthiest (Jenkins and Burkhead, 1994). Visual surveys in 2000 and 2001 found the population in the Chowan River drainage may be equally robust (Rosenberger and Angermeier, 2003; Rosenberger, 2007). Many populations are now affected by downstream impoundments, which alter riverine habitat and connectivity. Concerns about the status of *P. rex* were first vocalized in the 1970s, as chemical spills and proposed dams threatened the species, especially the population in the upper Roanoke River (Jenkins and Burkhead, 1994). Though *P. rex* was listed as Federally Endangered in 1989 (U.S. Fish and Wildlife Service, 1989), critical habitat has not been designated.

Percina jenkinsi, the Conasauga logperch, is one of the rarest fish in North America. It is known only from approximately 50 specimens taken from a 44-km reach of the Conasauga River, a tributary of the Coosa River in the Mobile Basin (Fig. 2), near the Georgia/Tennessee state line (Etnier and Starnes, 1993; Kuhajda et al., 2009). While no historical collections suggest that *P. jenkinsi* was previously more widely distributed (Thompson 1985), it is unusually restricted when compared to other Coosa River endemics. Most fishes in the Conasauga River are also shared with the adjacent Coosawattee and/or Etowah rivers (Mettee et al., 1996). The earliest survey of the upper Coosa River was in 1877, by which point agricultural development and extensive deforestation during the Civil War had dramatically altered the landscape (Jordan, 1877). *Percina jenkinsi* was not collected until the stretch of the Conasauga River it currently inhabits was first surveyed in 1969 (Thompson, 1985). It is hypothesized to be restricted by competition with a sympatric member of the subgenus, *Percina kathae* Thompson, which is widespread throughout the Mobile Basin (Thompson, 1985). Due to its extremely restricted distribution, *P. jenkinsi* was listed as Federally Endangered shortly after its description in 1985 (U.S. Fish and Wildlife Service, 1985).

The Conasauga River is known for a high number of endemic and imperiled aquatic species (Etnier and Starnes, 1993; Burkhead et al., 1997). While the Mobile Basin, and the Coosa River in particular, once supported the largest diversity of freshwater mollusks and snails in the world (Abell et al., 2000), the construction of six main stem dams, particularly Weiss and Logan Martin in the

1960s, caused one of the largest extinction events in the United States when at least 39 species of mollusks were lost (Folkerts, 1997; Neves et al., 1997). Many imperiled fishes found in the Conasauga River (*Cyprinella caerulea* (Jordan), *Noturus munitus* Suttkus and Taylor, *Etheostoma ditrema* Ramsey and Suttkus, *Etheostoma trisella* Bailey and Richards and *Percina antesella* Williams and Etnier) are now isolated from other extant populations in the Mobile Basin by impoundments, with little opportunity for dispersal. Though the Conasauga River has been designated as critical habitat for 11 federally listed species, and much of the headwaters drain National Forest, nonpoint-source pollution from agriculture and road construction continues to increase sedimentation and threaten the fauna (Burkhead et al., 1997; Parmalee and Bogan, 1998). Subsequent urban sprawl from Atlanta has further jeopardized the region; the Conasauga and Etowah rivers contain a higher proportion of imperiled aquatic species than any similarly sized system in the southeastern United States (Burkhead et al., 1997).

While there are no historical records indicating that either *P. rex* or *P. jenkinsi* ever occupied larger ranges, the occurrence of sympatric taxa with more widespread distributions suggests that their rarity may be relatively recent. If these species have only recently become rare, conservation strategies should seek to halt their recent decline by increasing available habitats to augment population size and prevent extinction (Hanski, 1998). However, if these species have always been spatially rare or geographically fragmented, conservation activities must be more carefully undertaken to preserve natural population size, structure, and dynamics. The objective of this study is to use genetic data to infer historical distributions, movement and population sizes, as well as to assess the relative conservation status of *P. rex* and *P. jenkinsi*.

METHODS

Logperch were collected by seining and/or electrofishing. Sampling localities and numbers collected are shown in Table 1. Five of the six management populations for *P. rex* from all four major systems were sampled (Rosenberger, 2007). Because the sample sizes for the management populations in the Smith River were small and are only separated by Philpott Reservoir (constructed in 1953), they were combined for these analyses. Each major system (Roanoke, Pigg, Smith, and Chowan) was therefore treated as a population.

Fish were anesthetized with MS-222, and approximately 1 cm² of the distal portion of the second dorsal fin was removed prior to release of the fish. Photo vouchers are available from the lead author. DNA was extracted, amplified, and sequenced using methods described in George et al. (2006). Sequences were verified by consensus between the two strands, edited and aligned by eye using BioEdit v5.0.9 (Hall, 1999). Veracity of all mutations was assessed

via examination of the electropherograms. For *P. rex*, haplotype H is accessioned as GenBank AF386556 and AY770857, haplotype A is accessioned as GenBank DQ493478 and DQ493523, and haplotype N is accessioned as GenBank DQ493479 and DQ493524. For *P. jenkinsi*, haplotype F is accessioned as GenBank AF386555 and AY770852, haplotype A is accessioned as GenBank DQ493480 and DQ493525 and haplotype B is accessioned as GenBank DQ493481 and DQ493526. All other haplotypes are available on GenBank as numbers EU293554–EU293585.

