

Neglected Taxonomy of Rare Desert Fishes: Congruent Evidence for Two Species of Leatherside Chub

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Abstract.—Conservation biologists rely heavily on taxonomy to set the scope for biological monitoring and recovery planning of rare or threatened species. Yet, taxonomic boundaries are seldom evaluated as falsifiable hypotheses that can be statistically tested. Here, we examine species boundaries in leatherside chub (Teleostei, Cyprinidae), an imperiled desert fish native to the Bonneville Basin and upper Snake River drainages of western North America. Recent molecular data hint that this fish could be composed of two distinct taxa that are geographically separated into northern and southern species. To formally test this hypothesis, we evaluated leatherside chub using several different categories of species concepts, including criteria dependent on phylogenetic, morphological, and ecological data. We found that leatherside chub is composed of two reciprocally monophyletic clades (candidate species) characterized by numerous fixed genetic differences for both mitochondrial and nuclear DNA markers; mtDNA sequence divergence between the two clades approached 8%. The candidate species also showed significant differences in cranial shape, revealed by morphometric analysis. Finally, controlled growth and foraging experiments using representative populations from each clade show that candidate species appear to be locally adapted to the thermal environments where they now occur. Combined, these three lines of evidence support the hypothesis that leatherside chub is composed of two species. Moreover, all lines of evidence place these two species within the genus *Lepidomeda*, a group consisting of three additional species of endangered spinedace fishes, and one extinct species, all native to the Colorado River system. Hence, we elevate the two clades of leatherside chub to distinct species status (*Lepidomeda copei* in the north and *L. aliciae* in the south), and argue that each warrants independent conservation and recovery action. [Conservation units; cryptic species; hypothesis testing; morphometrics; species concepts.]

Identifying species remains a controversial endeavor. Species debates persist on many fronts in evolutionary biology, ranging from philosophical exchanges about the biological “reality” of species (Avise and Walker, 2000; Hendry et al., 2000) to fundamental disagreement about which operational concepts most closely reflect the processes by which new species arise (Howard and Berlocher, 1998). As these debates continue, conservation biologists and wildlife managers face a difficult and pressing challenge—they must decide what constitutes a “good” species for conservation purposes, and then apply these criteria to establish species boundaries in rare or threatened taxa (Rojas, 1992).

Accurately defining species is critical to protecting biodiversity. Species continue to be the fundamental biological units that warrant legal protection under both national and international laws (e.g., US Endangered Species Act; Convention on International Trade in Endangered Species [CITES]). When such laws are invoked, species boundaries determine the biological scope of all subsequent monitoring and recovery efforts. Hence, neglecting taxonomy can unwittingly lead to population declines and in some cases, complete species extinctions (Daugherty et al., 1990). Species boundaries can also serve to protect the larger ecosystems that endangered species occupy. Moreover, species are the common currency used to determine centers of endemism and biodiversity hotspots, geographic areas typically viewed as having the highest priority for protection (Myers et al., 2000; Roberts et al., 2002). Hence, even conservation efforts focused on protecting ecosystems at regional or global scales rely heavily on how species are defined locally (Peterson and Navarro-Sigüenza, 1999).

In recent reviews, Sites and Crandall (1997) and Sites and Marshall (2003) argued that species propositions should be treated as biological hypotheses that can be explicitly tested. Yet practitioners attempting to evaluate species boundaries this way must contend with well over 25 unique species concepts, each emphasizing different biological criteria that in some way characterize the overall process of evolutionary divergence (Mayden, 1997). To make the task more manageable, we suggest that species definitions can be grouped into four general categories: (1) phylogenetic species concepts emphasizing shared evolutionary histories among populations; (2) similarity species concepts defined by common phenotypic features of the organisms, especially shared morphological traits; (3) ecological species concepts marked by adaptations to local environmental conditions; and (4) biological species concepts based on the ability of organisms to mate and produce viable offspring. Ideally, descriptions of species should address each of these four classes of concepts, but in practice this is seldom accomplished.

The desert fish, leatherside chub, provides a model system to examine species concepts as testable hypotheses in a conservation context. Leatherside chub has been widely viewed as a single contiguous species (commonly referred to as *Gila copei* or *Snyderichthys copei*), but one with a complex taxonomic history of being assigned and then removed from at least seven different genera (Miller, 1945). This rare minnow (family Cyprinidae) is native to small streams in the Bonneville Basin and upper Snake River drainages of western North America. Populations have declined dramatically over the past five decades, and the fish appears to be extinct now in three

(of eight known) drainage systems. Despite uncertainty regarding the relationship of leatherside chub to other fishes, management actions have assumed that just a single species exists. However, molecular genetic data have recently challenged the single species hypothesis, suggesting instead that leatherside chub is composed of two species, geographically isolated into northern and southern clades (Johnson and Jordan, 2000; Dowling et al., 2002). Given that taxonomy will frame subsequent conservation activities, we undertook a survey of genetic, morphological, and ecological data to test species boundaries in leatherside chub.

The purpose of this study is to examine leatherside chub using criteria from three different categories of species concepts: phylogenetic, similarity, and ecological. We first examined mitochondrial and nuclear DNA sequence data to reconstruct evolutionary relationships among leatherside chub populations relative to closely related spinedace fishes. Second, we compared cranial shape among the spinedace fishes and several populations of the two candidate species of leatherside chub. We found that leatherside chub clades had distinct morphotypes and that the degree of morphological divergence equaled or exceeded morphological variation found among several other spinedace species. Finally, we experimentally showed that representative populations from the two putative species have evolved differences in growth and feeding rates in response to the thermal environments where they live. Because the candidate species are reproductively isolated by allopatry, we did not explicitly test the biological species concept, although we do consider it in the discussion. Using criteria from the three applicable species concepts, we find that leatherside chub is composed of two unique species, each of which warrants independent conservation action.

METHODS

Geographic Distribution and Sampling

Historically, leatherside chub occurred in at least eight major drainage systems of the eastern Great Basin and upper Snake River (Fig. 1). Extant populations currently occupy five of these drainages: (1) Sevier River and its tributaries in southern Utah; (2) Utah Lake drainage system in central Utah; (3) Bear River drainage of southwestern Wyoming, southeastern Idaho, and northern Utah; (4) Goose Creek drainage of southern Idaho, a small tributary to the Snake River; and (5) the upper Snake River of northwestern Wyoming. Museum records show that leatherside chub also historically occupied (6) Beaver River of southern Utah; (7) Wood River system of southern Idaho; and (8) Ross Fork Creek of southeastern Idaho. Unfortunately, surveys conducted over the past decade have repeatedly failed to find leatherside chub in any of the latter three drainages, suggesting that these populations may now be extinct (Wilson and Belk, 2001; G. Smith, personal communication).

For the current study, we examined fish from seven of the eight historical drainages, relying on preserved museum samples for extirpated populations. Museum sam-

ples from the Beaver River were degraded and could not be used. Hence of the 17 areas where leatherside chub have been reported (Fig. 1), we collected and evaluated data from 15 populations. As outlined above, our study was composed of three parts, corresponding to three categories of species concepts being tested. However, data availability from the 15 populations examined varied for each part of the study—for example, fresh tissue samples for the DNA analysis could be obtained only from extant populations, whereas morphological data were most easily obtained from preserved museum collections. Consequently, statistical tests of species boundaries for each component of the overall study are based on subsets of the total 15 populations. Specific samples used in each part of the study are detailed below.

