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Molecular systematics and population structure in the North American endemic fish genus *Cycleptus* (Teleostei: Catostomidae)

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by

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Advisor: Guillermo Ortí

The overarching theme of this research was to investigate hierarchical levels of relatedness in natural populations of the cycleptid fishes (blue suckers), a widespread genus in North America that is of conservation concern throughout. Phylogenetic analysis of mitochondrial DNA sequences revealed that the two described *Cycleptus* species, *C. elongatus* and *C. meridionalis* are not reciprocally monophyletic, yet do not share any haplotypes. Although lineage sorting is incomplete, Bayesian coalescent analyses indicate that the two groups diverged early in the Pleistocene and have been reproductively isolated since. Whether they should be synonymized as *C.elongatus* and recognized as subspecies is open for debate. Due to differing morphological and allozyme profiles, the author is hesitant to call for this revision. Phylogenetic analyses also revealed that cycleptids in the Rio Grande are monophyletic and clearly divergent from the *C. meridionalis*/*C. elongatus* clade. Morphology is being revisited in Rio Grande specimens and a species description is underway. A novel suite of genus-specific, diploid microsatellite markers was also developed for population genetic analyses. Due to the tetraploid nature of these fishes, a single primer pair often

coamplifies multiple paralogous loci, limiting the usefulness of such markers; thus, a new technique was developed to isolate paralogous loci from one another. Genotypic data from isolated paralogs is consistent with functional diploidy and an allotetraploid origin for the family (Catostomidae). Population genetic analyses revealed significant isolation by distance and reduced allelic richness in the upper Missouri River, which has been fragmented by six dams and reservoirs for 50-60 years. These results are in contrast with those from the comparably-sized, but unimpounded, Mississippi River. These differences are noteworthy because only 5-6 generations have passed since the dams were constructed, rendering this one of the earliest detections of genetic effects of habitat fragmentation. Additional monitoring of this system with repeated genetic surveys is strongly recommended. Finally, rangewide population genetic analyses detected nine distinct subpopulations. Four occur in drainages outside of the Mississippi Basin while five occur within the basin. Whether or not the five intrabasin groups are ecologically exchangeable is unknown. Further study of specific subpopulations is encouraged.

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Where would I be without my beautiful wife, Gianna? Gianna, you are a blessing I do not deserve. I am not sure where I would be without you, but almost assuredly, it would not be here; that is, on the brink of receiving a doctoral degree at UNL. You have kept me going through all the challenges of graduate school in a very selfless and loving manner, and have taken on virtually all the 'home responsilities.' This degree really ought to have your name on it as much as mine because you did at least as much work - it was simply behind the scenes. Now that the crazy hours of graduate school are past, I am truly looking forward to getting to know you again and spending more time being a 'daddy' to our little blessings, Anna and William.

I was born and raised a Christian (and still am) and I would be remiss if I did not thank God for all the blessings in my life, especially an upbringing by parents that were and are the best role models one could have. Thank you, Mom and Dad, for raising me in the Christian faith and modeling honesty, kindness, and perseverance in the face of challenges – especially, in facing such challenges with a calm demeanor. Your examples – and your support and encouragement - were valuable to me throughout graduate school.

Speaking of families, I am also thankful for all the support from our extended families, especially my brother, Tim, and his wife, Betsy, who also live here in Lincoln. I can't count how many times they dropped whatever they were doing to help me - or us - out. It was always a great stress relief to come over and watch games, grill out, or just relax. Thank you for your generosity. I am also thankful for my in-laws, Tom and Georgie Hoem. While Gianna was a great support for me, you were a great support for

Gianna and helped ease the burden for both of us. The next time you come to visit, I will actually be able to spend time visiting rather than going off to the lab or hiding out writing somewhere. I am looking forward to that. Of course, all of our other brothers and sisters, nephews, nieces, aunts, uncles, and grandparents have all provided meaningful support throughout. Thank you very much.

What can I say about my advisor, Guillermo Ortí? When I came to UNL, I started out as a MS student under Royce Ballinger, who really helped get me in the door. When Royce left, Guillermo 'adopted' me even though I had very little knowledge of molecular techniques. I am forever grateful for that. I remember when I finished my Master's degree and was in the lab late one evening, I told Guillermo I wanted to pursue a PhD and thanked him for giving me a chance. He seemed very surprised and said, "Well, everyone deserves a chance." That may be true, but it certainly is not the way higher academia usually works. Not everyone gets a chance, especially with the limited background that I had. I feel very fortunate for having been allowed to learn molecular techniques – and the ways of graduate school - in your lab, Guillermo. What I appreciate most is how you treat all of your students with respect, not as mindless inferiors. In particular, I really appreciate the level of trust you placed in me; that is, how you allowed me to work fairly independently, but never had any doubts (at least I don't think) that I was making progress. Of course, I also have great respect for you as a scientist. You are one of those rare people who possesses top notch skills and scientist intellect, yet has a personality that I enjoy being around. In short, I think you were the perfect advisor for me.

In addition to Guillermo, I must express my sincere gratitude to all the members of my supervisory committee, Larry Harshman, Mary Liz Jameson, Guoqing Lu, and Etsuko Moriyama. All took time out of their busy schedules to meet with me, provide feedback on my project and proposals, and continually encourage me. They helped me navigate my way through the waters of graduate school and they continue to invest in me with letters, advice, and continued support. Thank you so much!

What are some of the things I will not miss about graduate school? I will not miss spending all night in the lab, often from 10:00pm to 6:00am. Neither will I miss spending night after night writing. I will not miss filling tip boxes, running the infamous 'black box' (BaseStation sequencer), or Sunday evening lab meetings for 101. However, I WILL miss all the great friends I have come to know in the Ortí lab: Paul Bates, Sara Brant, Chad Brock (a great person to talk philosophy with), Jeremy Brozek (we will get that paper out soon), Wei-Jen Chen (who helped me struggle through my MS), Federico Hoffman (who saved me from computer chaos on multiple occasions), Jeff Huebschman (always encouraging), Agustin Jimenez, Chenhong Li (my true confidant in the lab), Jason Macrander (a very hard worker), Federico Ocampo, Annie Paradis (I made it!), Cory Ross (another great friend – thanks for listening to me rant), Craig Sitzman (yeah! we are coauthors.), Julie Sommer (*Kurtus* STILL puzzles me), Michelle Steinauer, Obdulia Segura Leon (one of the nicest people I know), Adela Roa Varon (another one of the nicest people I know – on par with Obdulia), Stuart Willis, and Matt Bolek (my buddy from the parasit world who is going to make a great professor wherever he ends up).

If I had to single one person out in the lab, though, it would be Chenhong. I was a little unsure what to think when he joined the lab because he was so quiet. I am sure that is what people often think of me, too. What a great friend you have been, Chenhong! It's rare to meet someone who comes from such a vastly different culture, yet, shares so many common interests as well as a common mindset – that is, quiet determination. You know you have a great friend when you can communicate volumes without saying a single word. Chenhong, you set a high standard in the lab and it really pushed me – not in a negative way, but in a positive way. I hope the positive impact has been mutual. You have accomplished some amazing things. I can't believe you tamed the black box before it disappeared! You also got me to eat jellyfish, and now you can outfish me, too! I am going to have to do something about that. Seriously, I have no doubt that you will achieve all the goals you set. You are a tireless worker. It has been a real pleasure working with you on all our side projects and I look forward to collaborating on others in the future, wherever each of us ends up.

And with that, I will close this long journey. It has been pretty arduous at times, but also very satisfying. As I said in the beginning, I am a very blessed man and feel fortunate to have had the experience. I wonder which door will open next.

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INTRODUCTION

Rationale

The overarching theme of this research effort was to investigate hierarchical levels of relatedness in natural populations of the genus *Cycleptus* (blue suckers), an enigmatic group of freshwater fishes native to large rivers in the United States and Mexico. These hydrodynamic fishes are benthic in habit and attain lengths up to 93 cm and weights greater than 10 kg. Breeding colors range from olive blue in *C. elongatus* to 'almost black' in *C. meridionalis* while individuals from the Rio Grande (currently recognized as *C. elongatus*) have a more golden or brassy appearance (Burr and Mayden 1999). Prominent breeding tubercles cover much of the body of spawning males and are present in reduced numbers on females. Both recognized species are known to migrate long distances to spawn. Spawning occurs in fast current over gravel beds. *Cycleptus elongatus* has an approximate 10-year generation time (Becker 1983) while *C. meridionalis* may live considerably longer (Boschung and Mayden 2004). Although the genus is known from 22 states, populations are not considered stable in any one of them.

The research provides important contributions to a range of biological sub-disciplines, including systematics, molecular techniques, and conservation biology. For the chosen study taxon, the latter is of greatest urgency given the current status of the group. In carrying out this work, I have assembled one of the most expansive tissue collections for any single freshwater fish taxon in North America – both numerically and in terms of geographic coverage. Novel genetic markers were developed and employed to estimate phylogeny, demography, and phylogeographic patterns in the group.

At the time of this writing, there are approximately 20,000-28,000 described fish species and approximately 40% of these occur in freshwater systems (Eschmeyer 1998; Moyle and Cech 2000; Nelson 1994). In the past 100 years, anthropogenic modifications to freshwater lakes, rivers, and streams has have negatively impacted many species while others have faced direct threats from overexploitation (Duncan and Lockwood 2001; Richter *et al.* 1997). Furthermore, there are very few remaining systems that have not been subject to the introduction of nonindigenous species, often with catastrophic results for native taxa (Pimentel *et al.* 2000). Exotic fishes may compete directly for resources or, if closely related, may "swamp out" native stocks via introgressive hybridization (Koppelman 1994; Weigel *et al.* 2003). As a result of these cumulative threats, an estimated 20% of freshwater fish species are at risk for extinction (Leidy and Moyle 1998). In North America, trends of freshwater fish decline are consistent with other regions as approximately ¼ of all species are considered imperiled - and at least 10 species have recently become extinct (Williams *et al.* 1989).

North America harbors approximately 1000 freshwater fish species if the Mexican transition zone is included (Mayden *et al.* 1992; Moyle and Cech 2000). These fishes are distributed among 50 families and 201 genera, but almost 70% of the diversity lies within only four families: Cyprinidae (minnows, 34%), Percidae (perches and darters, 18%), Poeciliidae (livebearers, 8%), and Catostomidae (suckerfishes, 8%). The latter family, which includes the genus *Cycleptus*, is comprised of 80 species that are endemic to North America with the exception two: *Catostomus catostomus* (longnose sucker), which also occurs (naturally) in Asia, and *Myxocyprinus asiaticus* (Chinese sucker), which is restricted to the Yangtze Basin in China.

There are a number of motivating factors for the selection of *Cycleptus* as a study taxon. To begin with, the genus occupies an interesting phylogenetic position within Catostomidae. It has been hypothesized to be sister to the Asian genus *Myxocyprinus* (Ferris and Whitt 1978; Harris and Mayden 2001; Smith 1992). This relationship, if real, reflects an interesting biogeographic pattern that is repeated in paddlefishes (*Polyodon* + *Psephurus,* family Polyodontidae) (Grande and Bemis 1991), giant salamanders (*Cryptobranchus* + *Andrias,* family Cryptobranchidae), and alligators (family Alligatoridae) (Burr and Mayden 1999).

Secondly, there is comparative merit in studying cycleptid fishes. The Mississippi Basin has provided a relatively stable environment for freshwater fish evolution since the late Cretaceous / early Cenozoic (Briggs 1986). As a result, various basal actinopterygian lineages persist in the basin, including Semionotiformes (gars), Polyodontiformes (paddlefishes – one species), and Acipenseriformes (sturgeons). While it is blatantly incorrect from a "tree thinking" standpoint (O' Hara 1988) to group cycleptids with the aforementioned, these fishes are, in fact, commonly included with the others in a group termed the "old river ichthyofauna" of North America (Smith 1981). Perhaps this is due to co-occurrence (similar range) or a common ecology. In any event, several of these lineages are of considerable economic importance (e.g., caviar trade), but most are poorly understood in terms of evolutionary history (ex. Simons *et al.* 2001). Furthermore, a firm understanding of population genetic structure in these groups is complicated by the fact that many have already been translocated (i.e. "stocked" from one location to another) prior to the collection of any genetic data (see Gardner 2004; Rutledge 1989); thus, naturally occurring allele frequencies, for example, have been

obscured. *Cycleptus* is an important exception. Although they were subject to intense harvest in the early 1900s (Coker 1930) to my knowledge, none have been translocated.

Another, personally motivating challenge in the studying cycleptid population genetics regards the makeup of their nuclear genome. All catostomids, including the genus *Cycleptus*, are tetraploid. The family is thought to have an allotetraploid origin (genome duplication via hybridization), having descended from a cyprinid-like ancestor some 50 mya (Uyeno and Smith 1972). A major complicating factor is that while analytical techniques abound for codominant, diploid loci, polyploid data remain difficult to analyze and interpret, at least in a population genetics context (Pearse and Crandall 2004).

Microsatellite loci (short, tandemly repeated sections of DNA – e.g., CACACA) are the markers of choice for population studies due to their high mutation rate via slip-strand mispairing (Goldstein and Schlötterer 1999). As with any other taxon, these markers are interspersed throughout the *Cycleptus* genome. They may be amplified with primers that match the flanking region on either side of a given locus. Unfortunately, in a polyploid situation, the priming sites may be conserved across multiple sets of chromosomes; thus, in a tetraploid, four fragments may be amplified instead of two, rendering common analytical techniques useless or, at best, difficult. One way to get around this problem is to screen a greater number of loci in the hopes that at least some priming sites are not conserved across duplicate pairs of chromosomes. I have successfully used this approach. In addition, I have developed a method for circumventing the problem by isolating paralogous (co-amplifying) loci from one another (Chapter 3 (already published) - see Bessert *et al.* 2006).

A final rationale for the study of cycleptid fishes regards the general conservation status of the genus. They are widely distributed throughout the interior of central North America and extend southward into the Rio Grande basin, a range that includes 22 conterminous states, yet, as previously mentioned, populations are not considered stable in any one of them. At best, they have a natural heritage ranking of S3 (vulnerable) and at worst, SH (possibly extirpated). Most authorities subscribe to trends of population decline resulting from impoundments (blocked spawning routes) and pollution because *C. elongatus* is thought to be highly sensitive (Becker 1983; Pflieger 1997; Robison and Buchanan 1998). However, others suggest that these apparent declines are not an immediate cause for concern and may, in part, be an artifact of inadequate sampling, such as in remote areas of the Rio Grande Basin (Burr and Mayden 1999). Such is the impetus for further investigations such as this one.

Literature Review

In the established tradition of thesis preparation, I provide a literature review in the following pages. Rather than provide an exhaustive list of any publication that has ever mentioned cycleptid fishes, I have decided to winnow it down to only include literature that is relevant to the topics at hand – or that may be of future use to researchers building upon this work. For instance, simple redundant appearances on state lists with no additional information were excluded. The review begins with the original species description and concludes with contemporary studies, including the first publication from this dissertation.

Cycleptus elongatus was originally described by Charles Alexander Lesueur as *Catostomus elongatus* (Lesueur 1817) and placed within the family Catostomidae. His

effort was based on a dried specimen from the Ohio River that has since been reported lost or discarded (Burr and Mayden 1999). The species was subsequently transferred to a new subgenus *Cycleptus* (Rafinesque 1820). Approximately 25 years later, *Cycleptus* was elevated to generic status (Agassiz 1855).