Genetic diversity and population structure—Haplotype diversity (Nei and Tajima, 1981) and nucleotide diversity (Nei, 1987) were calculated using DNAsp (Rozas et al., 2003). DNAsp was also used to test for historical population changes and neutral mutation using Tajima's (Tajima, 1989) and Fu and Li's tests (Fu and Li, 1993). Haplotype networks were constructed using TCS 1.13, using combined ND2 and cyt *b* sequences with no ambiguous positions and a confidence limit of 95% (Clement et al., 2000). Networks were constructed separately for each species in order to represent multifurcating relationships between haplotypes.

Differentiation between populations of *P. rex* was examined in Arlequin by calculating pairwise f_{st} values and an AMOVA under a distance model of sequence evolution (Excoffier and Lischer, 2010). The correlation between pairwise f_{st} values and geographic distance was calculated using a Mantel test in Arlequin with 1000 permutations of the pairwise distance matrix to test for significance. River distances (km) for the Mantel test were measured from 1:100,000 scale basemaps (U.S. Geological Survey) of the drainage and follow the old river channel in impounded reaches. For river systems with multiple sampling sites, the most downstream locality was used to calculate distance.

Phylogenetic analysis—Incongruence-length difference analyses (Farris et al., 1994), as implemented in PAUP* (Swofford, 2002) with 100 replicates and uninformative characters removed (Lee, 2001), were used to test for homogeneity among cyt *b* and ND2 partitions. Relationships between 10 species in the subgenus *Percina* were inferred under parsimony analysis (MP), utilizing the heuristic search option in PAUP* with ACCTRAN and tree-bisection-reconnection during 100 replicates of random sequence addition. MP analyses were conducted with molecular characters unweighted and unordered. All minimal-length trees were kept, and zero-length branches collapsed. Support for individual nodes was assessed by performing 1000 jackknife replicates with 37% data deletion in each replicate and JAC emulation selected and calculating decay indices (Bremer, 1994) using TreeRot (v2, M. D. Sorenson, Boston University, Boston, MA, 1999, unpubl.). Additional members of the subgenus *Percina* were included in phylogenetic analyses (George et al., 2006), and outgroup taxa included *Percina macrocephala* (Cope), *Percina aurantiaca* (Cope), *Percina evides* (Jordan and

Copeland) and *Percina roanoka* (Jordan and Jenkins), as suggested by Near (2002) and Sloss et al. (2004).

RESULTS

Genetic diversity and population structure—Genetic variation within *P. rex* was high, with an overall haplotypic diversity (h) of 0.919 and nucleotide diversity (p) of 0.00367. Individual populations ranged from $h = 0$ –0.978, $p = 0$ –0.00314 (Table 2). Fourteen different haplotypes were recovered among the 29 *P. rex* analyzed. The population in the Pigg River was monomorphic while the other three populations contained at least one haplotype (Table 1). Pairwise distance values between the haplotypes ranged from 0.046% to 0.6%. The populations in the Roanoke and Smith rivers shared two haplotypes (F and I). No other haplotypes were shared among populations. Neither Tajima's D (0.063), nor Fu and Li's D^* (–0.037) or F^* (0.053) values were significant, indicative of demographically stable populations. An AMOVA using a distance model of evolution was conducted using the Roanoke and Chowan drainages as groups based upon their separation by Albemarle Bay. Significant genetic variation was recovered between and within populations, but not between groups ($f_{sc} = 0.498$, $f_{st} = 0.425$, $f_{ct} = 0.0767$, $p < 0.01$ for all but f_{ct}). Pairwise f_{st} values range from 0.31 (Pigg and Smith rivers) to 0.76 (Roanoke and Pigg rivers). The Mantel test did not reveal a significant correlation between geographic distance and pairwise f_{st} values for the populations ($Z = 1991.5$, $r = 0.143$, $p = 0.65$).

Six different haplotypes were recovered among the nine *P. jenkinsi* sequenced (Table 1), with pairwise distance values from 0.046% to 0.9%. While haplotype diversity was lower than that observed in *P. rex* ($h = 0.889$), nucleotide diversity was higher ($p = 0.00485$). In *P. jenkinsi*, significantly positive values of Tajima's D (2.17) and Fu and Li's F^* (1.76) indicate an excess of intermediate frequency haplotypes. The Fu and Li's D^* value of 1.38, while positive, was not significant.