Data Analyses

Phylogenetic species concept.—We used mitochondrial and nuclear DNA sequences to test leatherside chub species boundaries under the phylogenetic species concept (PSC) category. Our testable criteria under this concept were reciprocal monophyly (Moritz, 1994) and fixed diagnostic character differences (Davis and Nixon, 1992) between the two candidate species. Two previous studies provide mtDNA sequences for this test. Johnson and Jordan (2000) sequenced the complete cytochrome *b* gene (1140 bp) for 30 individual leatherside chub—3 fish from each of 10 populations (Fig. 1: populations 2, 5, 6, 9 to 11, 13 to 16). Dowling et al. (2002) sequenced the same gene in an additional four fish from four populations (Fig. 1: populations 2, 6, 10, 16). In the present study, we combined these two data sets (GenBank AF270885 to AF270914 and AF452084 to AF452087) to examine phylogenetic relationships among leatherside chub haplotypes relative to the closely related plagiogaster fishes (*Lepidomeda vittata*, *L. albivallis*, *L. mollispinis*, and *Meda fulgida*; AF452088 to AF452093). This approach combines the strengths of a detailed intraspecific phylogenetic analysis (Johnson and Jordan, 2000) with a broad interspecific analysis (Dowling et al., 2002). Both of these original studies suggested that leatherside chub was composed of two taxa: a northern clade comprising populations 2, 5, and 6; and a southern clade comprising populations 9 to 11 and 13 to 16.

We also generated nuclear DNA sequences to test species boundaries between the two candidate species of leatherside chub. The same populations sampled for the mtDNA work were included in the nuclear study. We examined two nuclear gene regions in 31 individual leatherside chub. We first sequenced a portion of the S7 ribosomal protein gene (861 bp of the first intron). This gene region was amplified by polymerase chain reaction (PCR) using primers S7RPEX1F and S7RPEX3R (Chow and Hazama, 1998). The thermal profile (1 min at 94°C; 1 min at 58°C; 1 min at 72°C) was repeated 39 times, followed by a 2-min extension at 72°C. We also sequenced a portion of the triosephosphate isomerase (TPI) gene (743 bp total from the fourth and fifth introns). The first intron was amplified by nested PCR.

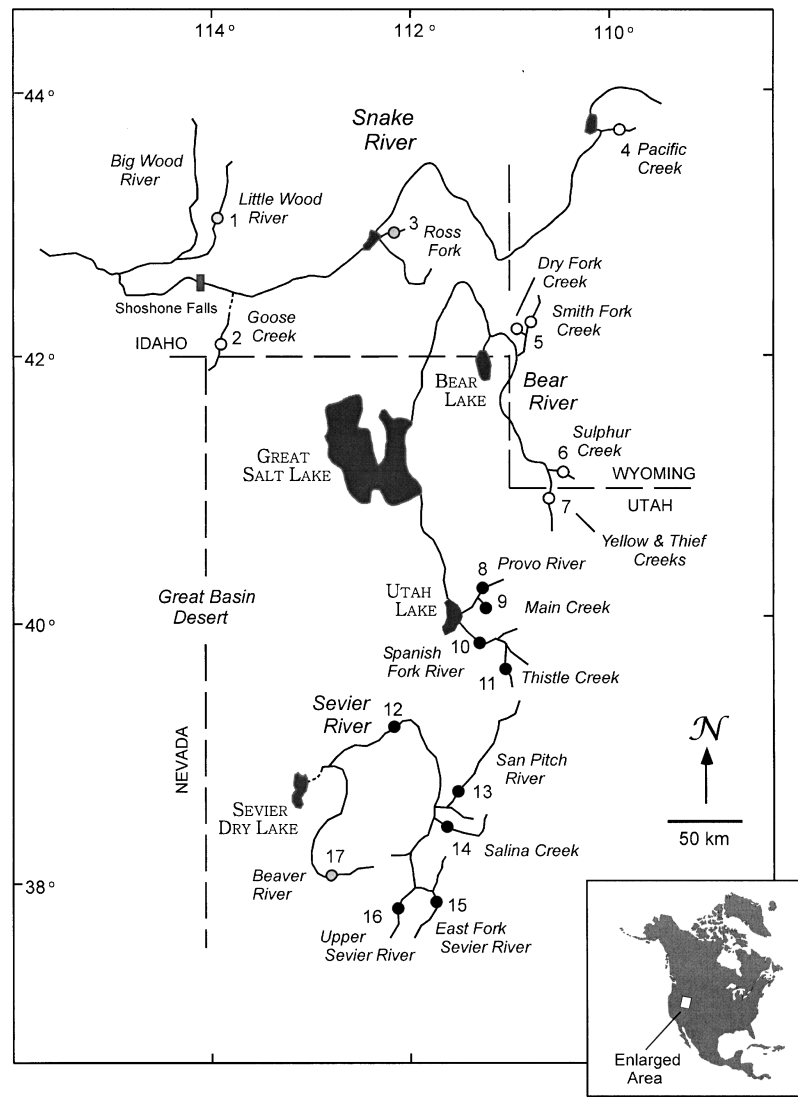


FIGURE 1. Geographic distribution map showing 17 populations of leatherside chub considered in this study (numbered circles). Shading reflects the authors' assignment of these populations into northern and southern leatherside species (see Results): Open circles identify extant populations in the northern leatherside chub species; solid black circles identify extant populations in the southern leatherside chub species; and gray circles identify populations now thought to be extinct. Morphological data indicate that populations 1 and 3 belonged to the northern species, and populations 17 belonged to the southern species.

We used primers TPI14F (Merritt and Quattro, 2001) and R3 (5'-TCCCGGAGCTTIGCATGCAC-3') to generate a PCR product that was then diluted (1:100) and used as template in a second reaction using primers TPI21F (5'-ACGGCGACAAAAGAGCATC-3') and R3. The thermal profile for the first reaction (30 s at 94°C; 30 s at 53°C; 1.5 min at 72°C) was repeated 30 times, followed by a 7-min extension at 72°C. The protocol for the second reaction was identical except that the annealing temperature was increased to 55°C. We amplified the fourth and fifth TPI introns using primers F2 (Quattro et al., 2001) and R3 using the same thermal profile as the S7 intron, except that the extension time was increased to 2 min and the annealing temperature was 57°C. For both S7 and TPI, purified double-stranded

PCR products were then used as template (~80 ng) in 10- μ l cycle sequencing reactions using Big Dye chemistry v. 3.1 (Applied Biosystems, Inc., Palo Alto, CA). For most individuals, we sequenced in both forward and reverse directions using the same primers listed above. Products of the cycle sequencing reactions were cleaned using the CeanSEQ dye terminator removal protocol (Agencourt Bioscience Corp., Beverly, MA) and visualized by electrophoresis using an ABI 3100 or ABI 377 automated sequencer (Applied Biosystems, Inc., Palo Alto, CA). Sequences are archived in the GenBank database (accession numbers AY825364 to AY825501).

We used similar analyses for both mtDNA and nuclear DNA data sets. We first reconstructed phylogenetic relationships among leatherside chub and spinedace

haplotypes under maximum parsimony and maximum likelihood criteria. Our intent with these analyses was to test if leatherside chub could be divided into two reciprocally monophyletic groups (Moritz, 1994). Both analyses were conducted using PAUP* 4.0b10 (Swofford, 1999). However, to justify combining the two nuclear markers as a single data set, we first compared trees generated when each marker was evaluated alone and when markers were combined; we found that the separate data sets and the combined data set all produced the same tree with respect to nodes supported by bootstrap values greater than 50%. In addition, by using the AIC output in MODELTEST 3.06 (Posada and Crandall, 1998), we found that sequence variation for each nuclear gene region was best described by the same model of molecular evolution (TrN+I) and that both markers had very similar estimates of model parameters. Finally, we ran a partition homogeneity test (100 replicates) and found no significant difference between the phylogenies inferred from the two nuclear data sets relative to differences in phylogenies constructed under random permutations of the data ($P = 0.11$). Hence, three lines of evidence justified combining the two nuclear data sets. For both mitochondrial and nuclear DNA analyses, we conducted an equally weighted maximum parsimony analysis using the heuristic search option with the starting tree generated through 10 replications of random stepwise addition and TBR branch swapping. A strict consensus was used to reconcile equally parsimonious trees. Support for each node was evaluated by 1000 bootstrap replicates (Felsenstein, 1985). Both maximum parsimony and maximum likelihood reconstructions were rooted using the sequences of *Meda fulgida*, a representative outgroup based on the phylogenetic hypothesis of Dowling et al. (2002).