An abundance of locality and additional descriptive records appeared throughout the mid-1800s to early 1900s, including: presence in Indiana (Jordan 1875); listing for Illinois (Nelson 1876); clarification of name (Jordan 1877); listing in North American catalog (Jordan 1878); presence in Cumberland River, Tennessee (Jordan and Brayton 1878); appearance in the Mississippi Valley (Jordan and Gilbert 1883); presence in the Baraboo River, Wisconsin (Hoy 1883); presence in the Kansas River, Kansas (Cragin 1885; Graham 1885); presence in Whitewater River, Indiana (Evermann 1886); presence in Ohio and Wabash Rivers (Jordan and Evermann 1886); uncommon in Ohio River (Henshall 1888); uncommon in Iowa (Meek 1890); presence in Cumberland River, Kentucky (Woolman 1892); presence at Falls of the Ohio River (Call 1896); uncommon in Missouri River (Evermann and Cox 1896); general description and Illinois distribution (Forbes and Richardson 1908); presence in Río Solado, Nuevo Léon, Mexico (Fowler 1913; Meek 1908); presence in Kiskeminetas River, Pennsylvania (Fowler 1913); description and summary of range (Jordan 1929); spring and fall migration patterns in the upper Mississippi River (Coker 1930); Illinois distribution reviewed (Donnell 1935).

From 1935-1945, coincidental with World War II, no studies were published on blue suckers. After that time, locality records continued but were intermixed with comments on population trends, anatomical studies, etc.: distribution in Minnesota (Eddy and Surber 1947); description of the Weberian ossicles in *Cycleptus* (Nelson 1948);

presence in Red River drainage, Oklahoma (Moore and Cross 1950); decline in main stem of upper Mississippi River (Barnickol and Starrett 1951); presence in Big Sioux River, Iowa (Harrison and Speaker 1954); presence in Big Muddy River, Illinois (Lewis 1955); comments on biology and range in Iowa (Harlan and Speaker 1956); presence in Texas Gulf Slope drainages (Hubbs 1957); presence in New Mexico (Koster 1957); distribution in Ohio (Trautman 1957); distribution in Mississippi (Cook 1959); presence in Neosho River, Kansas (Metcalf 1959); presence in lower Vermillion River and Missouri River, South Dakota (Underhill 1959); presence in the Big Blue River, Kansas (Minckley 1959); Cycleptus bones found at Bandelier National Monument, New Mexico – several hundred kilometers beyond their extant distribution in the state (Gehlbach and Miller 1961); studies of osteology and tuberculation (Branson 1962a; Branson 1962b); presence in the lower Missouri River (Fisher 1962); brain and lip morphology (Miller and Evans 1965); comments on spawning dates (Breder and Rosen 1966); update of Kansas distribution (Cross 1967); age and growth (Carlander 1969); Montana distribution (Brown 1971); occurrence in Lake Sharpe (Missouri River impoundment), South Dakota (Elrod and Hassler 1971); distribution in Missouri (Pflieger 1971); status in upper Mississippi River – uncommon (Smith *et al.* 1971); large numbers in tailwaters of Gavins Point dam, South Dakota (Walburg *et al.* 1971); threatened in six states (Miller 1972); occurrence in Río Bravo (Rio Grande) (Contreras-Balderas and Rivera 1972); karyotype – polyploidy (Uyeno and Smith 1972); distribution in Arkansas (Buchanan 1973); description of gill parasites (Leiby *et al.* 1973); Oklahoma distribution (Miller and Robison 1973); common in lower reaches of Chippewa and Red Cedar Rivers, Wisconsin (Christenson 1974); accounts from the Red and Sabine Rivers in Louisiana (Douglas

1974); updated Minnesota distribution (Eddy and Underhill 1974); presence in Kiamichi River, Oklahoma (Pigg and Hill 1974); considered rare in Kansas and Oklahoma (Platt *et al.* 1974; Robison *et al.* 1974); Kentucky distribution (Clay 1975); distribution and status update in Kansas (Cross and Collins 1975); description of biology and updated Missouri distribution (Pflieger 1975); threatened in Oklahoma (Hubbs and Pigg 1976); evolution of pectoral fins (Lundberg and Marsh 1976); loss of duplicate gene expression – return to functional diploidy, based on allozymes (Ferris and Whitt 1977); pharyngeal bone structure (Eastman 1977); qualities of bile salts as phylogenetic markers (Bussjaeger and Briggs 1978); catostomid phylogeny based on loss of duplicate gene expression - allozymes (Ferris and Whitt 1978); occurrence in the Tennessee River in Alabama (Etnier and Starnes 1979); threatened status in Indiana (McReynolds *et al.* 1979); Illinois range update (Smith 1979); structure of caudal skeleton (Eastman 1980); genetic variability compared to diploid teleosts – based on allozymes (Ferris and Whitt 1980); biology in upper Mississippi River (Rupprecht and Jahn 1980); threatened in Tennessee and Kentucky (Branson *et al.* 1981; Starnes and Etnier 1980); Holocene fossil record (Smith 1981); updated Ohio distribution (Trautman 1981); status in the lower Mississippi River (Guillory 1982); updated Minnesota distribution (Phillips *et al.* 1982); description of biology and Wisconsin distribution (Becker 1983); polyploidy confirmed with isozymes (Buth 1983); extirpated from Pennsylvania (Cooper 1983); presence in the Poteau River, Oklahoma (Lindsay *et al.* 1983); natural history (incl. spawning) from the Neosho River, Kansas (Moss *et al.* 1983); common along revetments in the lower Mississippi main stem (Pennington *et al.* 1983); present in the Green River, Kentucky (Retzer *et al.* 1983); distribution and abundance in Wisconsin (Fago 1984); tetraploidy (Ferris 1984);

phylogenetic position based on larval characters (Fuiman 1985); decline in Rio Grande (Williams *et al.* 1985); range reduced in Ohio drainage, member of 'old river' fauna (Burr and Page 1986); distribution in Gulf Slope drainages (Conner and Suttkus 1986); presence in Missouri River drainage (Cross *et al.* 1986); presence in the Rio Grande, Conchos, and Pecos rivers (Smith and Miller 1986); presence in Tennessee and Cumberland rivers (Starnes and Etnier 1986); found in proximity to wing dikes and revetments in middle Missouri River (Sandheinrich and Atchison 1986); member of 'old river' faunal group (Robison 1986); *Cycleptus* images on ancient pottery in New Mexico, far from extant range (Jett and Moyle 1986); collection from the Spring River, Arkansas (Baker and Armstrong 1987); diet in the Black River, New Mexico and distribution in New Mexico (Cowley and Sublette 1987a; Cowley and Sublette 1987b); updated Iowa distribution (Harlan and Speaker 1987); protected in the U.S. (Johnson 1987); doubtful from the Rio Grande in New Mexico (Propst *et al.* 1987); reproduction in upper Mississippi River (McInerny and Held 1988); description of biology and Arkansas distribution (Robison and Buchanan 1998); stranding and mortality below McAlpine dam, Ohio River (Pearson and Froedge 1989); species of special concern throughout range (Williams *et al.* 1989); range, status, and color image (Tomelleri and Eberle 1990); description and distribution in New Mexico (Sublette *et al.* 1990); not secure in Tennessee (Etnier and Starnes 1991); description, range map and habitat (Page and Burr 1991); distribution in Mississippi (Ross and Brenneman 1991); Wisconsin distribution (Fago 1992); phylogenetic position within Catostomidae, sister taxon to Myxocyprinus (Smith 1992); presence in James River, not secure in Dakotas (Berry *et al.* 1993); extirpated from Big Muddy River in Illinois (Burr and Page 1993); description, status in

Tennessee (Etnier and Starnes 1993); status in St. Croix River system, Wisconsin (Fago and Hatch 1993); status in the Wabash River, Indiana (Gammon 1993); status in Big Black and Yazoo Rivers, Mississippi (Holman *et al.* 1993; Jackson *et al.* 1993); present in the Arkansas River (Limbird 1993); rare in Kaskaskia River, Illinois (Larimore and Fritz 1993); present in western tributaries of the Missouri River, South Dakota (Ruelle *et al.* 1993); rare in the Kansas River system, Kansas (Sanders *et al.* 1993); presence in the Vermillion River, South Dakota (Schmulbach and Braaten 1993); healthy populations in the Yellowstone River, Montana (White and Bramblett 1993); present in the Lamine River, Missouri – spawning (Brown and Coon 1994); possible record in Virginia (Jenkins and Burkhead 1994); description of eggs and larvae (Kay *et al.* 1994); monotypic subfamily Cycleptinae (Nelson 1994); threatened status in Mexico (Contreras-Balderas *et al.* 1995); status update in Kansas (Cross and Collins 1995); distribution in West Virginia (Stauffer *et al.* 1995); new records in Illinois (Burr *et al.* 1996); Alabama distribution and biology (Mettee *et al.* 1996); update of Missouri distribution and comments on biology (Pflieger 1997); status below Miller's Ferry lock and dam, Alabama(Mettee and Shepard 1997); allozyme variation (Buth and Mayden 1998); stock dynamics in Yazoo River tributaries, Mississippi (Hand 1999); description and threatened status in New Mexico (Propst 1999); historic occurrence in Little Miami River, Ohio (Harrington 1999); formal description of *Cycleptus meridionalis*, summary opinion of conservation status – global populations not in imminent danger (Burr and Mayden 1999); negative impact of dams (Pringle *et al.* 2000); possible benefit from modifications to seasonal flow regime (Galat and Lipkin 2000); reproduction in backwaters of upper Mississippi River (Fisher and Willis 2000); life history characteristics in unchannelized Missouri River below Gavins

Point dam (Carreiro 2000); considered potential invader to Great Lakes (Cudmore-Vokey and Crossman 2000); gill parasites (Marcogliese 2001); recent accounts in Minnesota (Schmidt and Talmage 2001); allozymes in Rio Grande population distinct (Buth and Mayden 2001); use of main channel habitat in upper Mississippi River (Dettmers *et al.* 2001); Cycleptinae is either para- or polyphyletic (Harris and Mayden 2001); preference for fast current (Pegg and Pierce 2002); imperiled, but still occurs in upper Tennessee River (Butler 2002); swimming performance may give advantage in channelized waterways (Wolter and Arlinghaus 2003); stock characteristics in upper Yazoo basin, Mississippi (Hand and Jackson 2003a); spawning pattern in Missouri (Vokoun *et al.* 2003); abundance and biology in James and Big Sioux Rivers, North and South Dakota (Morey and Berry 2003); comparative abundance in two segments of upper Missouri River (Welker and Scarnecchia 2003); larval cycleptids in unimpounded upper Missouri River (Barko *et al.* 2004); larval densities in lower Missouri River (Galat *et al.* 2004); appearance in lower Missouri trawling efforts (Herzog 2004); status in Kansas (Haslouer *et al.* 2005); higher than expectected abundance in upper Kansas River, slow growth rates (Eitzmann *et al.* 2005); historic occurrence in middle and upper Rio Grande, New Mexico (Cowley 2006); included in general biogeographic study of Great Plains rivers (Hoagstrom and Berry 2006); larval blue sucker ecology in Mississippi River (Adams *et al.* 2006); development of molecular markers for population genetic studies (Bessert *et al.* 2006).

Ongoing Studiesin the Genus

It may be apparent to the reader that most of these records are due to coincidental encounters with cycleptid fishes during other endeavors. The papers that appear in the

following chapters are four of only a handful of studies that have focused exclusively on cycleptid biology. In addition to my efforts, there are two other ongoing investigations in the genus that are worthy of note. In Texas, BioWest consulting company, under the direction of Dr. Ed Oborny, is investigating various life history aspects of *C. elongatus* in the Colorado River. Those studies are intended to assess potential impacts of modifications to the lower Colorado River (flow diversion to provide water to large cities) on *C. elongatus* populations. Absolute range within the Colorado, migration patterns, and spawning areas are being defined via radio telemetry. I have assisted this group in one respect, using my molecular markers to confirm the identity of embryos from at least one purported spawning site. A second group, under the leadership of Dr. Trent Sutton at Purdue University, is investigating the age and growth characteristics of blue suckers in the Wabash River of Indiana. The Wabash River population is relatively healthy and is said to provide an important food base for larger predators. This is in contrast to most drainages throughout the global range, so the outcome of Trent's work will hold comparative interest for numerous state agencies.

Overview

The remainder of this dissertation is divided into four chapters, each with a particular focus and written to be published as a separate journal article. Chapter one examines intrageneric phylogeny using mitochondrial markers. It illuminates the divergence of the Rio Grande population from the two described species and provides an approximate time of divergence between the two major clades. The results of this work, along with previous morphological and allozyme studies (Burr and Mayden 1999; Buth and Mayden 1998; Buth and Mayden 2001), have provided the impetus to delineate

cycleptids from the Rio Grande as a new species. I am presently collaborating with Drs. Brooks Burr of Southern Illinois University (Carbondale) and Richard Mayden of St. Louis University on this work. Having personally visited a good portion of this species' range (including northern Mexico) and seen the effects of exotic salt cedar, golden algae, and drought, I may lobby to name it *Cycleptus speratus*. The Latin word *spero* means "to hope" or "to hope for," and the fishes and the people of this region are both in desparate need of some hope.

Chapter two characterizes the isolation and screening of a unique suite of microsatellite markers that I developed for population genetic studies in the genus. It also describes a new technique for isolating paralogous loci from one another. This can be a significant benefit to anyone attempting to use such markers in polyploid organisms. This work has already been published online (Bessert *et al.* 2006) and will soon appear in *Conservation Genetics*.

Chapter three details various measures of population structure in *C. elongatus* populations in the upper Missouri River. This drainage is unique relative to the remainder of the species range in that it has been subdivided by a series of six major dams and impoundments which have presumably isolated inter-reservoir populations for the last 50-60 years. Most of the population structural analyses show no startling aberrations with the exception of two: 1) The Missouri River populations show a pronounced signal of isolation by distance. This is in contrast to other continuous portions of the range (namely, the unimpounded Mississippi River); 2) Inter-reservoir samples from the upper Missouri show a significant reduction in allelic richness compared to samples from the lower Missouri and from throughout the range. These two

results may, indeed, be the first detectable deviations from normal / natural genetic patterns.

Finally, chapter four examines rangewide population structure using some 600 samples and 15 microsatellite markers. A Bayesian analysis of population structure shows nine distinct genetic clusters ('populations'). In addition to recognizing populations from disjunct drainages (Mobile Basin, Rio Grande Basin, Colorado River, and Sabine River) as distinct, there is clear structure within the Mississippi Basin as five distinct clusters are recognized there (i.e. a total of nine distinct genetic clusters from throughout the range). A second aspect of this chapter includes an investigation of the timing of divergence between *C. elongatus* and *C. meridionalis*, a pair of polyphyletic taxa in which lineage sorting is incomplete. A powerful coalescent approach was used to determine that these species diverged in early Pleistocene time. Associated results also reject the hypothesis of any recent gene flow, lending further support to the species designation of *C. meridionalis*.