Haplotype networks recovered four groups of haplotypes within *P. rex* (Fig. 3). The most diverse cluster contained nine haplotypes from the Roanoke and Smith rivers. Another was composed of a single haplotype found in individuals from both the Roanoke and Smith Rivers. The third contained two haplotypes found in individuals from the Pigg and Smith rivers, and the final contained two haplotypes from the population in the Chowan River. Analysis of *P. jenkinsi* haplotypes revealed two distinct clusters of three haplotypes each (Fig. 4), with up to 0.9% pairwise distance between the most divergent haplotypes, A and F. There was no geographic concordance with the distribution of these clades within their occupied range in the Conasauga River.

Phylogenetic analysis—The incongruence-length difference test revealed no significant heterogeneity between the individual genes ($p = 0.66$), thus, cyt *b* and

ND2 alignments were concatenated into a single data set totaling 2187 bp. Of these, 575 were variable and 397 were parsimony informative. Maximum parsimony analysis recovered nine trees (Fig. 5; length = 1011, CI = 0.694, RCI = 0.654). Consistent with previous analyses (Near, 2002; Near and Bernard, 2004; George et al., 2006), *P. rex* was recovered as the most basal logperch. All *P. rex* haplotypes formed a monophyletic group with 100% jackknife support. Support for groups within the tree was weak, except for strong support of a clade consisting of a monomorphic population in the Pigg River sister to a haplotype present in the population in the Smith River. A strong relationship between tree topology and geography was not recovered. While the populations from the Pigg River and the Chowan River formed monophyletic groups, haplotypes from the Roanoke River and Smith River were not resolved in a single clade. *Percina jenkinsi* was recovered as the second most basal species, sister to a clade containing the remaining members of the subgenus *Percina*. All six *P. jenkinsi* haplotypes formed a monophyletic group with 100% jackknife support. Within the species, two monophyletic clades were recovered, with 99% and 100% jackknife support and decay values of 4 and 10, respectively. Pairwise distance values between derived members of the subgenus (excluding *P. rex*, *P. jenkinsi*, and *Percina burtoni* Fowler) ranged from 0.78% (*Percina austroperca* Thompson and *P. kathae*) to 2.37% (*Percina carbonaria* (Baird and Girard) and *P. austroperca*).

DISCUSSION

The genetic signatures observed in *P. rex* and *P. jenkinsi* are quite different, and likely reflect the differing factors impacting genetic diversity in imperiled taxa. Genetic diversity in *P. rex* is high, particularly within the populations in the upper Roanoke and Smith rivers. These populations contain 11 of the 14 recovered haplotypes, which are distributed in three of the four clusters of the haplotype network (Fig. 3). Correspondingly, they also contain the highest nucleotide diversity within *P. rex* (Table 2). The upper Roanoke and Smith rivers are also the only populations with shared haplotypes (Table 1; Fig. 3). These numbers likely reflect the historical abundance and distribution of *P. rex* in the Roanoke River drainage. The recent discovery of *P. rex* in upstream intervening reaches of the Roanoke River drainage (Fig. 1), also suggests that dispersal would be facilitated between these populations.

The population in the Pigg River is fixed for haplotype J which is most similar to haplotype K found in the population in the Smith River. Given the geographic proximity of the Pigg River to the Roanoke and Smith rivers, the recent discovery of populations in intervening reaches, and the shared fauna with the upper Roanoke River (Jenkins and Burkhead, 1994), it is likely that the low genetic variation in the population in the Pigg River reflects either small sample size or a recent bottleneck. Only 15 individuals were captured during extensive surveys from this system from the 1960s through the 1980s

(Jenkins and Burkhead, 1994). The construction of Smith Mountain Reservoir (1963) and Leesville Reservoir (1963) likely has prevented immigration from supplementing this population and maintaining higher levels of diversity during bottleneck events (Fig. 1).

The population in the Chowan River also contained less genetic diversity. Only two haplotypes were recovered from the four individuals sampled. Recent surveys of this drainage indicate a large population of sub-adults (Rosenberger and Angermeier, 2003). Small sample size for genetic analysis is likely responsible for the lower recovered genetic diversity. The differentiation between populations in the Roanoke and Chowan river drainages suggests moderately long isolation, as supported by the presence of Albemarle Bay. Maintaining the population in the Chowan River drainage is extremely important due to the unique geographic position and divergent haplotypes.