Phylogenetic analysis under the maximum likelihood criterion requires an explicit model of molecular evolution. To identify the best-fitting evolutionary model for the cytochrome *b* gene, and for the nuclear S7 and TPI genes, we followed the procedure outlined in Posada and Crandall (1998). In brief, for each analysis we generated a neighbor joining tree calculated under a Jukes-Cantor model of evolution (Jukes and Cantor, 1969) and held this tree constant to evaluate the fit of 56 different models of DNA sequence evolution. Negative log-likelihood scores (generated in PAUP* 4.0b10) described the fit of each model to the observed sequence data; we selected the best-fitting simplest model from this set based on AIC scores calculated in MODELTEST 3.06 (Posada and Crandall, 1998). For the cytochrome *b* gene, this technique identified the GTR+I+G model (Tamura and Nei, 1993) as the best model ($-\ln$ likelihood score = 3152.65; AIC = 6325.3). For the S7 and TPI nuclear introns, the technique identified the TnN+I model as best model ($-\ln$ likelihood score = 2784.50; AIC = 5579.0). Hence, we used these respective models (including parameter estimates) to conduct a heuristic maximum likelihood search (again with 10 replications of stepwise addition and TBR branch swapping). Nodal support for the resulting trees was evaluated by 100 bootstrap replicates.

Given the extreme levels of divergence in mtDNA among candidate species, we also used these data in an analysis of molecular variance (AMOVA; Excoffier et al., 1992) to examine genetic structuring among leatherside chub populations. We tested for significant differentiation at the cytochrome *b* gene between the two candidate species by partitioning total genetic variation into: differences between the northern and southern leatherside chub species (Φ_{CT}); differences among populations within species (Φ_{SC}); and differences within populations across the total sample (Φ_{ST}). We conducted two additional AMOVAs to test for genetic structuring among drainages within each of the two candidate species, treating drainages as the grouping unit. Combined, these tests provide a way to gauge the level of divergence within species relative to divergence between the two candidate species. All analyses were run in ARLEQUIN 2.0 (Schneider et al., 2000).

Similarity species concept.—We used a geometric morphometric analysis to test leatherside chub species boundaries under the similarity species concept category. Our testable criterion under this concept was divergence in shape between candidate species relative to other members of the spinedace clade. According to the two-species hypothesis of Johnson and Jordan (2000), we predicted that fish from northern drainages (populations 1 to 7) would be morphologically distinct from those in southern drainages (populations 8 to 17). Also, we expected that the level of shape divergence between putative leatherside chub species would be comparable to that found among species of the genus *Lepidomeda*. Our goal was to include at least one population from each of the eight watersheds, using museum collections for drainages where leatherside chub appear now to be extinct. Unfortunately, museum samples from Beaver River (population 17) were of poor quality and could not be included. Populations 1 to 4, 6, 8, and 12 represented the remaining seven watersheds in our analysis. We also included samples from each species of *Lepidomeda* and from the outgroup *Meda fulgida*: *L. mollispinis mollispinis* (two populations); *L. albivallis* (two populations); *L. vittata* (two populations); *L. altivelis* (now extinct; one population); and *M. fulgida* (two populations). Descriptions of the original collections, sample sizes for each population, and museum identification numbers for these samples are provided in Appendix 1.

Our morphometric analyses focused on shape variation in cranial morphology, a trait not likely to be influenced by differences in preservation techniques among different museum samples. We restricted our study to include only individuals of adult size classes (standard length >60 mm for leatherside chub (Johnson and Belk, 1995) and *Lepidomeda* species; standard length >40 mm for *Meda fulgida*) to avoid the confounding influence of ontogenetic change in shape associated with juvenile development. Each fish was photographed in lateral view with a digital camera. From these images, we scored 10 anatomical landmarks for each individual. Two-dimensional landmark coordinates served as inputs to the shape analyses.

To describe shape variation among populations, we used the program TPSREGR (F. J. Rohlf 2002, <http://life.bio.sunysb.edu/morph>). This program employs a generalized orthogonal least-squares algorithm to align anatomical landmarks among individuals and to produce a “consensus,” or average shape, for the entire sample (Rohlf and Slice, 1990). The program then computes two measures of shape variation for each fish: a set of two uniform (affine) shape components; and a set of seven pairs of partial warp scores (describing nonuniform shape variation). These measures quantify the deviation of an individual from the overall consensus shape. The affine components represent changes in form that are geometrically uniform across the entire head of the fish, such as a general increase in depth or length relative to a consensus form; this measure is typically used to detect allometric variation in shape. Nonaffine shape variation—described by orthogonal partial warp scores—depicts the shape deviation of an individual relative to a consensus shape for specific landmark regions. In other words, partial warp scores describe nonuniform changes in the position of a subset of landmarks relative to other landmarks. We used both affine and nonaffine shape components to characterize differences among individuals, among populations, or between putative species.

We tested for differences in head morphology in two ways. First, we considered only the two putative species and tested for differences in head shape using a multivariate regression analysis. This required creating a dummy variable ($-1 =$ northern leatherside chub; $1 =$ southern leatherside chub) and assigning individual fish to these categories according to their population of origin. The two uniform variables and fourteen partial-warp variables (described above) were then regressed on the dummy variables to test for differences in head shape between the putative species. We assessed significance of shape differences using Wilk's Λ as test statistic. This analysis was executed using the program TPSREGR (F. J. Rohlf, 2002; <http://life.bio.sunysb.edu/morph>). Second, we tested for differences in head shape among the species considering each leatherside chub species and the spinedace species together (these taxa are listed in the phylogenetic analyses above). To do so, we used a data reduction technique to summarize shape variation among individuals in the form of relative warps. Relative warps (RW1, RW2, etc.) are orthogonal axes of shape variation generated from a principal components analysis of the uniform and nonuniform shape variables. For this analysis, we tested for differences between putative species by comparing RW scores among taxa using a multivariate analysis of variance (MANOVA; ProcGLM; SAS, 1997) with RW scores as dependent variables, and taxa as independent variables. We plotted the first two RW axes to note differences among species and to see which species could be statistically distinguished from one another. Calculations of RWs were executed using the program TPSRELW (F. J. Rohlf, 2002; <http://life.bio.sunysb.edu/morph>).

Ecological species concept.—We used growth and feeding rates to test leatherside chub species boundaries under the ecological species concept category. We predicted that populations from the northern species would show local adaptations to colder environments, whereas populations from the southern species would show local adaptations to warmer environments. Hence, ecological adaptation served as our testable criterion under this concept (Van Valen 1976; Templeton 1989). Specifically, we expected juveniles to maximize their growth rates at temperatures characteristic of their own natural habitat. We tested this hypothesis by measuring growth and feeding rates of young of year, juvenile fish (age 0) reared in one of four common-environment treatments (described below). Due to space constraints, we were forced to limit our experiment to two populations (Fig. 1: population 5, northern species; population 16, southern species). Thus, inferences regarding ecological species boundaries are based on limited numbers of populations.

The general experimental protocol and a subset of the growth data evaluated below were originally presented in Belk et al. (in press). Here we extend this original growth experiment by including data on two additional treatments using the same fish. In August 1998, we collected 80 young of year fish from each of the two locations. These fish were placed in common holding tanks in the laboratory and allowed to acclimate at room temperature (19°C) for 3 weeks. The full analysis included four temperature treatments (10°C , 15°C , 19°C , or 24°C) in a fully crossed factorial design with fish from both candidate species assigned to each of the temperature treatments. Although both populations could potentially experience any of these temperatures during a typical growing season in the wild, the two colder temperatures are more indicative of those experienced by the northern population and the two warmer temperatures are more indicative of those experienced by the southern population (Belk et al., in press). Space constraints required that we divide the experiment into two successive stages of two unique temperature treatments. Hence, growth rates were first measured on fish reared at 10°C and 19°C (Belk et al., in press) and then on fish reared at 15°C and 24°C (this study). The two parts of the experiment were separated by 4 weeks during which time fish were again held at room temperature (19°C) before being assigned at random to the second set of temperatures. We found that there was no interaction between treatments in the two successive stages ($F = 0.94$, $P = 0.33$). In other words, rearing temperature in the first stage had no effect on growth rate in the second stage, thus justifying the successive use of fish as outlined in the nested analysis below. For all treatments, we controlled for differences in initial size by including standard length as a covariate in the statistical analysis.