CHAPTER ONE

Molecular Systematics of the Freshwater Fish Genus *Cycleptus* (Teleostei: Catostomidae) as inferred from Mitochondrial DNA

Abstract

Fishes in the genus *Cycleptus* occur in large rivers throughout central North America. The genus was considered monotypic as *Cycleptus elongatus* for over 175 years until a sister species, *C. meridionalis*, was described from southeastern Gulf Coastal drainages based on morphological differences. Subsequent allozyme data revealed evidence for remaining polytypy within *C. elongatus*, but intrageneric relationships have never been assessed with direct genetic evidence. Complete mitochondrial control region sequences (920 base pairs) were collected from 151 specimens and cytochrome *b* sequences were collected from a subset of 48 specimens representing the known range of the genus. Results indicate polyphyly between the two described species while the Rio Grande population, currently recognized as *C. elongatus*, is monophyletic and clearly divergent from the rest. These results, in combination with previous data sets, support designation of the Rio Grande group as a distinct species.

Introduction

The blue sucker (*Cycleptus elongatus*) was originally described by Charles Alexander Lesueur (1817) as *Catostomus elongatus* from a dried museum specimen collected from the Ohio River. Three years subsequent, the species was placed within the subgenus *Cycleptus* (Rafinesque 1820) and twenty-five years later, *Cycleptus* was elevated to the level of genus (Agassiz 1855). For over 140 years the genus was considered monotypic as *Cycleptus elongatus* until Burr and Mayden (1999) described a sister species, *Cycleptus meridionalis*, from the Pearl and Mobile river basins (Mississippi and Alabama) based on an array of bimodally distributed morphological characters. Rio Grande specimens were divergent for some characters, most notably lip morphology and coloration, but not to the mutual exclusion of all other *Cycleptus* populations. A subsequent allozyme study (Buth and Mayden 2001) revealed further evidence for polytypy within *C. elongatus* because Rio Grande populations displayed unique profiles relative to those from the Mississippi Basin. Still, there have been no direct assessments of phylogeny with genetic (DNA sequence) data to this point.

Why do these fishes remain such an enigma? To be sure, cycleptid fishes have been the direct focus of only a handful of studies compared to most of their large riverine counterparts (e.g., sturgeons, paddlefishes). Although they occur over such a wide range, they are benthic in habit and their hydrodynamic morphology is associated with a preference for rapid current in main stem channels; thus, requiring heavy gear to collect them in reasonable numbers. In addition, unlike sturgeons and paddlefishes, which play a

major role in the caviar trade, cycleptids hold no contemporary economic value (although they were commercially harvested in the upper Mississippi during the early 1900's).

Why, then, should we seek a better understanding of cycleptid relationships? Aside from the basic value in fleshing out the tree of life, the study of cycleptid evolution may hold comparative merit for conservation of other, more heavily targeted taxa. They have likely been present since the early Cenozoic (Smith 1981); yet, have not radiated to the degree that other lineages have.

Given the extant distribution of these fishes, multiple hypotheses of unrecognized biodiversity (and relationships among) become apparent. The Mississippi basin is the most stable system inhabited by these fishes; thus, it is likely that cycleptids originated here and dispersed to other areas. The Rio Grande basin is the most disjunct point from the Mississippi basin; therefore, it is logical to hypothesize that each drainage between harbors a distinct, monophyletic clade; i.e. the Sabine (closest to the Mississippi) forms a basal branch from which the Colorado / Rio Grande clades diverged, etc. In this scenario, we would expect the Rio Grande population to be the most divergent from *C. elongatus* inhabiting the Mississippi basin. Conversely, the nature of this distribution is poorly known, especially given the difficulties in reconstructing drainage evolution along the Gulf slope (Conner and Suttkus 1986; Conner 1977). Another possibility is that cycleptids dispersed more recently along coastal margins during periods of reduced salinity resulting from Pleistocene glacial melt. In a recent study, Burr and Mayden (Burr and Mayden 1999) distinguished *C. meridionalis* from *C. elongatus* based on an array of discrete morphological differences, yet failed to do so with the Rio Grande, Colorado, or Sabine populations; therefore, if mtDNA divergence is concordant with morphological

divergence, we may expect the two described species to form reciprocally monophyletic clades with western Gulf slope populations nested within *C. elongatus*. Here, I approach these questions with the first molecular systematic study of the genus *Cycleptus* using mitochondrial markers.

Materials and methods

Samples

Tissue samples were collected from 151 specimens throughout the known range of the genus (see Materials Examined; Appendix 2). All are vouchered in the personal collection of the author and freely available upon written request. Acquisition of samples was challenging because it requires large river gear such as benthic gill nets and electroshocking devices; thus, assistance was solicited from a combination of more than 100 individuals representing 22 state agencies, two federal agencies, and 15 academic institutions. All samples were acquired in an approximate one year time span from November 2004 to November 2005. A small fin clip, approximately 1-2 cm^2, was excised from the anal fin of each specimen and fixed in 95% ethanol (EtOH) prior to shipment to the University of Nebraska.

DNA extraction

Genomic DNA was isolated from fin clips employing a phenol-chloroform extraction protocol (Sambrook *et al.* 1989) or the DNeasy® Tissue purification kit (Qiagen). Extractions were quantified with a GeneQuant II spectrophotometer (Pharmacia Biotech) and diluted to a standard concentration of 100ng/µl to facilitate consistency in PCR amplification.

Data collection

Primers Cyt_*b*-F (5'-ATGGCAAGCCTACGAAAAA-3) and Cyt_*b*-R (5'-GGCTCATTCTAGTGCCTTGTT-3') were designed from an alignment of other catostomid sequences acquired from GenBank (Table 1.1) and used to amplify 1020 base pairs of the cytochrome *b* gene (residues 16-356 of 380 total). A 25 µl cocktail was prepared for each as follows: 5.0µl 1mM dNTPs, 2.5µl 10X PCR buffer, 1.0µl 50mM $MgCl_2$, 1.0 µl 10mM Cyt_*b*-F primer, 1.0 µl 10mM Cyt_*b*-R primer, 0.3µl (0.5 units) Taq DNA polymerase (Invitrogen), 11.7 µl sterile ddH_2O, and 2.5µl (250 ng) DNA. Reactions were incubated for 31 cycles of 94º (60 sec), 54º (45 sec), 72 º (2 min) followed by a final 10 minute extension at 72 º. Residual primers were removed from PCR products via incubation with shrimp alkaline phosphatase (SAP) and exonuclease I at 37º C for 20 minutes. Cycle sequencing reactions were carried out according to a modified BigDye Terminator (Applied Biosystems) protocol. Each reaction contained the following: 1.6µl primer (1mM), 1.2µl BigDye RR mix, 1.0-2.0 µl PCR products. Cycle sequencing reaction conditions were as follows: 96°C (5 min), 99 cycles of 96°C (45 sec), 62°C (1 min), 72°C (2min) followed by a final extension at 72°C (2 min). Products were visualized with a MJ Research BaseStation 51 Automated DNA Fragment Analyzer. Internal primers were also designed and used in some cases to ensure quality signal strength throughout the fragment.

Primers FTTF (5' GCCTA AGAGCATCGGTCTTGTAA 3') and F12R (5' GTCAGGA CCATGCCTTTGTG 3') were used to amplify the mitochondrial control region (922 bp). A 25 µl cocktail was prepared similar to that described above for

cytochrome *b*. Reactions were incubated with a MJ Research thermal cycling regime: 10 cycles at 94º (60 sec), 55º (60 sec), and 72 º (60 sec) followed 21 cycles at 94º (60 sec), 53º (60 sec), 72 º (60 sec) and a final extension at 72 º (5 min). Products were prepared and sequenced according to the conditions highlighted above for cytochrome *b* or were shipped to the high throughput sequencing facility at the University of Washington (**http://www.genome.washington.edu/UWGC/**).

Analyses

Raw sequences were edited with Sequencher 4.2 (Gene Codes) or Contig Express (Invitrogen). Multiple alignments were performed with ClustalX (Thompson *et al.* 1997) and checked by eye for obvious misalignments. Since it is a protein-coding gene, cytochrome *b* sequences were translated and the polypeptide checked for erroneous stop codons. SplitsTree 4.0 (Huson and Bryant 2006) was used to identify identical haplotypes and collapse both data sets to exclude them. Hierarchical likelihood ratio tests (hLRTs) were performed with Modeltest 3.7 (Posada and Crandall 1998) and used to determine which of 56 models of molecular evolution best explained each data set.

Phylogenetic analysis of the cytochrome *b* data set was performed with PAUP 4.0 using both maximum likelihood (ML) and maximum parsimony (MP) criteria using *Myxocyprinus* and *Carpiodes* as outgroup sequences (accession numbers AB126083 and AB223007 in Table 1.1). For both analyses, the heuristic search option (100 random addition replications using tree bisection-reconstruction) was used to search for optimal trees. Bootstrap support was estimated with 100 pseudo-replicates for ML and 1000 pseudoreplicates for MP.

Phylogenetic analysis of the control region data set was conducted using a MP approach with PAUP 4.0. In addition, the best-fit model of sequence substitution (determined with Modeltest 3.07) was used in a Bayesian analysis conducted with MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). This software uses metropolis-coupled Markov chain Monte Carlo (MCMC) to explore tree space, seeking topologies with the highest likelihood. In using multiple heated chains with frequent swapping, tree space is explored much more quickly than with other approaches. At this time, it is the most computationally feasible approach for large data sets – at least if one wishes to implement a particular model of molecular evolution to correct for multiple hits. Two replicates were run with three heated chains and one cold chain with sampling every 100th generation. The progress of runs was checked periodically with the program AWTY ("Are We There, Yet?") (Wilgenbusch *et al.* 2004), a web tool to graphically assess convergence of the MCMC algorithm by plotting posterior probabilities of a given set of 20 splits during the course of a run (Figure 1.4). Once convergence occurs, these probabilities will remain fairly constant over the duration of a run (i.e. they 'flatten out').

In an attempt to gain greater resolution at shallow nodes, a final analysis was performed with concatenated sequences for 48 individuals in whom both regions were amplified. This analysis was also performed with MrBayes3.1.1. Data partitioning was applied to account for differences in molecular evolution between the two markers (using the APLYTO command). Again, two runs were carried out with multiple heated chains to efficiently explore tree space and convergence was monitored with AWTY.

Results

Molecular Evolution

Collapsing identical sequences reduced the control region set to 79 unique haplotypes from 151 individuals and for cytochrome *b*, a reduction to 20 unique haplotypes from 48 individuals. Sequence data may be downloaded directly from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/inde.g.,html) at the following accession numbers: cytochrome *b*: EF062360-EF062379; Control Region: EF062380-EF062458. Using the hierarchical likelihood ratio test criterion implemented in Modeltest, the TrN + I + G model (Kumar *et al.* 2004) was chosen for the control region data set. This model is a case of the GTR + I + G model where the parameters controlling the rates of the different types of transitions are equal. The resulting rate matrix parameter estimates were as follows: R(a) [A-C]=1.0000, R(b) [A-G]=30.9075, R(c) [A-T]=1.0000, R(d) [C-G]=1.0000, R(e) [C-T]=15.1074, R(f) [G-T]=1.0000; the proportion of invariable sites (I) was 0.8378; base frequencies were A=0.3239, C=0.2091, G=0.1578, and T=0.3092; gamma distribution shape parameter = 0.5416. The TrN+G model was chosen for cytochrome *b*. Rate matrix parameter estimates were R[A-C]=1.0000, R[A-G]=73.2022, R[A-T]=1.0000, R[C-G]=1.0000, R[C-T]=24.8720, and R[G-T]=1.0000; base frequencies were A=0.2605, C=0.2956, G=0.1716, and T=0.2724; gamma distribution shape parameter = 0.0153.

The cytochrome *b* gene was highly conserved in the 48 individuals examined. Among the 20 unique haplotypes, there was only a single non-synonymous substitution (Figure 1.1) that occurred in three individuals from the Rio Grande basin. Still, there were 38 variable sites among ingroup haplotypes. Phylogenetic analysis with ML

(Figure 1.2) and MP (not shown) criteria yielded consistent results. The inferred topology was interesting in two respects: 1) the hypothesis of reciprocal monophyly between the two described species (*C. elongatus* and *C. meridionalis*) was not supported; 2) instead, the two described species were polyphyletic (incomplete lineage sorting) and formed a sister clade to the monophyletic Rio Grande group (Figure 1.2). Mean pairwise sequence divergence within *C. meridionalis* (0.3%) and *C. elongatus* (0.4%) was virtually no different than the mean divergence between them (0.4%); however, each was markedly divergent from the Rio Grande group (2.0% and 2.1%, respectively, see Table 1.2).

The mitochondrial control region exhibited greater variability than cytochrome *b*, as mean pairwise differences within *C. meridionalis*, *C. elongatus*, and the Rio Grande clade reached 0.8%. Divergence between *C. meridionalis* and *C. elongatus* was slightly higher at 1.1%, while each was 2.5% and 2.7% divergent from the Rio Grande clade, respectively (Table 1.2). MP analysis of this data set resulted in 36 most parsimonious trees with a length of 157 steps (Figure 1.3). Inspection of split posteriors during Bayesian analysis of the control region data set indicated convergence at approximately 1.5×10^6 generations (see Figure 1.4), so the analysis was continued for another 4.5×10^6 generations. Posterior probabilities of nodes were estimated from all sampled generations after removal of the initial (1.5×10^6) burn-in and results were summarized with a 50% majority rule consensus tree (roughly equivalent to bootstrap support) (Figure 1.5). As with the cyt *b* analysis, MP topologies differed only slightly from Bayesian results at shallow nodes, while the overall patterns of *C. meridionalis* / *elongatus* polyphyly and divergence of the Rio Grande clade was retained.

Finally, the concatenated data set consisted of 48 individuals that, when collapsed, yielded 43 unique haplotypes. Bayesian analysis of the partitioned data set reached convergence at approximately 1.0 x 10^6 generations (as indicated with AWTY) and data collection was continued for another 3.0 x 10^6 generations. The analysis did result in slightly improved resolution at shallow nodes as two strongly supported *C. meridionalis* clades were recovered within *C. elongatus* (Figure 1.6).

Discussion

A fundamental goal of molecular systematics is to place biodiversity in a logical evolutionary context and to enable a better understanding of the underlying principles and processes. The same is true of morphogical systematics. Unfortunately, neither approach is without caveats. To be sure, morphological studies are extremely important in tracing the evolution of any number of specific physical traits - and are essential to the field of taxonomy. At the same time, morphology may not reveal the presence of lineages that diverged millions of years ago, especially in systems that are characterized by long-term environmental stability (Colborn *et al.* 2001). At the other end of the spectrum, molecular studies may also fail to reveal all relevant biodiversity (Simons *et al.* 2001). While it is true that any heritable divergence in morphology must be accompanied by an underlying change in the genetic code, identifying those changes within an entire genome can be a daunting task; thus, neutral markers (e.g., mtDNA control region, introns, and microsatellites), which may accumulate mutations in the absence of morphological divergence, have become a workhorse in studies of closely related taxa. In particular, mtDNA has become the common marker of choice because its haploid nature and lack of recombination facilitate rapid lineage sorting (Avise 2004).

Here, sequences from two mitochondrial regions were used to investigate phylogenetic structure in the genus *Cycleptus*. There are two known species in the genus, *C. elongatus* and *C. meridionalis*. They are geographically disjunct and they exhibit a mutally exclusive, bimodal distribution in a wide array of meristic and morphometric characters. Nevertheless, they fail to pass the test of reciprocal monophyly, even at the mtDNA control region. Should the taxonomy be revised to synonymize *C. meridionalis* under *C. elongatus*? If a strict phylogenetic species concept is applied (e.g., Mishler and Theriot 2000), the answer is "yes," but in the author's opinion, no. Indeed, Funk and Omland (2003) recently demonstrated in a survey of more than 2319 animal species assayed with mitochondrial markers that species-level paraphyly or polyphyly occurred in 23% of the cases. If not reciprocal monophyly, then what criterion should be used to delineate a new species? While it is not my intent to burden the reader with a long diatribe on species concepts (for a relevant discussion, see Wheeler and Meier 2000), I will say that no single species concept has yet placed an appropriate frame around each and every taxon (and only those taxa) we recognize as a 'species.' The inherent problem is that we attempt to put a box around a process that is a dynamic continuum. For instance, if we were to view the tree of life with a sliding window, that window could never be small enough to exclusively – and discretely - capture only those groups we recognize as species at any given point in time. While the biological species concept (Mayr 1942) is palatable to many who study sexually-reproducing organisms (and to non-biologists), it too, is difficult to test, as in this case.