One of the persistent questions about *P. rex* has been whether its disjunct distribution in the Roanoke and Chowan river drainages is due to recent extirpation or historical range restriction. Other sympatric and endemic taxa in the Roanoke and Chowan river drainages, such as *Moxostoma ariommum* Robins and Raney, *Noturus gilberti* Jordan and Evermann and *Ambloplites cavifrons* Cope, also exhibit a similar, though slightly expanded, distribution in the Piedmont. Jenkins and Burkhead (1994) hypothesized that the current distribution of *P. rex* is due to extirpation of many Piedmont populations in the past 150 years. If so, the absence of historical records from elsewhere in the middle Roanoke or Dan rivers may be tied to a paucity of collections from these areas prior to the 1960s. Since then, main stem habitats in much of this area have been impacted by impoundment of Smith Mountain Reservoir (1963), Leesville Reservoir (1963) and John H. Kerr Reservoir (1953) and subjected to considerable fluctuations in flow, thermal, and turbidity regimes. Though these results are inconclusive on the question of recent range contraction in *P. rex*, the shallow branches in the phylogram, non-significant Mantel test, and shared haplotypes between the Smith River and Roanoke River are indicative of recent gene flow. As suggested by these genetic data and new records for *P. rex* throughout the Roanoke River drainage, the species could have occupied a much wider distribution throughout the Piedmont region (Burkhead, 1983; Rosenberger, 2007; Roberts et al., 2009).

Despite this increase in known range size for *P. rex*, it is clear that connectivity is now a major conservation concern. There is currently no chance for dispersal between and even within most river systems due to impoundments in intervening reaches. Currently, the species is vulnerable, yet stable. Efforts at conservation should be targeted at increasing available habitat for all populations. The Roanoke River population appears to be currently stable and genetically healthy, but is of high importance due to its high genetic diversity. Protection of this reach also ensures the survival of a broad spectrum of other imperiled aquatic taxa with restricted distributions, including *N. gilberti* and *M. ariommum*.

In contrast to *P. rex*, the patterns of variation found within *P. jenkinsi* are much more unusual. The haplotype diversity within *P. jenkinsi* is lower than *P. rex*, which is unsurprising given its smaller range and population size. However, the nucleotide diversity of *P. jenkinsi* is higher than that seen in *P. rex*. Two distinct clades of haplotypes were recovered within *P. jenkinsi* (Fig. 5), and pairwise distance values for the most divergent haplotypes in these clades are 0.9%, compared to only 0.6% within *P. rex* and 0.78% between two described members of the subgenus (*P. kathae* and *P. austroperca*). There is no geographic or temporal congruence with the distribution of haplotypes in the Conasauga River. Divergent haplotypes were even recovered from the same collection (Table 1).

Because the range size and population size of *P. jenkinsi* is much smaller than that of *P. rex*, we expected that all measures of genetic diversity would be much lower given the impacts of rarity on genetic drift and fixation (Franklin, 1980). However, the high nucleotide diversity may still be indicative of a species at greater risk of decline than its congener. Significantly positive values from both Tajima's D and Fu and Li's F* tests indicate an excess of intermediate frequency haplotypes, suggesting either recent population reduction, collapse of two divergent populations into one, or balancing selection (Tajima, 1989). Of these three scenarios, the most likely is a recent population bottleneck, induced by human impacts on the Conasauga River and the rest of the upper Coosa River system within the past 400 years of European settlement.

Even within the past 50 years, local abundance of *P. jenkinsi* has been reduced. In 1969, the first year *P. jenkinsi* was collected, 20 specimens were vouchered in southeastern museums, including eight in a single collecting effort (Thompson, 1985). Over the past 20 years, numbers of *P. jenkinsi* observed during surveys of the Conasauga River has declined from 1–6 individuals per riffle to 1–3, and many riffles no longer support any *P. jenkinsi* (B.J. Freeman and M.C. Freeman, pers. comm.). Increased erosion and associated sedimentation, agricultural run-off, and excessive drought all threaten the system (Freeman et al., 1996). Human activities within the Conasauga River system could have resulted in a recent population bottleneck, resulting in two divergent clades of haplotypes based on random loss and drift. Similar to a recent bottleneck, these two clades could also be the result of a collapse of two populations into one. However, this seems less likely because haplotypes from both clades are distributed throughout the river, there is no data to support a more widespread distribution for *P. jenkinsi*, and there is no geological evidence for a barrier to gene flow within the Conasauga River.

Balancing selection can also result in a significantly positive Tajima's D value. If *P. jenkinsi* has always been restricted in its current distribution in the Conasauga River, assortative mating would have the benefit of minimizing inbreeding and maintaining diversity in a small population (Edwards and Hedrick, 1998). However, without evidence of balancing selection on the diploid genome, the

high nucleotide diversity and positive Tajima values recovered in *P. jenkinsi* are most likely due to a recent population bottleneck. Regardless of the cause of the high genetic diversity within *P. jenkinsi*, it is clear that recovery depends solely on reversing the recent declines of the species in the Conasauga River in order to preserve the current genetic diversity levels.