For each treatment, between 30 and 40 fish per population were individually assigned to 1-L plastic cups; the bottom of the cup had been removed and replaced with a 2-mm plastic mesh. Cups for each treatment were housed in a large (1198-L) aerated tank, providing uniform

temperature and water chemistry within treatments across individuals and between putative species. Fish from each population were measured at the beginning of the experiment and then randomly assigned to cups within a treatment. Fish were fed twice daily 0.4 g of trout food (~10% of individual body mass) for between 57 and 71 days (variation due to the time at which were introduced to the experiment). Individuals were measured at the end of the experiment and the change in standard length (mm) divided by the total number of days in the treatment was used to calculate the growth rate. The second experimental stage was identical to the first—the fish again being assigned at random to temperature treatments—except that the duration of treatment exposure was only 31 to 35 days. We tested for differences in growth rates using a nested ANCOVA, including population and temperature as main effects, with temperature nested in experimental stage, and initial body size as a covariate. Using this design, a significant interaction between population and temperature could provide evidence that populations are locally adapted to temperatures they most typically experience in the wild.

We conducted a second experiment to test for differences in temperature-specific foraging rates between the two putative species. We used 50 fish from each of the two populations, again focusing on young of year fish. We designed this experiment to measure foraging rates under three temperatures: 9°C, 19°C, and 25°C. These correspond to three of the four temperatures used in the growth experiment. All fish were held initially at room temperature (19°C). We then randomly assigned 20, 10, and 20 individuals from each species to the three respective temperature treatments. Fish were held in common tanks, but separated by putative species. Individuals were then assigned in random order and placed in an observation chamber that was visually isolated from other fish but still within the larger tank. Individuals were allowed to acclimate in this chamber for 90 min, after which we gave them 0.8 g trout food. Food presentation and observations of feeding rate were made from behind a blind so that the fish was never disturbed. The number of bites made by each fish over a 10-min period was recorded and the mean number of bites per minute served as the dependent variable in the statistical analysis. We tested for variation in foraging rate by ANOVA with population and temperature included in the model as main effects. We also tested for an interaction between population and temperature to see if populations showed similar changes in feeding behavior in response to changes in temperature.

RESULTS

Each of our species boundary tests—conducted under categories of phylogenetic, similarity, and ecological species concepts—supported the hypothesis that leatherside chub is composed of two distinct species.

Phylogenetic Divergence

Phylogenetic analyses of the mtDNA and nuclear DNA data sets both identified two reciprocally monophyletic clades of leatherside chub (Figs. 2, 3). For the mtDNA tree, one clade (hereafter referred to as the northern leatherside chub) was composed of six haplotypes that occurred only in the Bear River, Goose Creek, and upper Snake River drainages. The second clade (hereafter referred to as the southern leatherside chub) was composed of 12 haplotypes that occurred only in the Utah Lake and Sevier River drainages. Mitochondrial DNA sequence divergence between individuals from the northern and southern leatherside chub ranged from 7.8% to 8.2%. This contrasted sharply with low levels of sequence divergence found among individuals within each group: northern species (0.0% to 0.4%) and southern species (0.0% to 1.1%).

Maximum parsimony and maximum likelihood analyses of the mtDNA data produced similar results. The maximum parsimony analysis identified 1155 most-parsimonious trees of 342 steps (consistency index [CI] = 0.84; retention index [RI] = 0.93) that were combined into a strict consensus tree. A total of 24 characters supported monophyly of the northern species, whereas 26 characters supported monophyly of the southern species (Fig. 2). Maximum likelihood analysis also produced a well-resolved phylogeny ($-\ln$ likelihood score = 3167.52) that distinguished the northern and southern leatherside chub. Nodal support from bootstrap analyses was comparable under maximum parsimony and maximum likelihood criteria. This phylogeny rendered the two leatherside chub species paraphyletic, with the northern species being more closely related to the spinedace fishes (*Lepidomeda* species) than to the southern species.

Analysis of the nuclear DNA sequences also revealed two reciprocally monophyletic clades of leatherside chub (Fig. 3). The northern leatherside chub clade was composed of five distinct nuclear types that occurred only in the Bear River, Goose Creek, and upper Snake River drainages. The southern leatherside chub clade was composed of 12 nuclear types that occurred only in the Utah Lake and Sevier River drainages. Model-corrected sequence divergence between individuals from the northern and southern species ranged from 0.7% to 1.0%, revealing a slower rate of molecular divergence for the nuclear markers relative to the mitochondrial marker. The maximum parsimony analysis identified 167 most-parsimonious trees of 91 steps (consistency index [CI] = 0.87; retention index [RI] = 0.80) that collapsed into a strict consensus tree with several well-supported nodes. Maximum likelihood analysis also produced a well-supported phylogeny ($-\ln$ likelihood score = 2780.04) that distinguished the northern and southern species from one another. As with the mtDNA results, nodal support from bootstrap analyses was comparable under maximum parsimony and maximum likelihood criteria. However, unlike the mtDNA phylogeny, the deeper taxonomic relationship among the

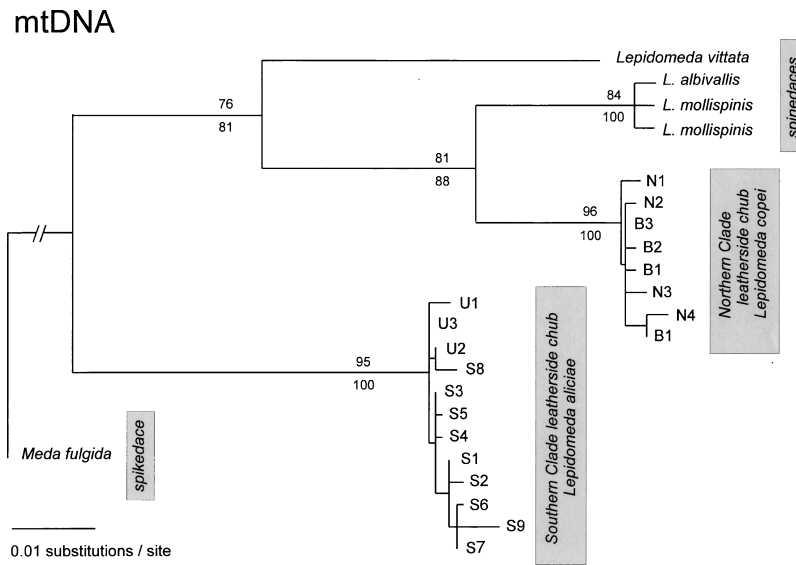


FIGURE 2. Maximum likelihood phylogeny of mtDNA haplotypes estimated under the GTR+I+G model of sequence evolution (see text) depicting relationships among haplotypes from two candidate species of leatherside chub and the closely related spinedace fishes (*Lepidomeda* species). Haplotypes are coded by the drainages where they occur (N = upper Snake River system; B = Bear River system; U = Utah Lake system; S = Sevier River system). Nodes supported on this tree are consistent with a strict consensus of 1155 most parsimonious trees (342 steps, CI = 0.84, RI = 0.93) from the maximum parsimony analysis. Numbers at each node indicate bootstrap support greater than 50% from maximum likelihood (above) and maximum parsimony (below). Shaded bars identify common names of taxonomic groups. Spikedace (*Meda fulgida*) is an outgroup taxon used to root the phylogeny.