The degree of morphological divergence between *C. elongatus* and *C. meridionalis* (Figure 1.7) (Burr and Mayden 1999) certainly suggests that they are on

independent evolutionary trajectories (Wiley 1978). So, too, does recent evidence for long-term genetic isolation between the two taxa (Chapter 4, p. 103-104).

Given the degree of genetic divergence and reciprocal monophyly between the Rio Grande and the *C. elongatus/meridionalis* clade, it is also clear that the Rio Grande clade has been genetically isolated for some time. In a recent study, Peng *et al.* (2006) analyzed mitogenomic data using a Bayesian relaxed clock approach (allowing for some rate variation among lineages) to estimate divergence times within Otocephali, a group that includes clupeomorphs (herrings, anchovies, etc.) and ostariophysans (minnows and suckers, characids, and catfishes). Their estimates were calibrated with five fossil records at various points in the tree. Five catostomid taxa were included in the data set. Among them were *Myxocyprinus asiaticus* and *Carpiodes carpio*, both of which have been hypothesized as sister to *Cycleptus* (Harris and Mayden 2001; Smith 1992). Peng *et al.* estimated a divergence time of 101 mya between the two taxa. This correlates to a divergence rate of approximate 0.17% / mya for cytochrome *b*. If we assume that a similar rate occurs in cycleptid fishes and apply it to the net sequence divergence (Nei 1987 p. 276) among the Rio Grande and *C. elongatus / meridionalis* clades (≈ 1.7%; also see Table 1.2), it translates to a mid-Miocene divergence at approximately 10 mya. This is not unreasonable given that one of the two aforementioned genera almost certainly harbors the closest extant relatives to *Cycleptus*. Of course, the most appropriate course would be to combine *Cycleptus* data with that from Peng *et al.* and rerun their analyses, but that is beyond the scope of this study.

Although cycleptid fishes inhabit a vast range and have been formally known for almost two centuries, limited attention has been placed on their systematics. Accurate

knowledge of these relationships advances our understanding of the evolution of these fishes and may shed light on unresolved patterns in other 'old river' ichthyofauna (Simons *et al.* 2001; Smith 1981). The phylogenetic patterns revealed here corroborate and advance previous findings of Burr and Mayden (1999) and Buth and Mayden (2001). The former recognized character differences in Rio Grande specimens (e.g., color patterns and lip papillae), but used caution in interpreting these as diagnosable traits since they are subject to environmental plasticity. Based on the previous recognition of *C. elongatus* and *C. meridionalis* as valid species and the phylogenetic pattern revealed here, renewed efforts are underway to study the morphology of these creatures, especially (lip) papillae structure and tuberculation patterns (Figure 1.8). A formal recognition of the Rio Grande clade as a distinct species is in progress. This will accurately reflect evolutionary history and bolster efforts to conserve these fishes.

Materials Examined

The list of specimens included in each analysis follows. Specimen numbers follow state (and river drainage) collected from; '>' indicates a continuous series of specimens. More detailed locality and collection information appear in Appendix 2.

Micochondrial control region

C. meridionalis. Alabama, (Alabama) MLB1-1>MLB1-30; Louisiana, (Pearl) MLB51-7; Mississippi, (Leaf) MLB4-1>MLB4-5.

C. elongatus. Arkansas, (Red) MLB2-1>MLB2-3, (White) MLB8-21, MLB8-28; Indiana, (Wabash) MLB9-7, MLB9-22, MLB9-27; Iowa, (Mississippi) MLB10-1; Kansas, (Kansas) MLB34-3; Kentucky, (Ohio) MLB24-1>MLB24-3; Louisiana,

(Mississippi) MLB40-1, MLB40-2, (Red) MLB51-5, MLB51-6, (Sabine) MLB58-1>MLB58-7; Minnesota, (Minnesota) MLB12-9, MLB12-10, (Mississippi) MLB13-2>MLB13-4; Mississippi, (Black) MLB59-1, MLB59-2; Missouri, (Missouri) MLB17-7; Montana, (Missouri) MLB18B-1>MLB18B-4, (Yellowstone) MLB20-23; Nebraska, (Missouri) MLB50-1>MLB50-4, MLB50-7>MLB50-9; North Dakota, (Missouri) MLB23-1, MLB23-2; Oklahoma, (Red) MLB33-31; South Dakota, (Missouri) MLB25-1, MLB25-2; Tennessee, (Cumberland) MLB28-2, (Duck) MLB51-2, (French Broad) MLB30-9, (Nolichucky) MLB30-1>MLB30-4, (Tennessee) MLB28-1; Texas, (Colorado) MLB45-1>MLB45-12, MLB45-14, MLB45-16, MLB45-17, MLB45-19, MLB45-20, MLB45-23>MLB45-25, MLB45-27>MLB45-30, (Sabine) MLB51-3, MLB51-4; Wisconsin, (Chippewa) MLB39-1, (Red Cedar) MLB39-29, (Wisconsin) MLB33-30, MLB51-1.

C. elongatus (Rio Grande clade). Mexico, (Conchos) MLB57-1; New Mexico, (Pecos) MLB22-1, MLB22-31>MLB22-34; Texas, (Rio Grande) MLB36-1>MLB36-20.

Cytochrome b

C. meridionalis. Alabama, (Alabama) MLB1-1, MLB1-2, MLB1-7, MLB1-9, MLB1-12, MLB1-19, MLB1-21; Mississippi, (Leaf) MLB4-3.

C. elongatus. Arkansas, (Red) MLB2-3; Indiana, (Wabash) MLB9-7; Kansas, (Kansas) MLB34-3; Kentucky, (Ohio) MLB24-2; Louisiana, (Mississippi) MLB40-1; Minnesota, (Minnesota) MLB12-9; Montana, (Missouri) MLB18B-4; North Dakota, (Missouri) MLB23-2; Tennessee, (Cumberland) MLB28-2, (French Broad) MLB30-9; Texas, (Colorado) MLB45-1, MLB45-2; Wisconsin, (Red Cedar) MLB39-30, (Wisconsin) MLB33-30.

C. elongatus (Rio Grande clade). Mexico, (Conchos) MLB57-1; New Mexico, (Pecos) MLB22-1, MLB22-31>MLB22-34; Texas, (Rio Grande) MLB36-1>MLB36>20.

Combined data set

See cytochrome *b* set above.

Acknowledgements

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Table 1.1 Taxa used for cytochrome *b* primer design. Sequence data listed below were aligned to identify conserved terminal regions in the catostomid cytochrome *b* gene.

Taxon	GenBank Accession Number	Source
Moxostoma erythrurum	AY253421	(Berendzen *et al.* 2003)
Moxostoma anisurum	AF454880	(Harris *et al.* 2002)
Thoburnia atripinnis	AF454911	(Harris *et al.* 2002)
Hypentelium roanokense	AY253420	(Berendzen *et al.* 2003)
Minytrema melanops	AF454879	(Harris *et al.* 2002)
Carpiodes carpio	AB126083	(Saitoh *et al.* 2003)
Carpiodes carpio	NC_00525	(Broughton *et al.* 2006)
Cycleptus elongatus	AF454868	(Harris *et al.* 2002)
Myxocyprinus asiaticus	AB223007	(Saitoh *et al.* 2005)

Table 1.2 Range and mean pairwise sequence differences within and among *Cycleptus* taxa and outgroups.

	Cytochrome *b*		Control region	
Taxa	Range	Mean	Range	Mean
ELO-ELO	0.002-0.006	0.004	0.001-0.014	0.008
MER-MER	0.001-0.005	0.003	0.001-0.016	0.008
CRG-CRG	0.001-0.005	0.003	0.001-0.013	0.008
ELO-MER	0.002-0.006	0.004	0.007-0.016	0.011
ELO-CRG	0.018-0.023	0.021	0.019-0.033	0.025
MER-CRG	0.019-0.022	0.020	0.020-0.035	0.027
ELO-OGR	0.146-0.159	0.109	--	--
MER-OGR	0.146-0.159	0.108	--	--
CRG-OGR	0.146-0.160	0.103	--	--

ELO = *Cycleptus elongatus*; MER = *Cycleptus meridionalis*;
CRG = proposed *Cycleptus sp.* from Rio Grande Basin
OGR = Outgroup: *Myxocyprinus asiaticus*; *Carpiodes cyprinus*

Figure 1.1. Cytochrome *b* translation in *Cycleptus* (amino acid residues 16-356). All substitutions were synonymous with the exception of a single point mutation at position 295 (shaded), which caused a change from serine to tyrosine in three of forty-eight individuals (all three from the Rio Grande Basin).

DALVDLPTPSNISVWWNFGSLLGLCLITQILTGLFLAM
HYTSDISTAFSSVAHICRDVSYGWLIRNIHANGASFFFI
CIYMHIARGLYYGSYLYKETWNIGVILLLLVMMTAFV
GYVLPWGQMSFWGATVITNLLSAVPYVGNELVQWIW
GGFSVDNATLTRFFAFHFLLPFVVAAATIIHLLFLHETG
SNNPAGINSDADKISFHPYFSYKDLLGFAAMLLALTSL
ALFSPNLLGDPENFTPANPLVTPPHIKPEWYFLFAYAIL
RSIPNKLGGVLALLFSILVLMVVPILHT KQRGLTFRPI
TQFLFWTLVADMIILTWIGGMPVEHPFIIIGQIASA

Figure 1.2. Maximum likelihood phylogram for *Cycleptus* based on 20 unique cytochrome *b* haplotypes and two outgroup taxa. Tree with highest ML score is shown. Numbers to the left of nodes indicate bootstrap values (50% majority rule) greater than 50. The analysis performed with PAUP 4.0 (Swofford 1998) under the TrN+G model of molecular evolution.

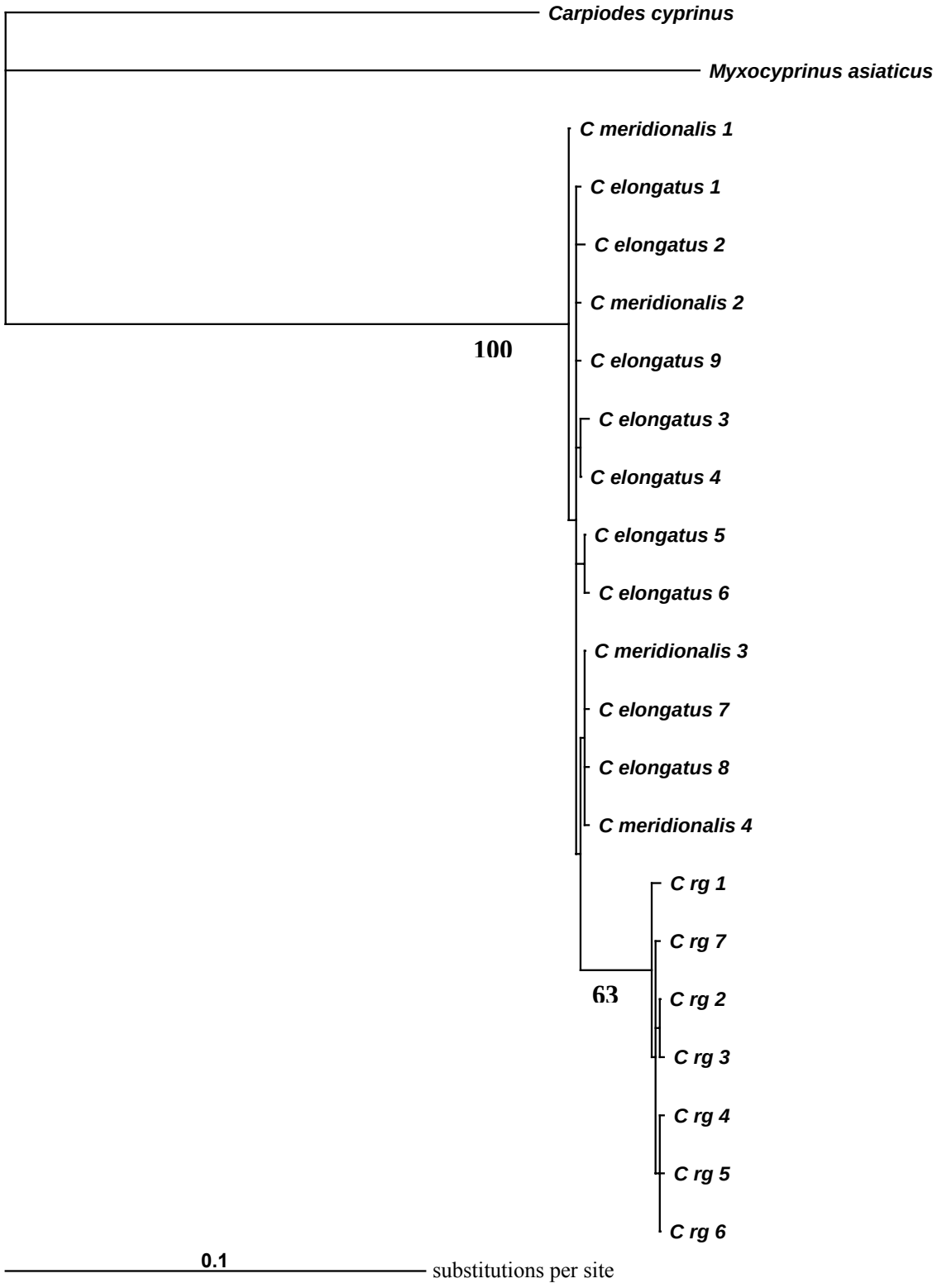


Figure 1.3. Strict concensus of 36 most parsimonious trees (157 steps) resulting from maximum parsimony analysis of the *Cycleptus* control region alignment. Numbers at nodes indicate percent recovery in 1000 bootstrap pseudoreplicates.