Comparative phylogenetic examination of the genetic diversity within these closely related logperch allows a better understanding of the conservation problems these species are facing. Within *P. rex*, recent habitat alteration has resulted in the reduction of gene flow between all extant populations. The recent discoveries of populations of *P. rex* in some intervening reaches help explain shared haplotypes and presumably recent gene flow between the populations in the Roanoke and Smith rivers. Conservation activities must target expanding the range of *P. rex* throughout the Roanoke River drainage in order to decrease fragmentation, augment declining populations, and restore patterns of movement. Limited translocation between populations in the Roanoke River may restore natural levels of gene flow and higher diversity values within populations. This species is more stable than *P. jenkinsi* as supported through data from range sizes, abundances in previous surveys, and this genetic data (Rosenberger and Angermeier, 2003; B.J. Freeman, unpubl. data).

In contrast, *P. jenkinsi* faces greater threats from its small population size, which is highly susceptible to extinction from stochastic events. Of the two species, it is in far greater need of conservation actions. While the headwaters of the Conasauga River are afforded protection as National Forest, the reach occupied by *P. jenkinsi* is entirely on private property. Poor sediment management practices in this reach may negate any benefits of upstream protection measures. Conservation efforts should focus on cooperative efforts to protect riparian buffers, keep livestock out of stream channels, and eliminate erosional "hot spots" both in the Conasauga River and its tributaries. These proposals are similar to those instituted for other imperiled species (e.g. Kuhajda et al., 2009). Any captive propagation efforts for *P. jenkinsi* must be conducted carefully to preserve its unique genetic structure.

The use of a phylogenetic framework in evaluating conservation status also helps illuminate another biological attribute of "extinction-prone" species (Terborgh, 1974). While ecological traits that predispose species to imperilment have been well-studied (Angermeier, 1995; Griffis and Jaeger, 1998; Pyron, 1999), there has been less examination of evolutionary factors that may contribute to the health of species. Evolutionary factors that affect species may include basic attributes such as the level of genetic diversity and degree of differentiation among populations (George et al., 2006), rates of differentiation within the group, or the age of the taxon. Purvis et al. (2000) noted a strong effect of phylogenetic diversity and clade age on risk of extinction. Older and depauperate clades

were more likely to go extinct than more diverse clades or than expected by random chance. *Percina rex* and *P. jenkinsi* were recovered as the oldest species within the subgenus, and the next most basal member, *Percina burtoni*, is also considered vulnerable (Warren et al., 2000). Within the genus, *Percina macrocephala*, *Percina lenticula* Richards and Knapp, *Percina antesella*, and *Percina cymatotaenia* (Gilbert and Meek) are all considered threatened or endangered by Warren et al. (2000) and are also recovered as more basal species in their respective clades (Near, 2002). Knowledge of the evolutionary history of a taxon will not only aid in current management, but may also aid in identification of extinction-prone species.

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Table 1. Collection data for specimens of *Percina rex* and *Percina jenkinsi* used in genetic analyses. Tissues are accessioned at the University of Alabama Ichthyological Collection (UAIC), or at the Saint Louis University Museum (STL). The haplotype column corresponds to Figures 3 and 4, with the number of individuals with that haplotype in parentheses.

Site Number	Locality	Population	Accession No.	Number	Haplotypes
<i>Percina rex</i>					
1	N. Fk. Roanoke River, Montgomery Co., VA	Roanoke	UAIC 13539.01	2	B (1) C (1)
2	S. Fk. Roanoke River, Montgomery Co., VA	Roanoke	UAIC 13538.01	4	A (1) B (1) D (1) I (1)
3	Roanoke River, Montgomery Co., VA	Roanoke	UAIC 7932.15	1	H (1)
3	Roanoke River, Montgomery Co., VA	Roanoke	UAIC 13465.01	3	E (1) F (1) G (1)
4	Big Chestnut Creek, Franklin Co., VA	Pigg	UAIC 13540.01	5	J (5)
5	Smith River, Patrick Co., VA	Smith	UAIC 13466.01	3	F (1) I (1) L (1)
6	Town Creek, Henry Co., VA	Smith	UAIC 13467.01	7	F (1) K (6)
7	Stony Creek, Dinwiddie Co., VA	Chowan	UAIC 13468.01	1	M (1)
8	Stony Creek, Dinwiddie Co., VA	Chowan	UAIC 13541.01	3	M (2) N (1)
<i>Percina jenkinsi</i>					
1	Conasauga River, Polk Co., TN	Conasauga	UAIC 13464.09	1	C (1)
2	Conasauga River, Polk Co., TN	Conasauga	STL 1008.01	1	B (1)
3	Conasauga River, Polk Co., TN	Conasauga	UAIC 13489.06	2	A (1) D (1)
3	Conasauga River, Polk Co., TN	Conasauga	STL 1009.01	1	E (1)
4	Conasauga River, Murray Co., GA	Conasauga	STL 1010.01	1	A (1)
5	Conasauga River, Murray Co., GA	Conasauga	STL 1011.01	1	A (1)
6	Conasauga River, Murray Co., GA	Conasauga	STL 1012.01	1	C (1)
6	Conasauga River, Murray Co., GA	Conasauga	UAIC 11680.01	1	F (1)

Table 2. Diversity indexes for *Percina rex* and *P. jenkinsi* cytochrome *b* and ND2 sequences used in this study. Number of individuals (N), number of haplotypes (Hn), haplotype diversity (Hd), and nucleotide diversity (π).