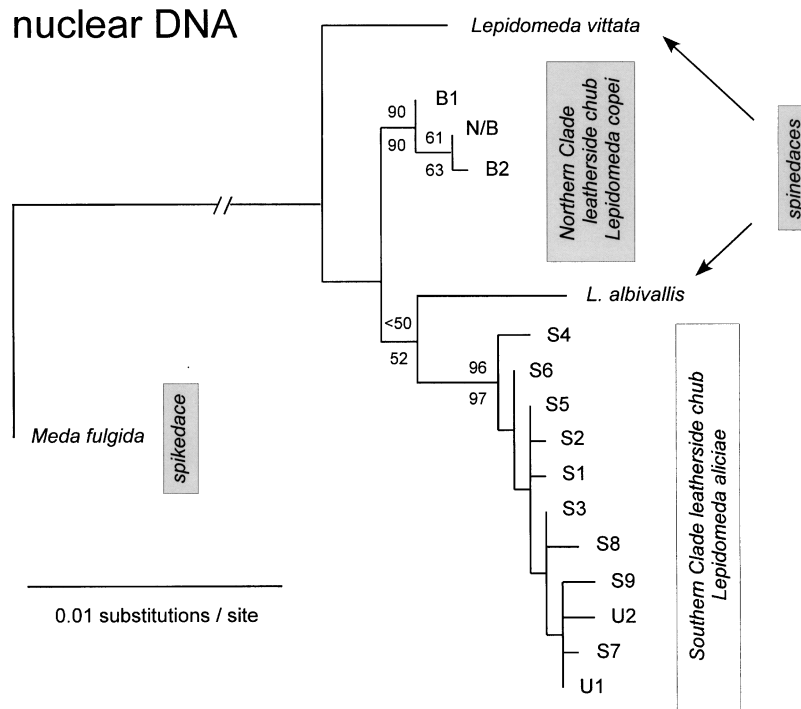


FIGURE 3. Maximum likelihood phylogeny of nuclear DNA genotypes estimated under the TrN+I model of sequence evolution (see text) depicting relationships among nuclear types from two candidate species of leatherside chub and the closely related spinedace fishes (*Lepidomeda* species). Nuclear genotypes are coded by the drainages where they occur (N = upper Snake River system; B = Bear River system; U = Utah Lake system; S = Sevier River system). Nodes supported on this tree are consistent with a strict consensus of 167 most parsimonious trees (91 steps, CI = 0.87, RI = 0.80) from the maximum parsimony analysis. Numbers at each node indicate bootstrap support greater than 50% from maximum likelihood (above) and maximum parsimony (below). Shaded bars identify common names of taxonomic groups. Spikedace (*Meda fulgida*) is an outgroup taxon used to root the phylogeny.

TABLE 1. Analyses of molecular variance (AMOVA) showing distribution of mtDNA genetic variation among leatherside chub populations at three scales: (1) total sample of northern and southern leatherside chub species combined; (2) northern leatherside chub alone; and (3) southern leatherside chub alone. Reported Φ -statistics are analogous to Wright's F -statistics. Values in parentheses show the percent of variation explained by each hierarchical contrast. Numbers within brackets identify populations that make up each group (ID numbers correspond to those in Fig. 1).

	Between groups Φ_{CT}	Among populations within groups Φ_{SC}	Within populations Φ_{ST}
Total sample	0.96* (95.9%)	0.61* (2.5%)	0.98* (1.6%)
Groups: Northern leatherside chub [2,3,6] Southern leatherside chub [9–11,13–16]			
Northern leatherside chub	0.59* (58.8%)	0.07 ns (2.9%)	0.62* (38.3%)
Groups: Goose Creek Drainage [2] Bear River Drainage [3,6]			
Southern leatherside chub	0.41† (40.5%)	0.47* (28.3%)	0.69* (31.2%)
Groups: Utah Lake Drainage [9–11] Sevier River Drainage [13–16]			

* $P < 0.01$; † $P < 0.05$; ns = not significant.

spinedace and leatherside chub species was poorly resolved with the nuclear DNA markers (Fig. 3).

AMOVA results of the mtDNA data set were consistent with the phylogenetic analyses, revealing strong genetic structuring between northern and southern regions. Grouping populations by candidate species (Goose Creek and Bear River drainages versus Utah Lake and Sevier River drainages), we found that 96% of the total genetic variation among haplotypes could be explained between species; the remaining 4% was partitioned evenly among and within populations (Table 1). In contrast, genetic structuring observed within each leatherside chub species was more evenly distributed. In the northern species, most genetic variation occurred between drainages (59%), with almost no variation found among populations within drainages (3%) and moderate variation occurring within individual populations (38%). In the southern species, genetic structuring was also strong between drainages (41%), but there was approximately equal variation occurring among populations within drainages (28%) and among individuals within populations overall (31%). In all three AMOVAs, the between-group variance component (V_a)—between species in the first test and between drainages in the second and third tests—was larger than predicted by a random distribution of haplotypes among locations (Fig. 4).

Morphological Divergence

Each leatherside chub species has a distinct cranial shape. Fish from the northern species on average had deeper heads with shorter noses than fish from the southern species. These species-specific morphotypes were statistically different from one another as measured by multivariate multiple regression of uniform and non-uniform shape components (Wilk's $\Lambda = 0.356$, $P < 0.001$). The expanded analysis considering all taxa revealed clear differences in cranial shape among the four *Lepidomeda* species, *Meda fulgida*, and the two leatherside chub species (Wilk's $\Lambda = 0.014$, $P < 0.001$). The relative warp plot clarified this pattern. Relative warps one and two (RW1 and RW2) explained 32% and

23%, respectively, of the total shape variation in cranial morphology among individuals. Populations from the northern versus southern species were well separated in relative warp space, particularly along the first warp axis (Fig. 5). In fact, *Lepidomeda mollispinis* had a cranial shape intermediate to the two leatherside chub species, again demonstrating the distinctiveness of each of these taxa. The second warp axis did not distinguish among the *Lepidomeda* species and leatherside chub, but clearly distinguished this group from the outgroup *Meda fulgida*.

Life History Divergence

Leatherside chub grow at different rates under different temperatures (Fig. 6). Populations from both species showed a significant increase in growth rate as a positive function of temperature (Table 2). At colder temperatures, fish from the northern population grew more rapidly than fish from the southern population. However, this advantage was reversed at high temperatures, where fish from the southern populations grew faster than fish from the northern population (Fig. 6). This was demonstrated statistically by a significant interaction

TABLE 2. Analysis of covariance results for the common environment growth and feeding experiments. Fish were reared at four different temperatures to test for differences in growth rates and at three different temperatures to test for differences in foraging rates. The primary focus of each experiment was to test for differences between populations of the two candidate species.

Effect	df	F	P
Growth experiment			
Population	1	0.5	0.48
Temperature (Stage)	3	81.7	<0.01
Population \times Temperature (Stage)	3	9.1	<0.01
Initial body size	1	0.1	0.71
Error	248		
Foraging experiment			
Population	1	10.8	<0.01
Temperature	2	10.7	<0.01
Population \times Temperature	2	3.7	0.02
Error	88		