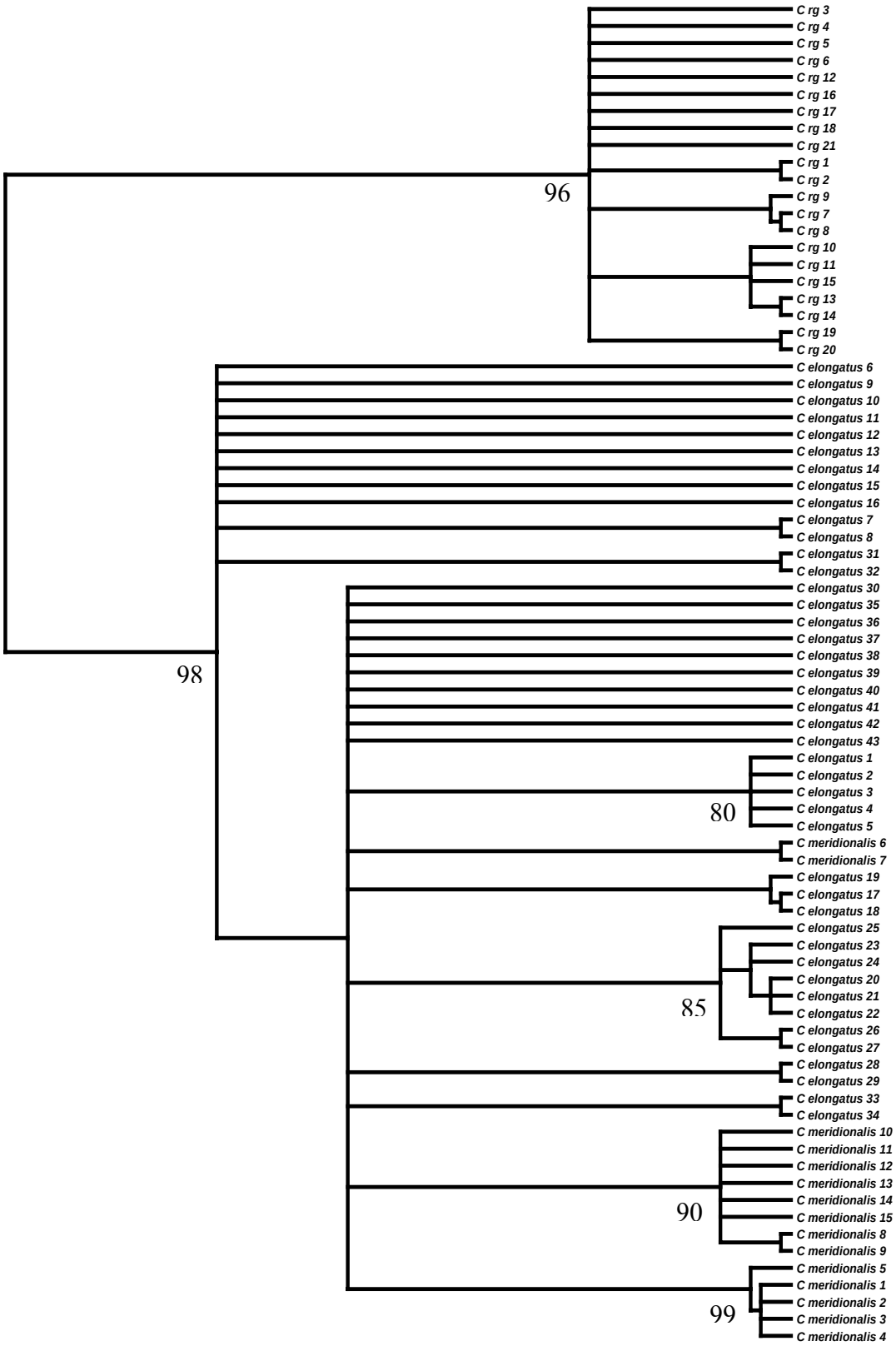


Figure 1.4. Visual exploration of MCMC convergence with the software AWTY (Wilgenbusch *et al.* 2004). Each line represents the posterior probability of splits 1-20 during the course of a Bayesian phylogenetic analysis of mtDNA control region sequences performed with MrBayes3.1.1 (Ronquist and Huelsenbeck 2003). In this case, convergence occurred slightly beyond 1.5 x 10^6 generations (X axis on the following page).

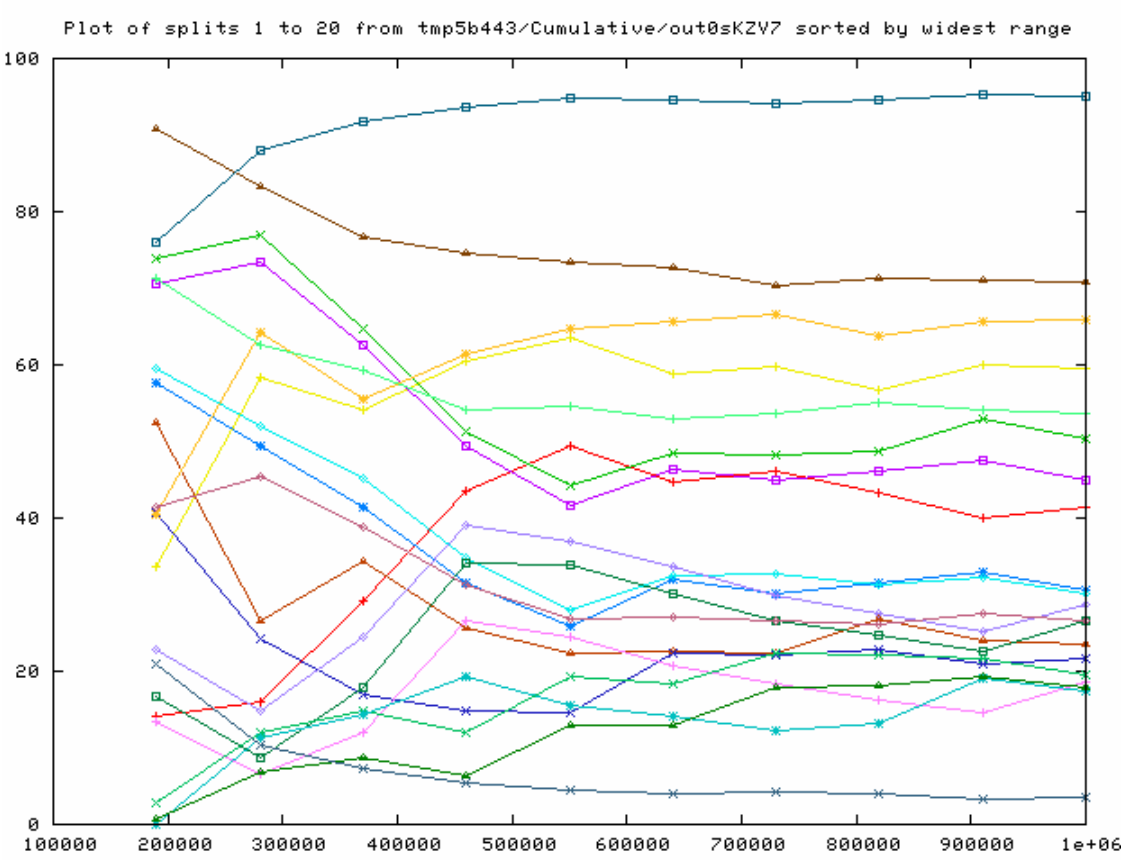


Figure 1.5. Unrooted fifty percent majority rule phylogram resulting from Bayesian analysis of 79 unique mitochondrial control region haplotypes identified among 151 individuals. See text for specific models and prior distributions. Numbers at nodes indicate frequency of occurrence among sampled (post burn-in) tree space for highly supported clades (≥85). *C. elongatus* haplotypes from the Rio Grande basin are termed 'C rg.'

Figure 1.6. Unrooted fifty percent majority rule phylogram resulting from Bayesian analysis of 43 unique concatenated (cytochrome *b* + control region) haplotypes identified among 48 individuals. Data was analyzed using a partitioned model approach. See text for specific models and prior distributions. Numbers at nodes indicate frequency of occurrence among sampled (post burn-in) tree space for highly supported clades (>85). Haplotypes from the Rio Grande basin are termed 'C rg.'

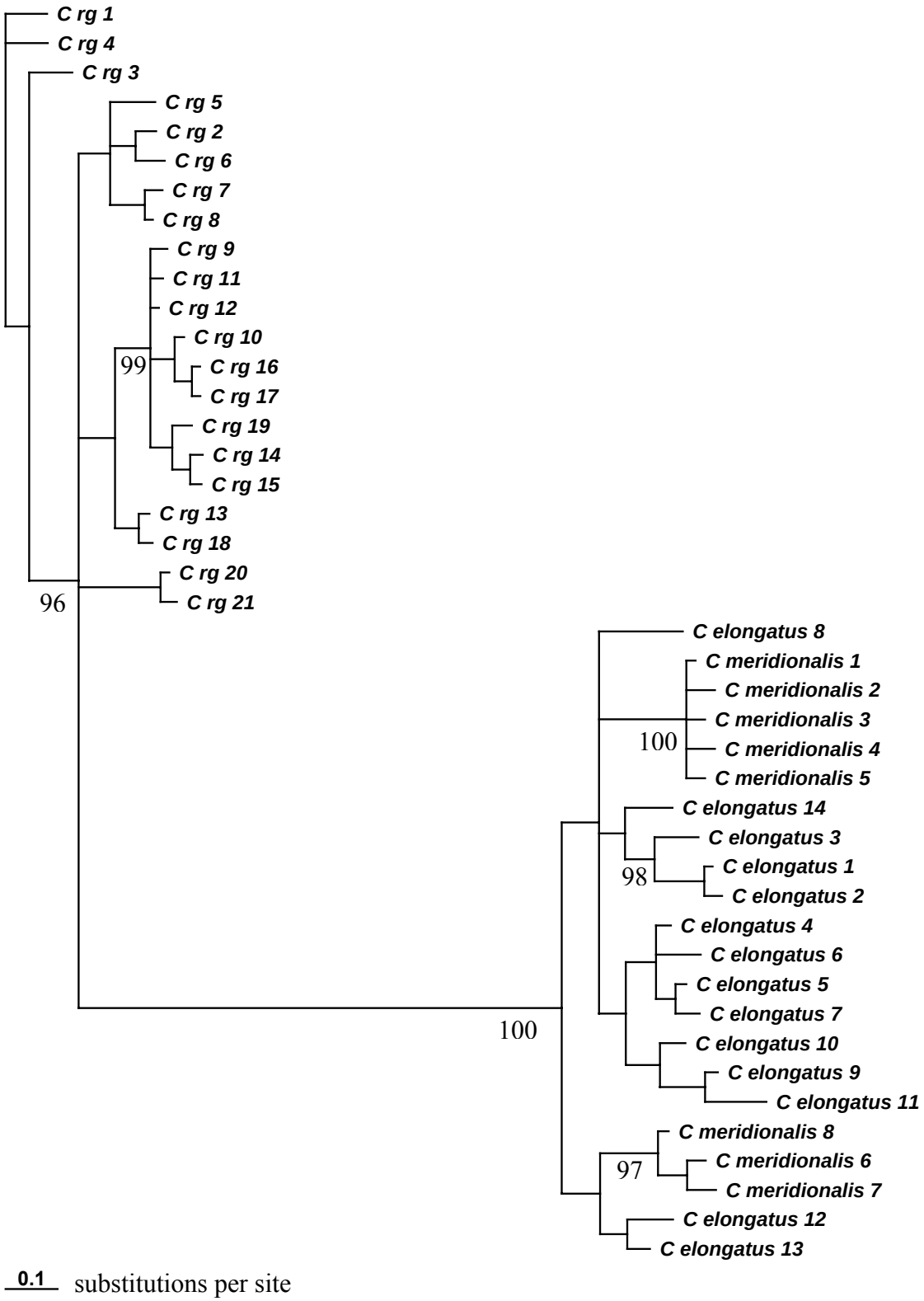
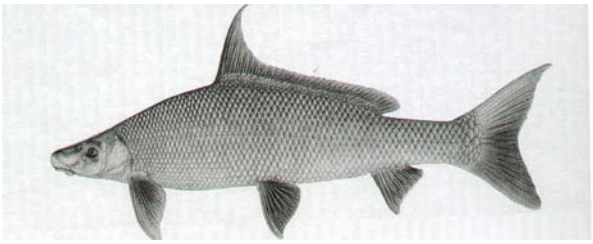


Figure 1.7. General morphological comparison of *C. elongatus* (top) and *C. meridionalis* (bottom). Images by Joseph R. Tomelleri. High resolution images available from the artist at: joe@americanfishes.com , phone 913-383-9771.



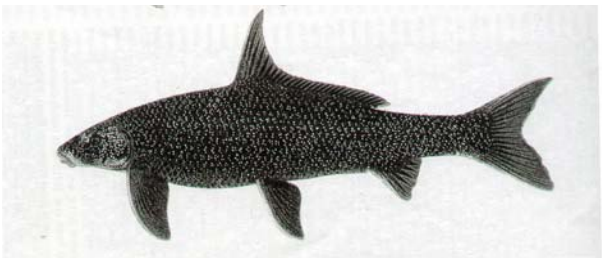
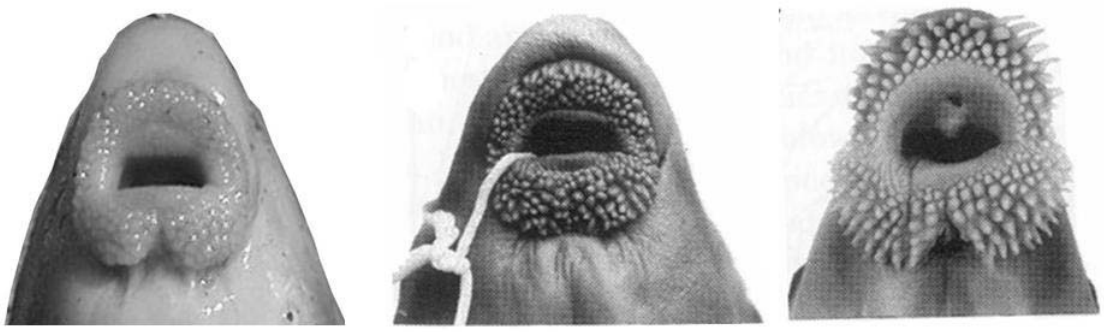


Figure 1.8. Lip morphology comparison in the three species of *Cycleptus*: a) *Cycleptus elongatus*, Missouri River, Nebraska (photo M. Bessert); b) *Cycleptus meridionalis*, Pearl River, Lousiana; c) *Cycleptus sp*, Rio Grande, Texas; (b) and (c) from Burr and Mayden (1999); also, (d) tuberculation in *C. elongatus*, French Broad River, Tennessee. Note that (a), (c), and (d) are from live specimens.

(a) **(b)** **(c)**



(d)

CHAPTER TWO

Microsatellite loci for allotetraploid blue sucker fish (*Cycleptus elongatus*, Catostomidae) with tests of cross-species amplification and isolation of paralogous loci

Abstract

The blue sucker (*Cycleptus elongatus*) is a widespread North American catostomid fish that appears to be declining throughout much of its range. Here, we describe the isolation and characterization of eleven microsatellite loci developed for population genetic studies in the genus. We show that an additional step of cloning and sequencing can be useful in isolating paralogous loci that often co-amplify in polyploid organisms. Finally, we present results of cross-species amplifications tests in nine other taxa, including four catostomids.

Keywords: Catostomidae, *Cycleptus elongatus*, microsatellite, polyploidy, paralog

Introduction

Cycleptus elongatus (Catostomidae: Cypriniformes) are large fishes that inhabit main stem river channels throughout the Mississippi and Rio Grande basins as well as several disjunct gulf coastal drainages in North America. Although distributed over such a vast range, populations are not considered stable in any of 21 states where they occur. The species is endangered or extirpated in four states (New Mexico, West Virginia, Ohio, and Pennsylvania) and is a candidate for federal listing (Elstad and Werdon 1993). Some authorities suggest that these apparent trends are an artifact of inadequate sampling (Burr and Mayden 1999). Here, we describe the isolation and characterization of eleven microsatellite loci developed for population genetic studies in the species.