Species/Population	N	Hn	Hd	π
<i>Percina rex</i>				
Roanoke River	10	9	0.978	0.0020
Pigg River	5	1	0.000	0.0000
Smith River	10	4	0.644	0.0031
Chowan River	4	2	0.500	0.0005
Total	29	15	0.919	0.00367
<i>Percina jenkinsi</i>				
Conasauga River	9	6	0.889	0.00485

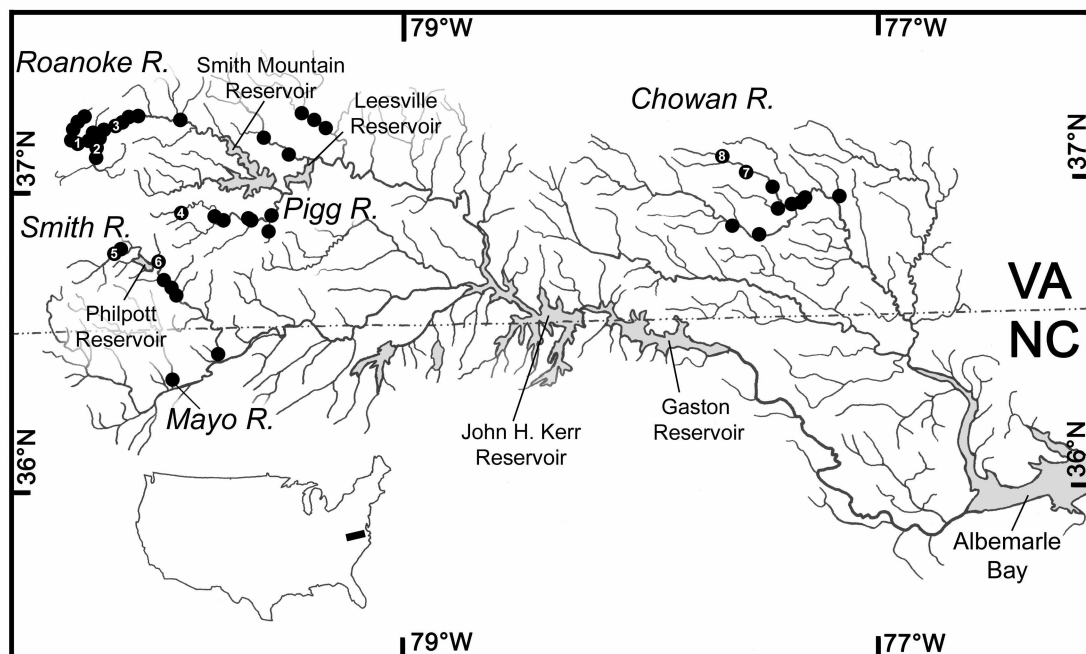


FIGURE 1. Distribution of *Percina rex* in the Roanoke and Chowan river drainages. Black dots with numbers correspond to those listed in Table 1. Solid dots represent additional extant localities.