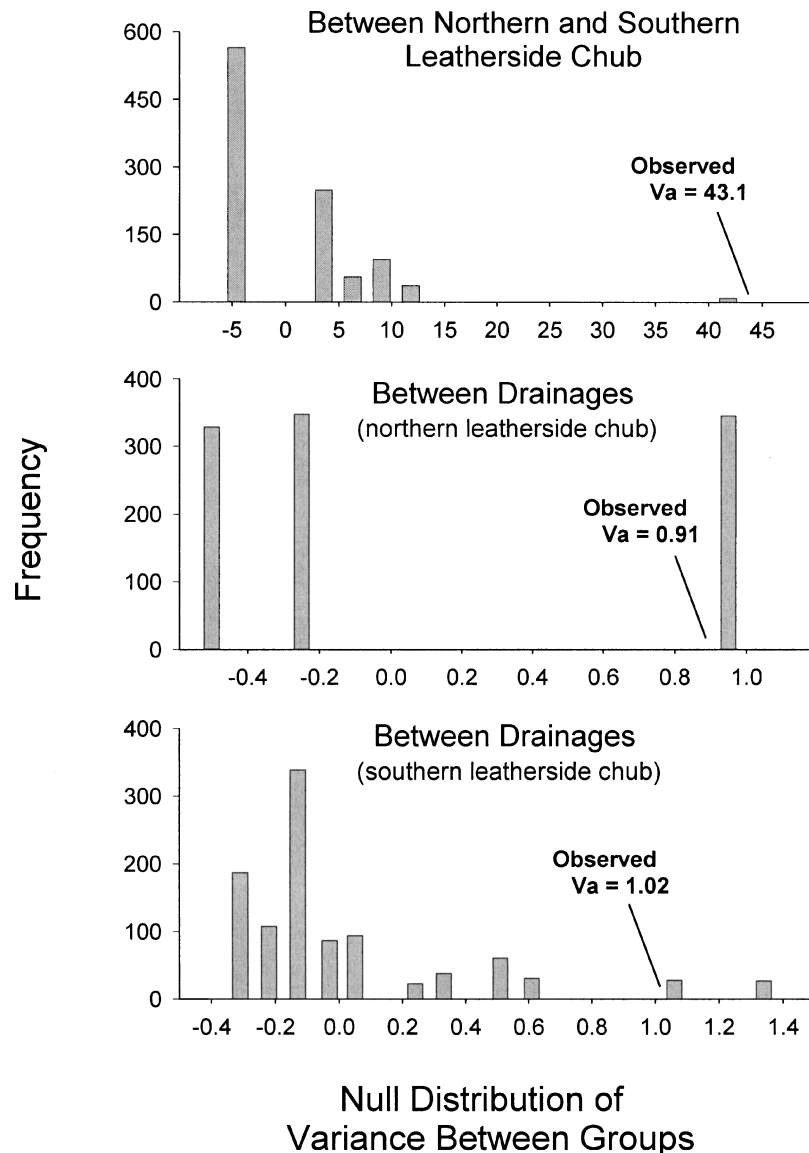


FIGURE 4. Histograms of genetic variance between randomly constructed groups of leatherside chub relative to actual observed values for specified groups. Panels show group comparisons: between northern and southern leatherside chub (upper); between Goose Creek and Bear River drainages within northern leatherside chub (middle); and between Utah Lake and Sevier River drainages within the southern leatherside chub (lower).

between temperature and population (Table 2) and visually by crossing reaction norms (Fig. 6).

Leatherside chub from the two populations also foraged at different rates under different temperatures (Table 2). The northern population fed more rapidly than the southern population at low and intermediate temperatures, but did not differ from the southern population at the highest temperature (Fig. 6). The southern population showed increased foraging activity with each increase in temperature, resulting in the highest feeding rate at the 25°C treatment. In contrast, the northern population fed most rapidly at the intermediate 19°C treatment. Overall, the northern population fed more rapidly than its southern counterpart (Table 2).

DISCUSSION

Tests of Species Boundaries

Many scientists have emphasized the importance of accurately defining species in conservation biology (Soule, 1990; Rojas, 1992; Soltis and Gitzendanner, 1999). Yet, formal tests of species boundaries remain uncommon (Sites and Crandall, 1997; Sites and Marshall, 2003). This trend is alarming given well-known cases where mistakes in taxonomy have led to misguided intervention or to neglect eventually resulting in species extinctions (Avice and Nelson, 1989; Daugherty et al., 1990). One perceived difficulty in testing species boundaries is the unresolved debate on how species should be

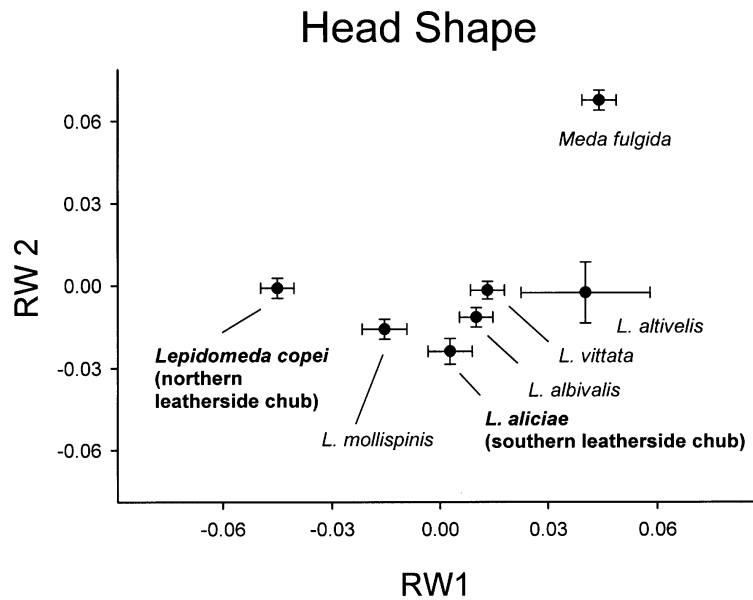


FIGURE 5. Characterization of morphological variation in head shape among all *Lepidomeda* species, including the two species of leatherside chub. Relative warp scores (± 1 SE) for each species are plotted along the first two relative warp axes (axis units are arbitrary) showing differences in head shape among species. The first RW axis represents a shift from a short-snout, deep head phenotype at the left to a longer-snout, shallow head phenotype at the right.

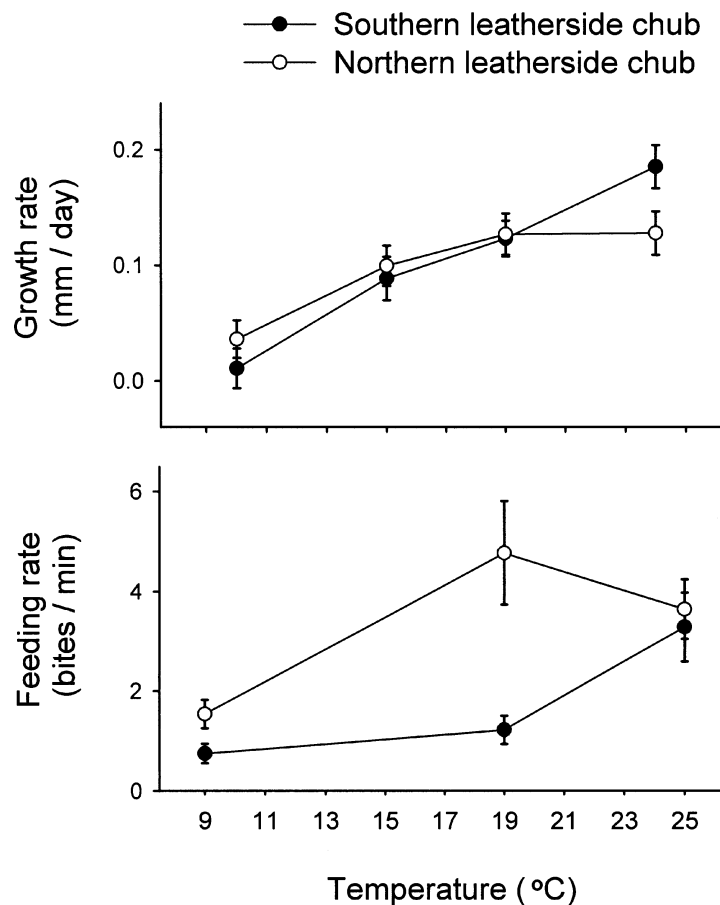


FIGURE 6. Results of common environment rearing experiments showing the effects of temperature on growth rate (upper panel) and feeding rate (lower panel) in juvenile fish compared between the two candidate species of leatherside chub.

TABLE 3. Current evidence for two distinct leatherside chub species. The common names 'northern leatherside chub' and 'southern leatherside chub' identify these two groups. Candidate species are evaluated under three categories of species concepts: phylogenetic species; morphological species; and ecological species. Within each species, populations are identified by water drainage. ID numbers correspond to those in Figure 1.