Materials and methods

Genomic DNA was isolated from hypaxial muscle tissue using a standard phenol-chloroform extraction protocol (Sambrook *et al.* 1989), followed by ethanol precipitation. Purified samples were sent to Savannah River Ecology Laboratory (SREL - University of Georgia) for microsatellite enrichment with GATA, CATA, CA, and CT probes. The enrichment protocol is summarized by Hauswaldt and Glenn (2003). Subsequent revisions may be obtained directly from Travis C. Glenn (glenn@srel.edu).

Enriched DNA was amplified with the following cocktail: 2.0µl of 1mM dNTPs, 2.5µl of 10X PCR buffer, 2.5µl BSA (250ng/µl), 1.3µl SuperSNX-24 linker (10 µM) provided by SREL, 1.0µl of 50mM $MgCl_2$, 0.3µl (0.5 units) of Taq DNA polymerase (Invitrogen), 13.4µl sterile ddH_2O, and 2.0µl eluted DNA fragments from SREL. Cycling conditions were: 95°C (2 min), 35 cycles at 95°C (20 sec), 60°C (20 sec), 72°C (1.5 min), followed by a final extension at 72°C (30 min). PCR products were cloned using the pGEM-T-Easy Vector System I (Promega). PCR-based screening was performed to determine insert length in approximately 200 positive clones. Inserts were amplified using flanking primers M13F and M13R according to the following conditions: 96°C (5 min), 30 cycles of 96°C (45 sec), 60°C (1 min), and 72°C (1min) with a final extension of 72°C (5 min). Products were electrophoresed on a 2% agarose gel to visualize insert length. Approximately 100 clones with inserts > 350bp were selected for sequencing.
Clones were sequenced in one direction with a BigDye Terminator cycle sequencing reaction (Applied Biosystems) using the M13F primer. Complimentary (reverse) sequences were obtained for fifty-nine clones that contained microsatellite sequences using the M13R primer. Complimentary pairs were aligned and edited with Sequencher™ 4.2.2 (Gene Codes Corp.).

PrimerSelect© (DNASTAR Inc) was used to design primer pairs for 19 candidate loci that possessed adequate flanking regions (at least 50bp) and at least seven repeat units of the microsatellite motif. PCR optimization was conducted using a gradient thermal cycler (MJ Research PTC-200) with an annealing step of 48-66°C to determine optimum

annealing temperature. Reaction mixtures had a total volume of 10µl and contained 2.0µl of 1mM dNTPs, 1.0µl of 10X PCR buffer, 0.4µl of 50mM $MgCl_2$, 0.4µl 10mM forward primer, 0.4µl 10mM reverse primer, 0.1µl (0.5 units) of Taq DNA polymerase (Invitrogen), 4.7µl sterile ddH_2O, and 1.0µl (≈100ng) DNA. Reactions were denatured at 94°C for 2 minutes, then carried out for 30 cycles at 94°C (30 sec), annealing temperature (30 sec), 72°C (40 sec), followed by a final extension of 72°C (2 min). Fifteen loci amplified cleanly. For these, an ABI Prism 310 Genetic Analyser (Applied Biosystems) was used to score allele lengths in 30 individuals collected from a contiguous 150 km stretch of the upper Missouri River, Montana, USA.

Results and Discussion

Six loci exhibited tetrasomic genotypes (more than 2 alleles) while the other nine were disomic (Table 2.1, loci Ce35-215). The number of alleles per individual was always one or two and the number of alleles per locus ranged from 1-27 with a mean of 10.2 alleles per locus. Three loci (Ce49, Ce126, and Ce215) deviated significantly from Hardy-Weinberg (HW) equilibrium as determined by the HW probability test implemented in GENEPOP 3.1 (Raymond and Rousset 1995).

Although sophisticated techniques abound for population genetic analysis of diploid, codominant markers (Pearse and Crandall 2004), there remains a paucity of methods to analyze polyploid data. Therefore, we sought to increase the number of diploid markers by isolating two co-amplifying (paralogous) loci from one another. A candidate

locus (primer pair Ce13F: 5'-GTTCTGGGACTTAACAAAGGGATTT-3', Ce13R: 5'-ACAAACATGAGGTATCAAGTAGTCTAA-3') was amplified by PCR and the products from a single individual that possessed four alleles of distinct length (at least 10 bp different from one another) were cloned and sequenced. Pair-wise length differences (in base pairs) for each combination of alleles were as follows: {1,2}=13; {1,3}=25; {1,4}=55; {2,3}=12; {2-4}=42; {3,4}=30. Allele sequences were aligned to identify regions that differed between paralogs and new primers (Ce13S and Ce13L, Table 2.1) were designed at these sites. Each new primer pair amplified only two alleles in the sequenced individual and paralog identities were confirmed by comparing allele lengths to those amplified by the original Ce13 primer pair. Ce13S products differed by 55 bp, corresponding to pair {1,4}; Ce13L products differed by 12 bp, corresponding to pair {2,3}.

Loci Ce13S and Ce13L were also helpful in determining chromosomal inheritance patterns in *C. elongatus*. In an organism of autopolyploid origin, a multivalent formation (of more than two chromosomes) may occur during meiosis (Wua *et al.* 2001); thus, some individuals would fail to inherit at least one priming site for one of the two loci (resulting in no amplification at that locus). Every individual in this screening set possessed either one or two alleles and the results did not differ significantly from HW expectations (Table 2.1). These results confirm functional diploidy in *C. elongatus* and are consistent with an allotetraploid origin as suggested by Uyeno and Smith (1972).

Finally, cross-species amplifications were attempted to determine primer efficacy in nine other taxa. Reactions were conducted in single individuals with positive and negative controls. All products were verified by genotyping as described previously. As expected, some loci are well-conserved within Catostomidae (Table 2.2). In conclusion, these novel markers will be useful for population genetic studies within the genus and may have broader utility within Catostomidae.

Acknowledgements

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Table 2.1 Primer sequence, repeat motif, and allelic diversity for eleven microsatellite markers in *Cycleptus elongatus*, each screened in 30 individuals. T_a, annealing temperature; k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity

Locus/ GenBank Accession no.	Primers (5'⟶3')	Repeat motif	Size (bp)	T_a (°C)	k	H_O†	H_E
Ce13S DQ401677	F: ATAACAACTTGTCATGCATTCCTGA R: CCGAGGACAGCGGTTTAAAATAT	$(GATA)_{20}$	114-186	60	24	0.900	0.942
Ce13L DQ401678	F: GTAACAATTTTTCATGCATTCCTGGA R: GCCGAGGACAGCGGTTTA	$(GACG)_5(GATG)_3(GATA)_4$	132-154	60	6	0.800	0.687
Ce35 DQ401679	F: CTTCACACCCAGCTCAAGTCACAT R: TGGCAGCCTAAGCTTAATGCTCTA	$(GA)_{17}$	123-151	60	11	0.833	0.856
Ce49 DQ401680	F: TTTAAGATTTTCTTCCTTCGACTAA R: GAATGTGCCCGTGCGCATGAACA	$(CAA)_7$	107-113	60	3	0.533*	0.538
Ce52 DQ401681	F: ATGACAGCATCCATGCACATTTA R: GTTTCCATGGATACCAATTTACCC	$(CAAT)_8CACT(CAAT)_{13}$	229-241	64	4	0.433	0.534
Ce63 DQ401682	F: CCAAAAGCGTCTTGAAATGTTCA R: CAGACGGCGAGAGGAGATGGA	$(GT)_8(GA)_{14}$	145-255	64	18	0.733	0.829
Ce104 DQ401683	F: CACACCCATTACGGCAGGATTA R: GATACAGCAATGAGCTTTCATAACACA	$(CT)_{18}$	145-153	60	5	0.567	0.491
Ce126 DQ401684	F: TTCGCTCTCCGTCCCTTTCATTCT R: TGGAGAGCGAAAAAGAGACATTATCA	$(CT)_{12}$	155-179	60	9	0.967**	0.832
Ce146 DQ401685	F: AACCCAAAAATGAAAATTGTGTTA R: TGCTCGCTATTAAGAGACTCTGATT	$(GATA)_8$	150-164	60	4	0.733	0.697
Ce195 DQ401686	F: ACATTGCGATTAATTGCATTCATT R: TCCATCCTCTTCTGCCATTACATT	$(CATA)_2CG(CATA)_{18}$	243	60	1	0.000	0.000
Ce215 DQ401687	F: TTGTCACACCTTTATGGGATTCAT R: CACTCTCAATAGCGAAATGTAGTTCTT	$(TATC)_{16}C(TATC)_{14}$	211-287	60	27	0.767***	0.963

†Three loci deviated significantly from Hardy-Weinberg equilibrium: Hardy-Weinberg equilibrium probability test, GENEPOP 3.1 (Raymond and Rousset 1995). *P=0.036, **P=0.020, ***P=0.010.

Table 2.2 Cross-taxon amplification to determine priming site conservation. Numbers below each locus represent actual genotypes (allele lengths in base pairs) in a single individual of the given taxon. "X" indicates no successful amplication. All reactions were conducted under conditions optimized for *C. elongatus*.

Taxon	Locus Ce13S	Ce13L	Ce35	Ce49	Ce52	Ce63	Ce104	Ce126	Ce146	Ce195	Ce215
Cycleptus meridionalis	99/123	145/145	X	113/113	202/202	159/173	152/152	175/185	X	245/245	179/213
Carpiodes cyprinus	155/175	X	131/146	113/113	171/171	X	127/127	155/155	148/148	245/245	X
Catostomus commersoni	200/204	X	X	113/113	200/200	X	140/140	154/156	X	X	X
Moxostoma macrolepidotum	X	X	X	113/113	X	X	X	X	X	245/245	X

Additional taxa for which all amplification attempts failed: *Pimephales promelas, Ameiuras melas, Prochilodus lineatus, Lepomis cyanellus,* and *Culaea inconstans.*

CHAPTER THREE

Population genetic structure of the Blue Sucker (*Cycleptus elongatus* Lesueur, 1918) in the Upper Missouri River: genetic effects of habitat fragmentation

Abstract

In recent times, anthropogenic modifications have caused profound changes to North American waterways. Channelization and impoundment have homogenized seasonal flow regimes and obstructed upstream migration of many large riverine fishes. The blue sucker, *Cycleptus elongatus*, is a large catostomid fish that occurs in main stem rivers throughout the Mississippi basin of North America. Although not federally listed as threatened or endangered, populations are not considered stable in any of 21 states where they occur. Included in this range is the Missouri River, which flows more than 3200 kilometers from its headwaters in Montana to its confluence with the Mississippi River at St. Louis, Missouri. Historically, *C. elongatus* was distributed continuously throughout the mainstem Missouri and its major tributaries, but from 1952-1963, six major impoundments were constructed on the upper Missouri by the US Army Corps of Engineers. The resulting reservoirs have inundated and fragmented riverine habitat from Yankton, South Dakota to the headwaters in Montana. *Cycleptus elongatus* still occurs in the remnant unimpounded stretches between reservoirs; however, little is known of reproduction, recruitment, and whether inter-reservoir populations are genetically isolated. In order to assess genetic diversity and connectedness, 231 individuals from nine sites in the drainage were genotyped at 15 variable microsatellite loci. Genetic data

were used to calculate traditional summary statistics and fixation indices. In addition, four techniques were used to investigate population structure. The results indicate shallow, 'graded' structure from the lower reaches toward the headwaters. Mantel tests revealed a highly significant pattern of isolation by distance. This is noteworthy because such a pattern does not exist in the unobstructed Mississippi River main stem, a river of comparable size. These results are consistent with reduced intradrainage gene flow in the Missouri River and may represent the first traces of impoundment effects on genetic structure. This information will assist governing agencies in making well-informed decisions regarding conservation and management of *C. elongatus* in the Missouri River basin.

Introduction

The past 100 years have witnessed a great deal of anthropogenic change to natural waterways in North America. Some rivers have been impounded to store water and regulate flow while others have been channelized to facilitate navigation. In addition, locks and dams have been constructed on many others to further control water levels for navigation. Migration and reproduction in many riverine fishes has been negatively impacted by such modifications because routes to historical spawning grounds have been compromised (Ickes *et al.* 2001; Jungwirth *et al.* 1998; Laroche and Durand 2004).

The blue sucker, *Cycleptus elongatus*, is a large catostomid fish native to main stem rivers throughout the Mississippi basin and is one of the most widespread lotic fish taxa in North America. Its elongate, hydrodynamic body form, with paired fins that are anteriorly rounded, enables it to maintain its position in swift current with little energy expenditure (Wolter and Arlinghaus 2003). Historically, the species occurred in 21 conterminous states, but it is now endangered or extirpated in four (New Mexico, West Virginia, Ohio, and Pennsylvania) (NatureServe 2005) and is a candidate for listing at the federal level (Elstad and Werdon 1993). The range of *C. elongatus* has diminished greatly over the past 100 years, and it is thought that the impoundment of main stem rivers has played a major role (Boschung and Mayden 2004; Robison and Buchanan 1998). In the upper Missouri drainage, *C. elongatus* inhabited virtually all reaches of the mainstem Missouri River from its confluence with the Mississippi River at St. Louis to the far northern headwaters of the Missouri and Yellowstone rivers in Montana (Brown 1971; Underhill 1959), a distance of more than 3200 kilometers.

From 1952-1963, seven major impoundments were constructed on the upper Missouri River by the Army Corps of Engineers in an effort to control flooding and provide hydroelectric power (Hesse *et al.* 1982). The resulting reservoirs have inundated and fragmented large riverine habitat from Yankton, South Dakota to the Missouri River headwaters in Montana, reducing mainstem riverine habitat in the intervening stretches by some 70% (estimate based on length of reservoirs at full pool – see USACE 2001). Following construction of the dams, blue suckers and other migratory species have amassed in the tailwaters at times coinciding with seasonal spawning movements (Eitzmann *et al.* 2005; Walburg *et al.* 1971). Blue suckers are, indeed, riffle spawners that swim up tributaries to reach suitable habitat (i.e. flooded gravel bars).

Although *C. elongatus* still occurs in the reaches between reservoirs, it is unknown whether these represent viable (sustainable) populations and whether any gene flow occurs between them. At best, there is enough reproduction and recruitment to maintain genetic diversity and allow for at least some level of downsream gene flow and at worst there is little to no reproduction and recruitment and complete genetic isolation between inter-reservoir stretches. Here, the fundamental working hypothesis was that little, if any, downstream gene flow occurs because adults are not suited to the lentic characteristics of reservoirs and probably avoid them. Also, larval drift from tributaries above reservoirs may settle to the bottom or be consumed by predators before it can pass through floodgates or turbines, thus limiting gene flow and recruitment.

From this point hereafter, the term "population" refers to the evolutionary paradigm set forth by Waples and Gaggiotti (2006), that is, a population is defined as, "*a group of individuals of the same species living in close enough proximity that any*

member of the group can potentially mate with any other member." In this context, the following hypotheses regarding impoundment effects were tested in Missouri River populations: 1) inter-reservoir populations in the upper Missouri River will show an overall reduction in genetic diversity compared to those in open sections of the lower river; 2) genetic variance between populations will be non-negligible, arbitrarily accounting for at least 5% of the total variance observed (due to reduced gene flow and increased effects of genetic drift); 3) Missouri River populations will show a significant pattern of isolation by distance due to a lack of homogenizing gene flow; and 4) inter-reservoir populations will show a signal of recent decline in number as evidenced with an excess in observed heterozygosity.