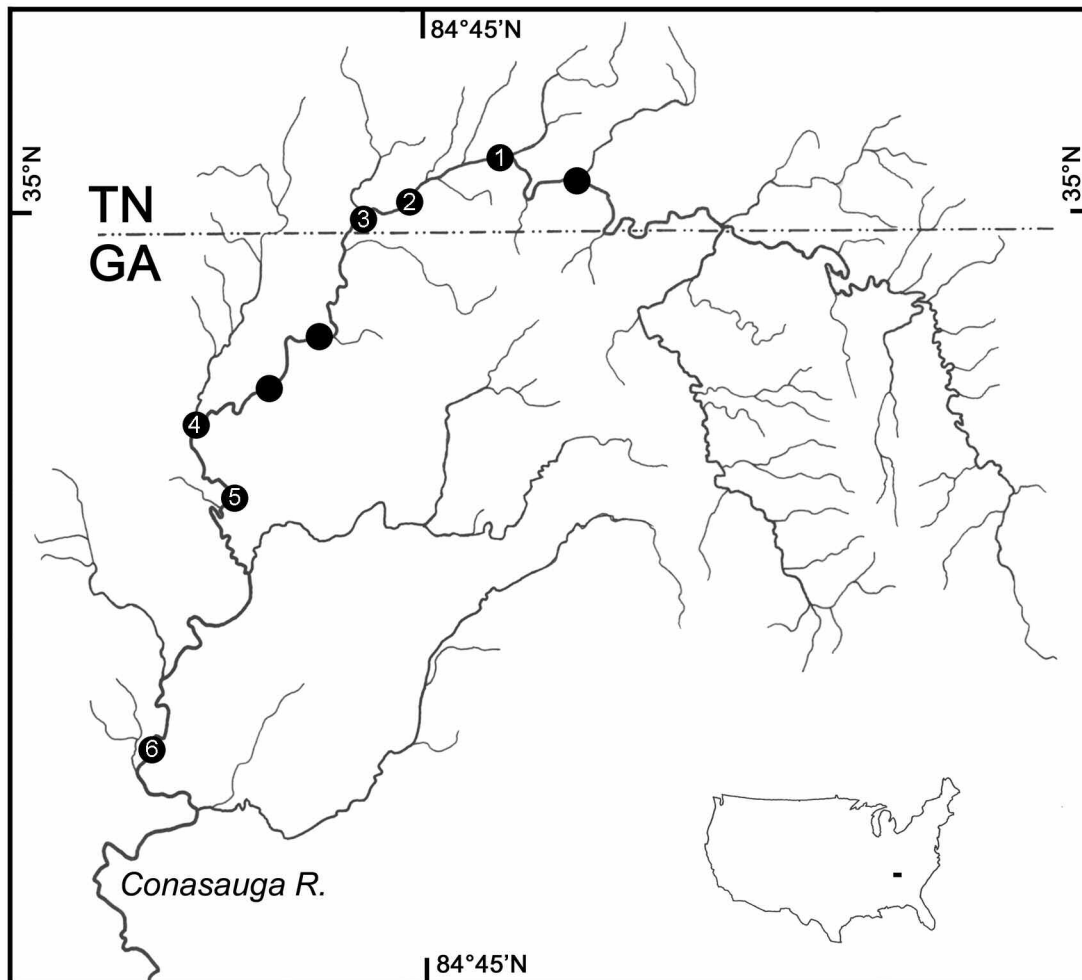


FIGURE 2. Distribution of *Percina jenkinsi* in the Conasauga River system. Black dots with numbers correspond to those listed in Table 1. Solid dots represent additional extant localities.

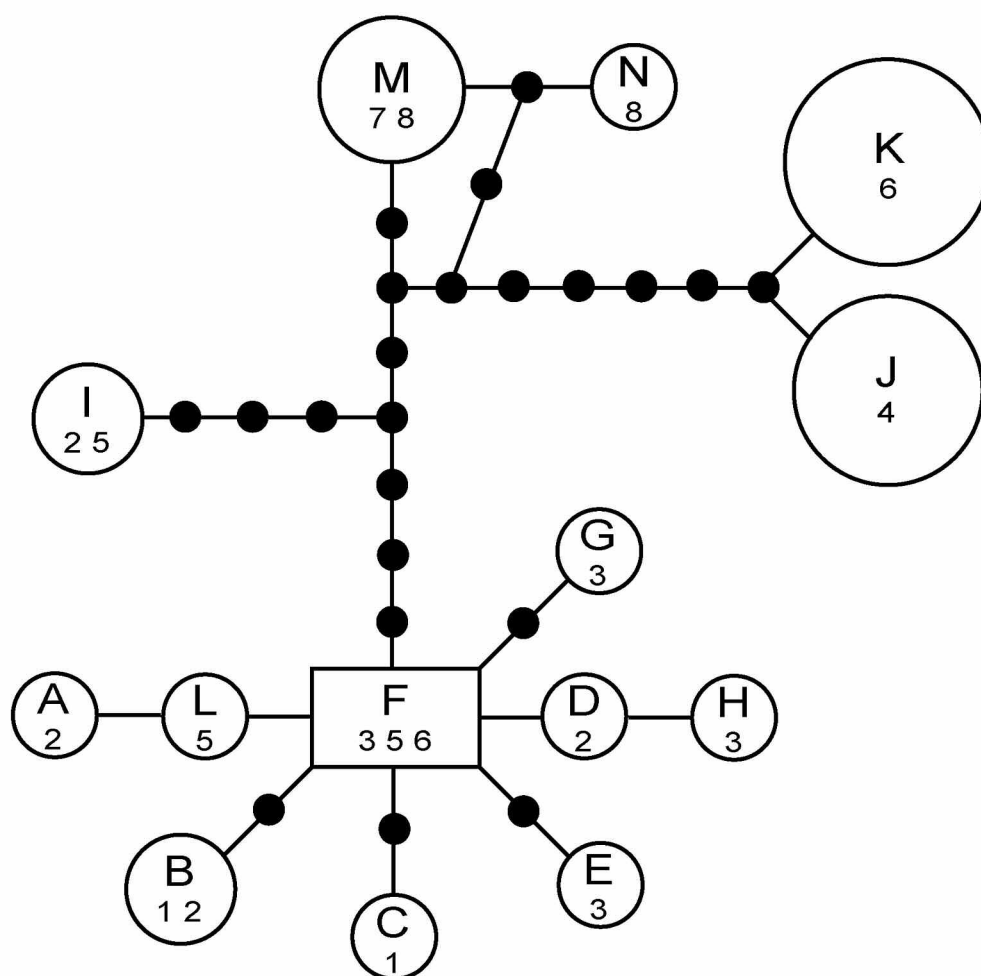


FIGURE 3. Haplotype network for *Percina rex* representing 14 haplotypes from 29 individuals within the Roanoke and Chowan river drainages. Circle size reflects the frequency of haplotypes, solid lines represent one mutational event and small black circles represent theoretical haplotypes. Haplotype letters correspond with those listed in Table 1. Numbers below the haplotypes represent site numbers as listed in Table 1. The ancestral haplotype, F, is denoted by a square.