	Phylogenetic species mtDNA and nuclear DNA clades ^{1,2}	Morphological species Head shape morphology ²	Ecological species Life history feeding rate ² and growth rate ^{2,3}
Northern leatherside chub			
<i>Wood River Drainage</i>			
1 Little Wood River [†]	—	Deep	—
<i>Goose Creek Drainage</i>			
2 Goose Creek	Northern	Deep	—
<i>Portneuf River Drainage</i>			
3 Ross Fork River [†]	—	Deep	—
<i>Upper Snake River Drainage</i>			
4 Pacific Creek	Northern	Deep	—
<i>Bear River Drainage</i>			
5 Dry Fork and Smith Fork Creeks	Northern	—	Fast feeding/rapid cold growth
6 Sulphur Creek	Northern	Deep	—
7 Yellow and Thief Creeks*	—	—	—
Southern leatherside chub			
<i>Utah Lake Drainage</i>			
8 Provo River	—	Shallow	—
9 Main Creek	Southern	—	—
10 Spanish Fork River	Southern	—	—
11 Thistle Creek	Southern	—	—
<i>Sevier River Drainage</i>			
12 Sevier River	—	Shallow	—
13 San Pitch River	Southern	—	—
14 Salina Creek	Southern	—	—
15 East Fork Sevier River	Southern	—	Slow feeding/slow cold growth
16 Upper Sevier River	Southern	—	—
<i>Beaver River Drainage</i>			
17 Beaver River [†]	—	—	—

¹Published results from Johnson and Jordan (2000).

²Results from this study.

³Published results from Belk et al. (in press).

[†]Data collected from historic museum collections. No extant populations are currently known from this drainage.

*This population was recently discovered in these two small tributaries to the Bear River and has yet to be analyzed to determine species placement. Inclusion here with northern leatherside chub is based solely on geography, pending further analyses.

defined (Mayden, 1997). This "species problem" presents a particular challenge to conservation managers when alternative species concepts suggest different conservation strategies (Peterson and Navarro-Sigüenza, 1999). Clearly, evaluating species boundaries under a set of alternative concepts should precede all management efforts that rely in any way on taxonomy.

In our study, we examined taxonomic boundaries in leatherside chub under three different categories of species concepts. We tested for two distinct species using the following criteria: reciprocal monophyly and fixed diagnostic character differences in nuclear and mtDNA under the phylogenetic species concept category; statistically distinguishable and diagnostic cranial shape under the similarity species concept category; and local adaptation in growth and foraging rates under the ecological species concept category. We present testable and repeatable evidence for species divergence between candidate species based on molecular markers (Figs. 2, 3), cranial shape (Fig. 4), and in growth and foraging rates (Fig. 5). In short, we found complete congruence among our results evaluated under each of the three species concepts categories (Table 3), clearly supporting the hypothesis that leatherside chub is composed of two allopatric species.

Our approach in this study is similar to the cohesion species concept promoted by Templeton (2001), particularly in the sense that defining a cohesion species is also based on support from several kinds of data. Strictly defined, a cohesion species is "an evolutionary lineage or set of lineages with genetic exchangeability and/or ecological interchangeability" (Templeton, 2001). Delineating species boundaries under this concept requires rejecting two null hypotheses. The first is that the geographic sample of populations comes from a single evolutionary lineage. In our study, this is clearly not the case. Our sample contains two phylogenetically distinct clades (based on both mtDNA and nuclear DNA markers) that are geographically distinct from one another—that is, they are not genetically exchangeable. The second test is based on direct comparison of ecological data among candidate taxa. Again, our comparisons of experimental growth data and cranial shape data demonstrate that the leatherside chub species are distinct from one another, and therefore not ecologically exchangeable. Hence, each leatherside chub species meets the 'cohesion species' criteria based on the formal hypothesis-testing framework for this species concept.

Earlier studies that noted high levels of mtDNA variation among the northern and southern leatherside chub species maintained the possibility that leatherside chub was actually only a single species, but that the variation in mtDNA among populations was due to mitochondrial introgression into northern populations via hybridization with a *Lepidomeda* species. Our nuclear DNA data reveal that this is not the case as these two forms exhibit reciprocal monophyly and can be discriminated by several diagnostic nucleotides. In fact, based on the mtDNA phylogeny, the two species of leatherside chub actually appear to be nonsister taxa within the genus *Lepidomeda*, each of which is distinct from other members of this genus in lacking the prominent dorsal spine that otherwise characterizes this group. Our morphometric data are consistent with this conclusion. Of particular note is the fact that *L. mollispinis* has a cranial shape that is intermediate to the leatherside chub species. Moreover, the degree of divergence in cranial shape between leatherside chub species is comparable to the level of divergence among other species in the genus *Lepidomeda*. However, the nuclear DNA phylogeny is unclear on this issue of paraphyly, given the lack of resolution among taxa at deeper nodes of the tree (Fig. 3).

We did not test leatherside chub species boundaries under the biological species concept category. This omission may seem odd given the pervasive habit biologists have of using reproductive isolation as the primary criterion to delineate species. However, an important line of evidence suggests that reproductive isolation would be an inappropriate test in this particular case. Several western North American minnow species are known to readily hybridize (Dowling and DeMarias, 1993; Dowling and Secor, 1997). In fact, some research suggests that hybridization may be an important mechanism by which new minnow species arise (Dowling and Secor, 1997; Gerber et al., 2001). Consequently, it would not be surprising to find that viable hybrids could be generated from any of the crosses among the taxa considered in this study (leatherside chub and spinedaces included). Yet, by all other criteria, we would clearly recognize these as distinct species. Hence, not all species concepts are equally applicable in defining species boundaries in all taxa. In this study, we apply the three categories of species concepts that best fit the biology of this group of fishes.

Taxonomic Revision

All evidence suggests that leatherside chub is composed of two distinct species. Moreover, both cranial shape data and molecular data reveal that these two species clearly fall within the genus *Lepidomeda*. Consequently, we suggest the following taxonomic revisions. First, each leatherside chub species should hereafter be recognized as a member of the genus *Lepidomeda*. The precise taxonomic relationship among all species within this genus is not clear from the present work. However, two of us (Johnson and Dowling) have a molecular phylogeny project underway that should resolve this ques-

tion. This new placement removes leatherside chub from the monotypic genus *Snyderichthys* (*S. copei*: Simons and Mayden, 1997) and from its earlier and more commonly known placement in the genus *Gila* (*G. copei*: Robins et al., 1990; Sigler and Sigler, 1996). Second, the species of leatherside chub that occupies tributaries to the upper Snake River and Bear River drainages should hereafter be referred to by the common name 'northern leatherside chub' and by the scientific name *Lepidomeda copei* as the type specimen for *copei* was collected at a "tributary of Bear River at Evanston, Wyoming" (Jordan and Gilbert, 1881). The clade of leatherside chub that occupies Utah Lake and Sevier River drainages and associated tributaries should hereafter be referred to by the common name 'southern leatherside chub' and by the scientific name *Lepidomeda aliciae*. This designation marks the reevaluation of *aliciae* first described in 1881 (Jouy). The type locality is Provo River at Utah Lake, a site where southern leatherside chub appear now to be extinct. These new common names retain the term 'leatherside chub' to reflect the smooth, leather-like skin typical of each species, but add the terms 'northern' and 'southern,' respectively, to specify the natural geographic distributions of these two species relative to one another (Fig. 1).

Conservation Implications

Our findings clearly indicate that leatherside chub must be managed as two distinct species. Prior to this study, managers could have considered translocating fish from healthy populations of one region to augment dwindling or extirpated populations in another. Numerous lines of evidence now suggest that such translocations across species would be misguided. For example, we found that individuals from the southern species grow more slowly at colder temperatures than individuals from the northern species; the opposite pattern was true of individuals from the northern species reared at warmer temperatures (Fig. 5). If fish from the northern and southern species are capable of reproducing, then homogenizing effects of introgression via translocations could depress individual growth rates in northern or southern fish, potentially increasing susceptibility to predators, over-winter mortality risk, or other size-dependent ecological interactions.