Materials and methods

Study location and individual collection

A total of 231 blue suckers were collected from six main stem localities and three tributaries of the Missouri River from November 2004 to November 2005 (Figure 3.1, Table 3.1). To ensure adequate representation throughout, I specifically targeted all inter-reservoir populations and employed stratified sampling in the lower reaches (below Lewis and Clark Reservoir, the first impoundment). Fish were captured using hoop nets, gill nets, and electroshocking devices. A small (1 cm^2 or less) fin clip was removed from each fish and preserved in 95% EtOH for subsequent genetic work. All tissues are vouchered in the personal collection of the author (see Appendix 2) and are freely available upon written request.

DNA preparation and amplification

DNA was extracted from tissue samples using either a standard phenol-chloroform protocol (Sambrook *et al.* 1989) or a DNeasy® Tissue purification kit (Qiagen). DNAs were eluted in either water of buffer (EB) supplied by Qiagen. A small number of samples were randomly selected and (1-2 µl) electrophoresed through a 1% agarose gel to check for quality (high molecular weight). All samples were quantified with a GeneQuant II spectrophotometer (Pharmacia Biotech) and a portion of each was diluted to a working stock concentration of 100ng/µl. The remainder of each elution was placed in -70º C for long-term storage. As with tissue samples, DNA samples are maintained in the personal collection of the author housed at the University of Nebraska (lab of G. Ortí) and are freely available upon written request.

Eleven microsatellite loci previously isolated from *Cycleptus elongatus* (Bessert *et al.* 2006) were chosen for this study. In addition, sixteen primer pairs designed for other catostomid taxa were screened and optimized in *C. elongatus.* This resulted in the addition of four more loci: Mox294, Mox306, Mox329 (Lippe *et al.* 2004), and Dlu4235 (Tranah *et al.* 2001).

In order to increase the efficiency of data collection, primer pairs were screened for potential cross-reactivity in multiplex reactions using the software AutoDimer (Vallone and Butler 2004) and two multiplex reactions were optimized according to guidelines provided by Henegariu *et al.* (1997). If two loci (in the same reaction) were labeled with the same fluorophore, special care was taken that the allelic size ranges were non-overlapping. In this way, I was able to successfully amplify all 15 loci in only two reactions, as follows (also see Table 3.2). Note that all primers were at 10µM "working stock" concentrations. Reaction "A" (Figure 3.2) contained the following: 1.60 µl

dNTPs (1mM), 1.20 µl PCR reaction buffer (10X), 0.80 µl MgCl (50mM), 0.30 µl 13S-F*, 13S-R, Ce52F*, and Ce52R, 0.32 µl Ce35F*, Ce35R, Ce126F*, Ce126R, Mox329F*, and Mox329R, 0.50 µl Ce215F* and Ce215R, 0.48 µl Mox306F* and Mox306R, 0.20 µl Ce104F* and Ce104R, 0.12 µl Taq DNA polymerase (Gibco BRL), and 1.0 µl DNA (=100ng) for a total volume of 10.20 µl. Thermal cycling conditions were as follows: 94°C (1 min), 30 cycles of 94°C denature for 30 sec, 55.8°C anneal for 40 sec, and 65°C extension for 2 minutes, followed by a final extension of 65°C for 5 minutes and a holding temperature of 4°C. Reaction "B" (Figure 3.3) contained the following: 1.60 µl dNTPs (1mM), 1.20 µl PCR reaction buffer (10X), 0.88 µl MgCl (50mM), 0.26 µl 13L-F* and 13L-R, 0.44 µl Dlu4235F* and Dlu4235R, 0.30 µl Ce49F* and Ce49R, 0.48 µl Ce63F* and Ce63R, 0.28 µl Ce146F* and Ce146R, 0.32 µl Ce195F* and Ce195R, 0.54 µl Mox294F* and Mox294R, 0.14 µl Taq DNA polymerase (Gibco BRL), and 1.0 µl DNA (=100ng) for a total volume of 10.06 µl. Thermal cycling conditions were the same as those for reaction "A" except the annealing temperature was 54.8° C. Note that 'pigtail' modifications were added to the 5' end of reverse (unlabeled) primers for loci Ce35, Ce63, and Ce104 in order to reduce stutter and ease scoring (see Brownstein *et al.* 1996). For the specific labeling scheme of each reaction, see Table 3.2.

Sample preparation and data collection

Prior to genotyping, PCR products were purified with a Mini-Elute (Qiagen) or Microarray PCR purification kit (Telechem International, Inc.) to remove residual unincorporated dyes that can obscure allele signatures in an electropherogram (see Butler 2002). Products were prepared for electrophoresis by mixing (0.5 µl PCR product) with

0.5 µl LIZ 500 size standard (Applied Biosystems) and 9.0 µl deionized formamide. Samples were denatured at 95° C for 3 minutes and quenched on ice for two minutes prior to capillary electrophoresis on an ABI 310 fragment analyzer. Allele sizes were scored with GeneMapper 3.7 (Applied Biosystems) and the data transformed to GenePop format (Raymond and Rousset 1995) with GMCONVERT 0.32 (Faircloth 2006).

Genotypic data were checked for typographical errors, evidence of null alleles (Hardy-Weinberg equilibrium), and adherence to the Stepwise Mutation Model (Ohta and Kimura 1973) with MicroChecker 2.2.3 (VanOosterhout *et al.* 2004) and MSAnalyzer 4.0 (Dieringer and Schlötterer 2002). Additional transformations of the data set to other formats was performed with CONVERT 1.3.1 (Glaubitz 2004).

Data Analysis – Summary statistics and fixation indices

In addition to observed and expected heterozygosities for each locality, deviations from Hardy-Weinberg equilibrium and pairwise F_{ST} estimates (Weir and Cockerham 1984) were calculated for each locality using GenePop3.3 (Raymond and Rousset 1995). The program FSTAT 2.9.3.2 was used to calculate the average allelic richness (A) at each locality (Goudet 1995; Goudet 2002). This is a useful method for comparing samples of different sizes because it scales all results to the smallest sample. Since 12 loci did not deviate from expectations of the stepwise mutation model (SMM) (Ohta and Kimura 1973; Valdes *et al.* 1993; Wehrhahn 1975), they were used to calculate *rho*, an estimator of R_{ST} values (Valdes *et al.* 1993), with RSTCALC (Goodman 1997) and to perform pairwise comparisons. Note that Bonferroni corrections were applied to adjust for multiple pairwise comparisons (Rice 1989). Pairwise t-tests were performed to test for

differences in mean allelic richness between free ranging populations (sites A-D) and impounded populations (E-I).

Population structure

In order to assess the distribution of genetic variation within and among collection sites as well as that between impounded (sites E-I) and unimpounded (sites A-D), an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was performed with Arlequin 3.1 (Excoffier *et al.* 1992; Schneider *et al.* 2000). This test was executed under the standard model ("different alleles are considered mutationally equidistant from each other") as well as the stepwise mutation model. Two loci were excluded from the latter due to inconsistencies with the SMM (gaps in allele frequency distribution).

Another useful method that does not require *a priori* assumptions regarding population structure is the Bayesian clustering technique implemented in STRUCTURE 2.0 (Pritchard *et al.* 2000). Here, genetic data is used to define the number of subpopulations (k) in the absence of any locality data. The method assumes Hardy-Weinberg equilibrium (HWE) within subpopulations and seeks combinations of individuals that maximize HWE in a pre-determined number of populations. To use the method, one performs several runs at values of k from 1 to n where n = the maximum number of subpopulations to be considered. Assuming quality control in preparation and performance of these runs (unambiguous data, quality markers, adequate burn-in, etc.), the k value eliciting the highest mean posterior probability over several runs indicates the true number of genetic subdivisions within the data set. Four simulations were executed at value of k from one to nine under the admixture model with independent allele

frequences and λ fixed at 1; that is, the model assumes that allele frequencies in each subpopulation are independent from one another (i.e. independent draws from a distribution defined by λ). A set of preliminary simulations was performed to determine appropriate burn-in and run lengths. Real time plots of α (parameter for the degree of admixture) and other parameter estimates were monitored to determine when convergence occurred. A conservative burn-in period of 250,000 generations was selected for subsequent simulations and was followed by 1,000,000 generations of data collection. Posterior probabilities for *k* were computed based on the mean log likelihood of the data from 4-5 simulations at each value of *k* (see Pritchard and Wen 2004).

Finally, I referred back to the summary statistics and performed several analyses to determine whether the data fit a model of isolation by distance (IBD) (Wright 1943). This model predicts that gene flow will be negatively correlated with geographic distance between populations. When the homogenizing effects of gene flow are reduced, the effects of drift become more pronounced and distant populations tend to diverge at neutral genetic markers. In order to test for IBD, central localities (CL) for each sampling area were estimated from locality plots of each individual collected, and a matrix of linear distances between the central locality for all pairs of populations was calculated (Table 3.5). The web-based software package IBDWS (Jensen *et al.* 2005) was used to calculate a matrix of pairwise genetic differentiation between populations ($F_{ST}/1-F_{ST}$) (Rousset 1997) and to perform a Mantel test with 10000 random permutations between the matrix of linear distance and the matrix of pairwise genetic differentiation between populations. Additional Mantel tests were also performed between each of the

following matrix combinations: genetic differentiation vs ln (linear distance); ln (genetic differentiation vs linear distance; and ln (genetic differentiation) vs ln (linear distance).

Demography

BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) was used to investigate the possibility of recently reduced effective population sizes in the sampling areas. The method compares gene diversity (expected heterozygosity, H_e) with the expected equilibrium gene diversity (H_{eq}) that is computed from *na* (the observed number of alleles) under mutation/drift equilibrium. If a significant number of loci show an excess in gene diversity then the population has likely undergone a recent bottleneck. A Wilcoxon sign-rank test is used to make this determination. The two-phase mutation model (TPM) was used because it more realistically describes microsatellite evolution than either the strict stepwise mutation (SMM) or infinite alleles (IAM) model. In the TPM, a 95% frequency of stepwise mutations was assumed with a 12% variance of multiple-step mutations.

Results

Marker screening

One locus, Ce63, showed a heterozygote deficite across all sample sites. This pattern is consistent with the presence of null alleles; thus, we did not use this locus in any analyses. In addition, two loci, Mox329 and Ce215 had significant gaps in their allele frequency distributions. While they did not deviate from HW expectations, the mutational pattern clearly deviated from a stepwise (SMM). Consequently, these two markers were withheld from any analyses that assumed the stepwise model.

Summary statistics and fixation indices

Summary statistics are presented in Table 3.1. Note that site B was excluded from these analyses due to a very small sample size (n=6). For the remaining sites, gene diversity (=observed heterozygosity) was only slightly reduced in impounded populations, and not significantly so ($mean_{A\text{-}D} = 0.701$, $mean_{E\text{-}I} = 0.697$; $t = 0.544$, $df = 6$, $p = 0.61$). In a similar site comparison of mean allelic richness, the upper Missouri did prove to be significantly reduced in comparison to the lower Missouri ($mean_{A\text{-}D} = 7.31$ $mean_{E\text{-}I} = 6.75$; $t = 2.68$, $df = 6$, $p = 0.036$), supporting the hypothesis of reduced genetic diversity in the impounded populations. When this analysis was extended to include additional unimpounded sites from throughout the Mississippi basin (see Chapter 4, Table 4.1), the difference was even greater ($mean_{others} = 7.31$, $mean_{E\text{-}I} = 6.75$; $t = 3.68$, $df = 15$, $p = 0.002$). Pairwise R_{ST} comparisons appear in Table 3.3. Following Bonferroni correction, five significant pairwise differences remained. Four of these occurred between pairs that were among the most distant from one another, hinting at the possibility of isolation by distance.

Population structure

Both analyses of molecular variance revealed that more than 98% of the total variation occurred within populations, that is, within sample sites (Table 3.4). Roughly 1% occurred among populations above and below the lowest impoundment (sites A-D vs E-I), while a very modest amount (est. 0.38% and 0.31%) occurred between populations; thus, refuting hypothesis 2.

For the Bayesian analysis with STRUCTURE 2.0 (Pritchard *et al.* 2000), the setting of $k = 2$ yielded the highest mean posterior probability over 4-5 separate

simulations for each value of *k* from 1-5. The results of the single run with the highest posterior probability appear in Figure 3.4. Pritchard and Wen (2004) suggest caution in interpreting results when a value of $k = 3$ or less is indicated; however, the slightly graded pattern here is valid as the number of individuals assigned to each cluster is unequal. A questionable signal would include an approximately equal number of individuals in each cluster (Pritchard and Wen 2004). Interestingly, this pattern is unique to the Missouri River when analyzed as part of a range-wide data set (see Chapter 4, Figure 4.3), further suggesting that it represents real structure.

Mantel tests for isolation by distance were highly significant ($p < 0.001$) for all four matrix combinations, i.e. genetic distance and ln(genetic distance) vs geographic distance and ln(geographic distance). Results for ln(genetic distance) vs geographic distance are presented in Figure 3.5. Slopes and negative correlations were similar for the other three tests, indicating a clear pattern of IBD in the Missouri River.

Demography

Multilocus frequencies of observed heterozygosity were slightly higher than expected at seven of the nine sampling locations; however, no recent bottlenecks were detected at any of the sample sites (see Table 3.5).

Discussion

Is there any reason to believe that *C. elongatus* populations in the Missouri River are declining – or that dams have had any genetic impact to this point? At first glance, the answer is unclear. Gene diversity is somewhat uniform throughout. In addition, no significant heterozygosity excess was detected in any of the sampled areas; thus, with this

method, there is no evidence for any recent bottlenecks (Table 3.5). However, allelic richness differed significantly between populations above and below the lowest impoundment (see Table 3.1). A reduction in allelic diversity is frequently associated with invasive (Genton *et al.* 2005) or founding populations (Ramstad *et al.* 2004), but may also indicate population decline (Faugeron *et al.* 2004).

Should we expect to see any deviations from 'normal' (undifferentiated) genetic patterns given the brief time frame of approximately 60 years? To be sure, the array of molecular tools employed is powerful enough to detect any of these effects, if present (Table 2; mean number of alleles (*na*) = 122 in each population); however, it is also important to consider the generation time for *C. elongatus*, which is approximately 10 years (Becker 1983). Given the accompanying lag time, detection of a significant bottleneck (Cornuet and Luikart 1996) would indicate an extreme situation for the affected populations, and this is not the case (Table 3.5).

Conversely, a highly significant pattern of isolation by distance was detected among Missouri River populations (Table 3.6, Figure 3.5). While this may seem intuitive or trivial given that sampled populations span a distance of nearly 3,050 river kilometers, cycleptid fishes are known to migrate hundreds of kilometers to spawning grounds (Mettee and Shepard 1997). In an unobstructed waterway of equal length, it is conceivable that these behaviors could mitigate distance effects and elicit a population signature of - or tending toward - panmixia. In this light, it is worthy to note that the majority of main stem tributaries (e.g., Red, Missouri, Ohio, and Tennessee) within the Mississippi Basin have been impounded at some point (Benke and Cushing 2005) while the main stem Mississippi itself has not. A long series of locks and dams was constructed

over a distance of several hundred kilometers in the upper Mississippi, but fish passage is still possible through these corridors (Ickes *et al.* 2001).

To test for potential differences between the two major rivers, equivalent genotypic data was collected from an additional 135 individuals from 6 sites on or near the Mississippi's main stem (Table 3.7; also see Figure 4.2, Chapter 4) and combined with data from the lowest Missouri River site (Table 3.1, site A) for a total of 172 individuals distributed over seven sites spanning 2,493 unobstructed river kilometers. The results of a Mantel test for isolation by distance comparing genetic differentiation and geographic distance in this data set were nonsignificant (Figure 3.6). The other three possible matrix combinations - genetic differentiation vs ln (linear distance); ln (genetic differentiation) vs linear distance; and ln (genetic differentiation) vs ln (linear distance) - were tested and also proved nonsignificant.