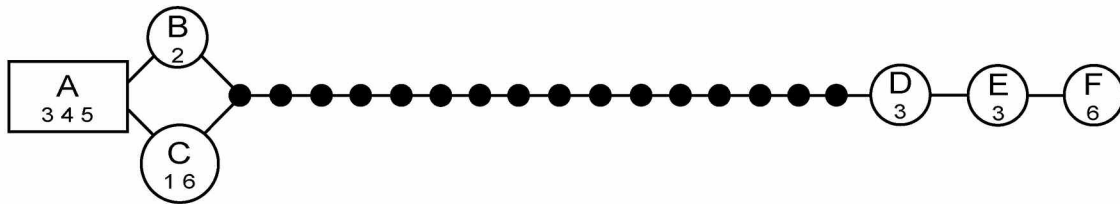


FIGURE 4. Haplotype network for *Percina jenkinsi* representing 6 haplotypes from 9 individuals within the Conasauga River. Circle size reflects the frequency of haplotypes, solid lines represent one mutational event and small black circles represent theoretical haplotypes. Haplotype letters correspond with those listed in Table 1. Numbers below the haplotypes represent site numbers as listed in Table 1. The ancestral haplotype, A, is denoted by a square.

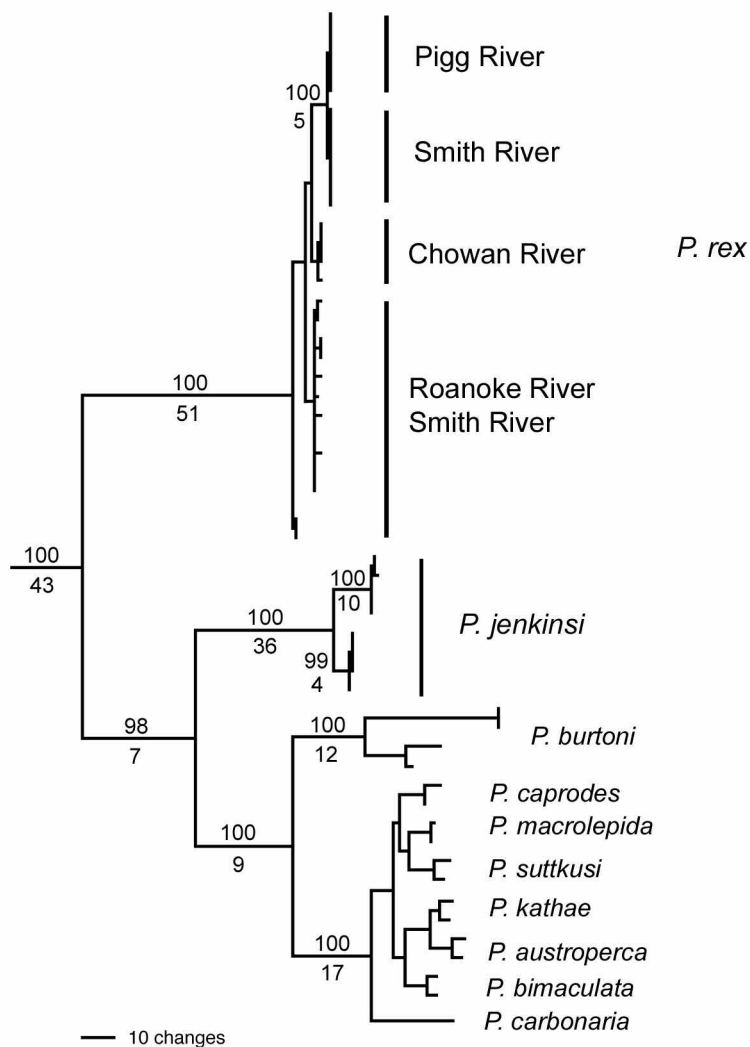


FIGURE 5. Phylogram representing one of nine topologies recovered by parsimony analysis of combined *cyt b* and ND2 datasets for the subgenus *Percina*. Jackknife support values of greater than 80% are shown above branches, and Bremer decay values greater than two are shown below branches for the basal nodes and within *P. rex* and *P. jenkinsi*.