Northern and southern species also differ in their patterns of population subdivision. Genetic variation between drainages was substantial in both taxa, but in the northern species differences between drainages accounted for almost 20% more of the total variation than between drainages in the southern species (Table 1). Johnson and Jordan (2000) suggested that this pattern could be due to greater historic gene flow in the southern species during the late Pleistocene when Utah Lake and Sevier River drainages were connected via Lake Bonneville. Higher levels of fragmentation in the northern species could also be the result of historic extinctions caused by volcanic activity that exterminated fishes from vast regions of the upper Snake River prior to the Pleistocene (Hubbs and Miller, 1948). Our data show

that individual populations are often genetically distinct (Table 1). Consequently, recovery and restoration efforts in both species must be applied at a fine scale, focusing on specific populations within individual drainage systems.

Museum records show that both species of leatherside chub have declined dramatically from historic levels (Appendix 1). However, our analyses now reveal that the northern species is extremely rare, with extant populations known from only four general locations (Table 3). Based on qualitative measures of sampling effort, we have found that populations of northern species are relatively small compared to populations that compose most of the southern species (Belk and Johnson, unpublished data). However, no estimates of actual population sizes or demographic trajectories have been made from either species.

Given our findings, we recommend three conservation priorities for the two species of leatherside chub. First, exhaustive field surveys must be made to identify all extant populations of the northern species. This effort should begin with the Little Wood River and Ross Fork Creek, two locations where historic populations have been documented but now appear to be extinct. The recent rediscovery of northern leatherside chub in Pacific Creek and in upper Bear River tributaries provides hope that historic locations might still house extant populations. Managers must also identify and sample appropriate habitats from other streams throughout the upper Snake River drainage (above Shoshone Falls) and in the Bear River drainage where overlooked populations might now exist. Second, monitoring efforts must be implemented across multiple populations in both the northern and southern species to assess the demographic stability of populations. We must know if populations are growing or declining, and what kind of variation exists in population sizes over time. Such data will be critical to predict the long-term viability of dwindling populations. Finally, we need to identify and experimentally test the effects of anthropogenic agents in shaping evolutionary and demographic patterns in each species. Irrigation projects have fragmented and modified most leatherside chub habitat in both species. These water projects have frequently been accompanied by the introduction of exotic game fishes that prey upon leatherside chub and displace individuals to marginal habitats (Walser et al., 1999). How these factors impact the ecological persistence and evolutionary trajectories of leatherside chub species remains unclear.

SUMMARY

Given the fundamental importance of taxonomy in conservation and recovery planning, it is essential that conservation boundaries be evaluated as testable hypotheses (Sites and Marshall, 2003). The ongoing species debate offers conservation practitioners a set of operational criteria derived from different species concepts that can be applied to specific taxonomic problems. An advantage of employing multiple criteria is that concor-

dance (or discordance) among species concepts can provide insight into the processes by which evolutionary divergence has occurred. In the case of leatherside chub, each species concept we evaluated was supported by our data. Moreover, our data show that each leatherside chub species is a member of the genus *Lepidomeda*. Hence, based on several testable criteria, we feel confident in designating two distinct leatherside chub species and recommend conservation and management actions that recognize this point. The rarity of the northern leatherside chub species suggests a need for immediate conservation and recovery action.

ACKNOWLEDGMENTS

We are grateful for collecting permits and cooperation from state wildlife management agencies in Idaho, Utah, and Wyoming. Special thanks to Jerry Smith at the University of Michigan and Jerry Finan and Sandra Raredon at the United States National Museum, for help in accessing preserved fish specimens from their respective museums. Jess Jordan and Eralee Jordan conducted the foraging experiment and Derek Houston helped with the growth experiment. James Rhydderch helped develop PCR protocols for *S7*; Anne Kelsen assisted with generation of sequences for *TPI*; Joe Quattro kindly provided *TPI* primers prior to their publication. The manuscript was improved by helpful comments from Louis Bernatchez, Stevie D. Jordan, Leon Perrie, Chris Simon, and Robin Waples. Our work was funded in part by a generous grant from the Maki Foundation to JBJ and MCB, by a US National Research Council Research Associateship Award to JBJ, by Brigham Young University to MCB, and by the National Science Foundation to TED.

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First submitted 29 July 2002; reviews returned 2 November 2002;

final acceptance 7 July 2004

Associate Editor: Peter Lockhart

Appendix 1. Museum identification numbers and sample sizes for fish examined in the morphometric study. Codes for museums: UMMZ = University of Michigan Museum of Zoology; NMNH = United States National Museum of Natural History (Smithsonian).

Lepidomeda copei: northern leatherside chub

- Population 1: Little Wood River, Idaho—UMMZ 130454 ($n = 1$), UMMZ 130466 ($n = 18$), UMMZ 130467 ($n = 6$), UMMZ 130468 ($n = 4$), NMNH 48041 ($n = 13$)—total ($n = 42$).
 Population 2: Goose Creek, Idaho—Collected and photographed by Utah Division of Wildlife Resources; specimens currently unavailable ($n = 3$).
 Population 3: Ross Fork, Idaho—NMNH 048056 ($n = 2$).
 Population 4: Pacific Creek, Wyoming—UMMZ 136922 ($n = 9$).
 Population 6: Sulphur Creek, Wyoming—Photos taken of live fish, none collected ($n = 6$).

Lepidomeda aliciae: southern leatherside chub

- Population 8: Provo River, Utah—NMNH 41632, NMNH 125138 ($n = 10$).
 Population 12: Sevier River, Utah—UMMZ 141443 ($n = 24$) and NMNH 174851, NMNH 63218 ($n = 13$).

Lepidomeda mollispinis mollispinis: Virgin spinedace

- Santa Clara River, Utah—UMMZ 162849 ($n = 30$).
 Santa Clara River, Utah—UMMZ 141674 ($n = 18$).

Lepidomeda altivelis: Pahranaagat spinedace

- Ash Spring, Nevada—UMMZ 125005 ($n = 7$)

Lepidomeda albivallis: White River spinedace

- White River, Nevada—UMMZ 132180 ($n = 28$)
 Preston Big Springs, Nevada—UMMZ 124977 ($n = 29$)

Lepidomeda vittata: Little Colorado spinedace

- East Clear Creek, Arizona—UMMZ 179572 ($n = 30$)
 Little Colorado River, Arizona—UMMZ 137082 ($n = 29$)

Meda fulgida: spikedace

- Taylor Creek, New Mexico—UMMZ 118179 ($n = 20$)
 Grant Gila River, New Mexico—UMMZ 124748 ($n = 20$)

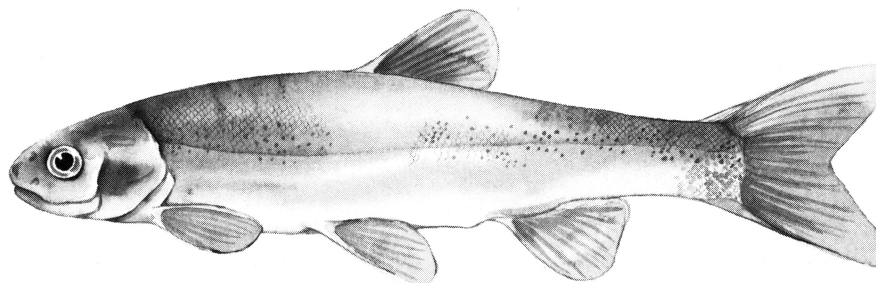


FIGURE. Drawing of a “typical” leatherside chub generated prior to the discovery that this fish is actually composed of two distinct species, a finding supported by molecular, morphological, and ecological data. Image taken from *Fishes of the Great Basin: A Natural History* by William F. Sigler and John W. Sigler. Copyright © 1987 by the University of Nevada Press. Reproduced with permission of the University of Nevada Press.