While this case provides only a single - albeit expansive - comparison with the Missouri River, it warrants concern. A recent study in gallinaceous birds with a similar generation time (10 years) revealed declines in genetic diversity and concomitant shifts toward a metapopulation structure with isolation by distance after only 50 years of anthropogenic fragmentation (Segelbacher *et al.* 2003). In the Missouri River, these highly significant correlations may be the first detectable signal of genetic change due to isolation by impoundment. Careful monitoring of populations in the upper Missouri with periodic combinations of physical and genetic surveys is encouraged.

Acknowledgements

A major portion of this work was generously supported by a State Wildlife Grant from the Nebraska Game and Parks Commission. Other significant sources included the Center for Great Plains Studies, the UNL School of Biological Sciences, and the Initiative for Ecological and Evolutionary Analysis. Again, a special note of thanks is extended to the many people who assisted with sample collection (see listed individuals and agencies in Appendix 2) as well as all the members of the Orti lab, who patiently allowed exclusive access to our fragment analyzer for weeks on end.

Table 3.1 Site-specific summary statistics; CL = central locality (latitude/longitude) of sampling area; *n* = number of individuals sampled; *na* = number of alleles; Gene diversity ($H_{O)}$ = observed heterozygosity; H_E = expected heterozygosity; A = allelic richness.

	River / State	CL	*n*	*na*	Gene diversity (H_O)	H_E	A
A	Missouri / Missouri	38º68'48" N 90º66'96" W	30	140	0.700	0.688	7.340
B	Kansas / Kansas	39º11'20" N 96 º31'03" W	6	62	0.576	0.729	-
C	Missouri / Nebraska	40º81'49" N 95º84'43" W	38	138	0.704	0.745	7.290
D	Platte / Nebraska	41º05'13" N 96º10'83" W	23	128	0.698	0.661	7.311
E	Missouri / South Dakota	42º76'47" N 97º98'81" W	29	142	0.717	0.692	7.271
F	Missouri / North Dakota	47º25'42" N 101º24'34" W	14	96	0.690	0.698	6.343
G	Yellowstone / Montana	46º43'36" N 96º31'03" W	30	134	0.691	0.732	6.629
H	Missouri / Montana	48º03'42" N 106º91'72" W	31	132	0.699	0.727	6.902
I	Missouri / Montana	47º73'71" N 109º61'83" W	29	125	0.686	0.675	6.591
totals			n = 231	total k = 194	mean = 0.698	0.702	mean = 7.554

Table 3.2. Locus name, repeat type, allelic size range in screening population, fluorophore label, and color of fifteen microsatellite markers used in this study.

	Locus	Repeat type	Allelic size range (bp)	Fluorophore	Color
Reaction "A"	Ce13S	tetra	120-192	6-FAM	blue
	Ce35	di	124-140	PET*	red
	Ce52	tetra	233-245	6-FAM	blue
	Ce126	di	166-178	NED*	yellow
	Ce215	tetra	215-303	NED*	yellow
	Mox306	tetra	175-227	VIC*	green
	Mox329	tetra	158-218	PET*	red
	Ce104	di	144-152	VIC*	green
Reaction "B"	Ce13L	tetra	142-162	6-FAM	blue
	Dlu4235	tetra	131-187	PET*	red
	Ce49	tri	106-109	6-FAM	blue
	Ce63	di	152-268	VIC*	green
	Ce146	tetra	144-156	NED*	yellow
	Ce195	tetra	240-244	NED*	yellow
	Mox294	tetra	227-271	PET*	red

*Proprietary dyes from Applied Biosystems, Inc.

Table 3.3. Pairwise R_{ST} comparisons for all collection sites in the Missouri River. Bold text indicates significant differences after Bonferroni corrections for multiple comparisons.

R_{ST}									
Significance after Bonferroni correction		A	C	D	E	F	G	H	I
	A	-	0.0019	-0.0028	0.0014	0.0078	***0.0126**	0.0113	*** 0.0572**
	C		-	-0.0003	-0.0026	0.0127	0.0072	***0.0118**	*** 0.0488**
	D			-	0.0057	0.0136	0.0057	0.0074	*** 0.0686**
	E				-	-0.0052	-0.0038	-0.0029	0.0263
	F					-	-0.0110	0.0043	0.0120
	G	*					-	-0.0009	0.0258
	H							-	***0.0572**
	I	*	*	*				*	-

Table 3.4. AMOVA summary under the standard model (A) and the microsatellite model B), which assumes the SSM model of microsatellite evolution. The group level refers to (1) impounded and (2) unimpounded populations.

A)

Source of variation	degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among groups	1	17.177	0.05165 Va	1.05
Among populations within groups	7	40.303	0.01871 Vb	0.38
Within populations	437	2128.168	4.86995 Vc	98.58
Total	445	2185.648	4.94031	

Fixation Indices
FST : 0.01424
FSC : 0.00383
FCT : 0.01045

B)

Source of variation	degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among groups	1	13.270	0.03944 Va	0.98
Among populations within groups	7	31.978	0.01237 Vb	0.31
Within populations	437	1739.923	3.98152 Vc	98.72
Total	445	1785.170	4.03333	

Fixation Indices
FST : 0.01285
FSC : 0.00310
FCT : 0.00978

Table 3.5. Results from tests for genetic bottlenecks (i.e. tests for significant heterozygote excess) performed with BOTTLENECK 1.2.02 (Cornuet and Luikart 1996); H_O, observed heterozygosity; H_E, expected heterozygosity; P, p-value for one-tailed test of heterozygote excess.

Collection site	H_E	H_O	P
A	0.688	0.700	0.596
B	0.729	0.576	--
C	0.745	0.704	0.095
D	0.661	0.698	0.271
E	0.692	0.717	0.213
F	0.698	0.690	0.188
G	0.732	0.691	0.892
H	0.727	0.699	0.776
I	0.675	0.686	0.122

Table 3.6. Matrix of linear pairwise distances between populations (sampling areas) based on the central locality (CL) for each. Units are in (river) kilometers.

Site	A	B	C	D	E	F	G	H	I
A - Missouri River, MO	x	644	700	795	1183	1955	2681	2599	3021
B - Kansas River, KS		x	507	602	990	1762	2488	2406	2828
C - Missouri River, NE			x	95	483	1255	1981	1899	2321
D - Platte River, NE				x	420	1193	1192	1918	2258
E - Niobrara River, SD					x	772	1498	1416	1838
F - Missouri River, ND						x	726	644	1065
G - Yellowstone River, MT							x	526	948
H - Missouri River, MT								x	422
I - Missouri River, MT									x

Table 3.7. Matrix of linear pairwise distances from samples on or near the mainstem Mississippi River. Site numbers correspond to Table 4.1 and Figure 4.2 in Chapter 4. Units are in river kilometers.

Site	8	11	20	16	17	18	19
8 – Mississippi River, LA	x	827	1456	1844	2029	2227	2493
11 – Hatchie River, TN		x	790	1178	1363	1561	1827
20 – Missouri River, MO			x	726	911	1109	1374
16 – Mississippi River, IA				x	185	383	649
17 – Wisconsin River, WI					x	278	544
18 – Chippewa River, WI						x	314
19 – Minnesota River, MN							x

Figure 3.1. Sampling area for nine localities (A-I) in the Missouri River basin. Letters correspond to localities listed in Table 3.1.

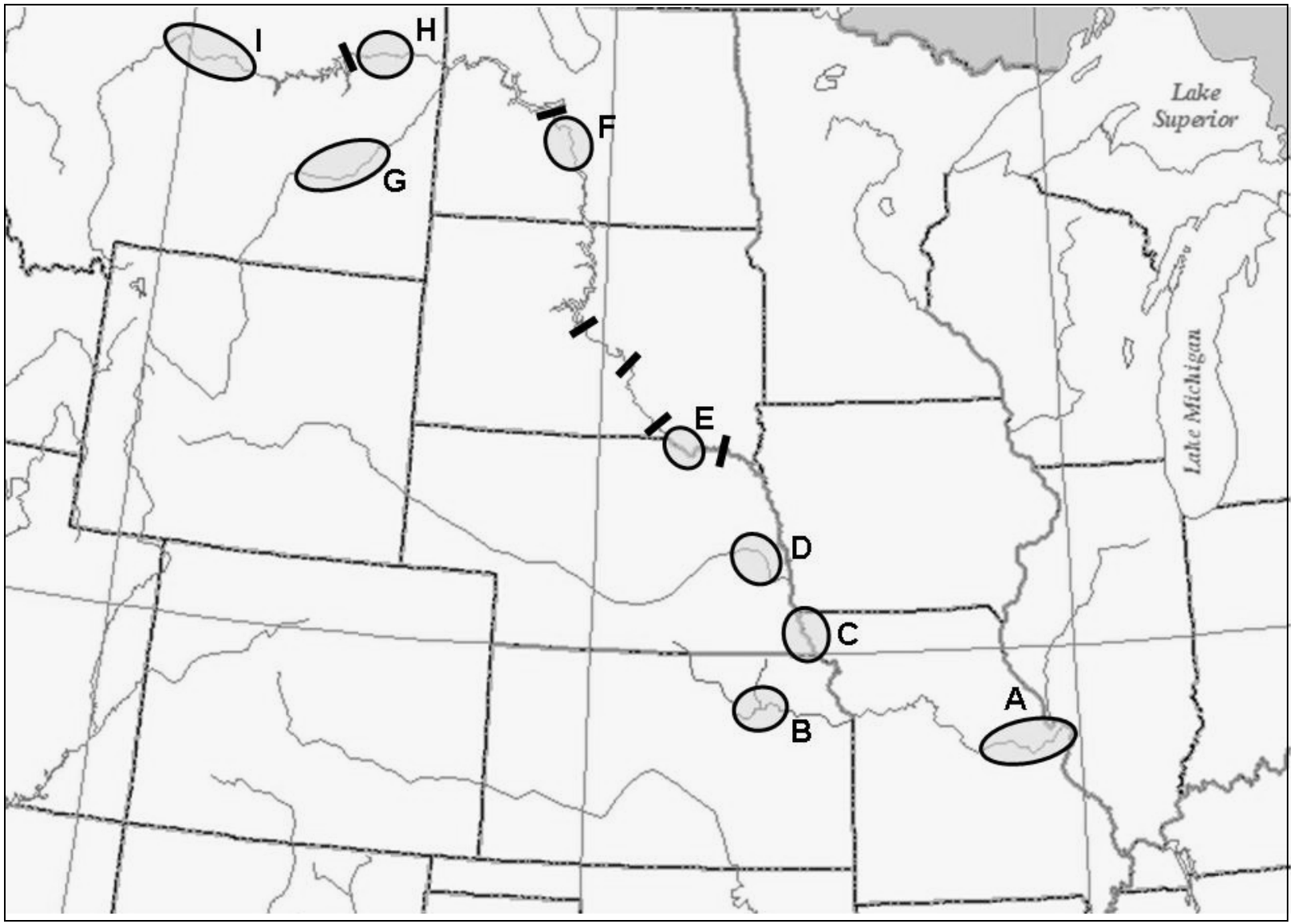


Figure 3.2. GeneMapper screen image of multiplex "A" reaction for individual 17-16. Eight co-amplified loci appear in the top four lines with LIZ500 internal size standard appears on the bottom line. True allele peaks are circled.

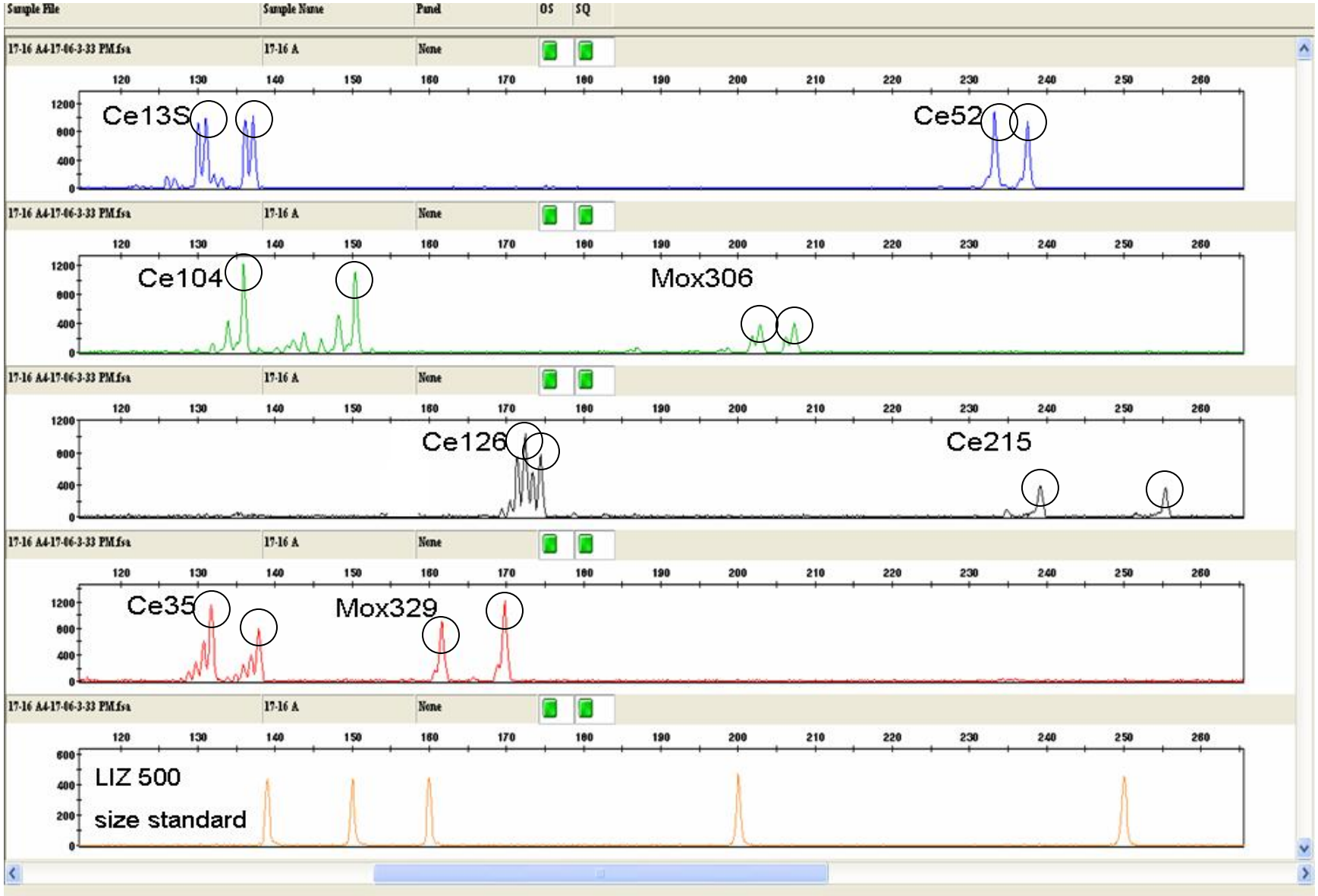


Figure 3.3. GeneMapper screen image of multiplex "B" reaction for individual 24-. Eight co-amplified loci appear in the top four lines with LIZ500 internal size standard appears on the bottom line. Again, true allele peaks are circled.

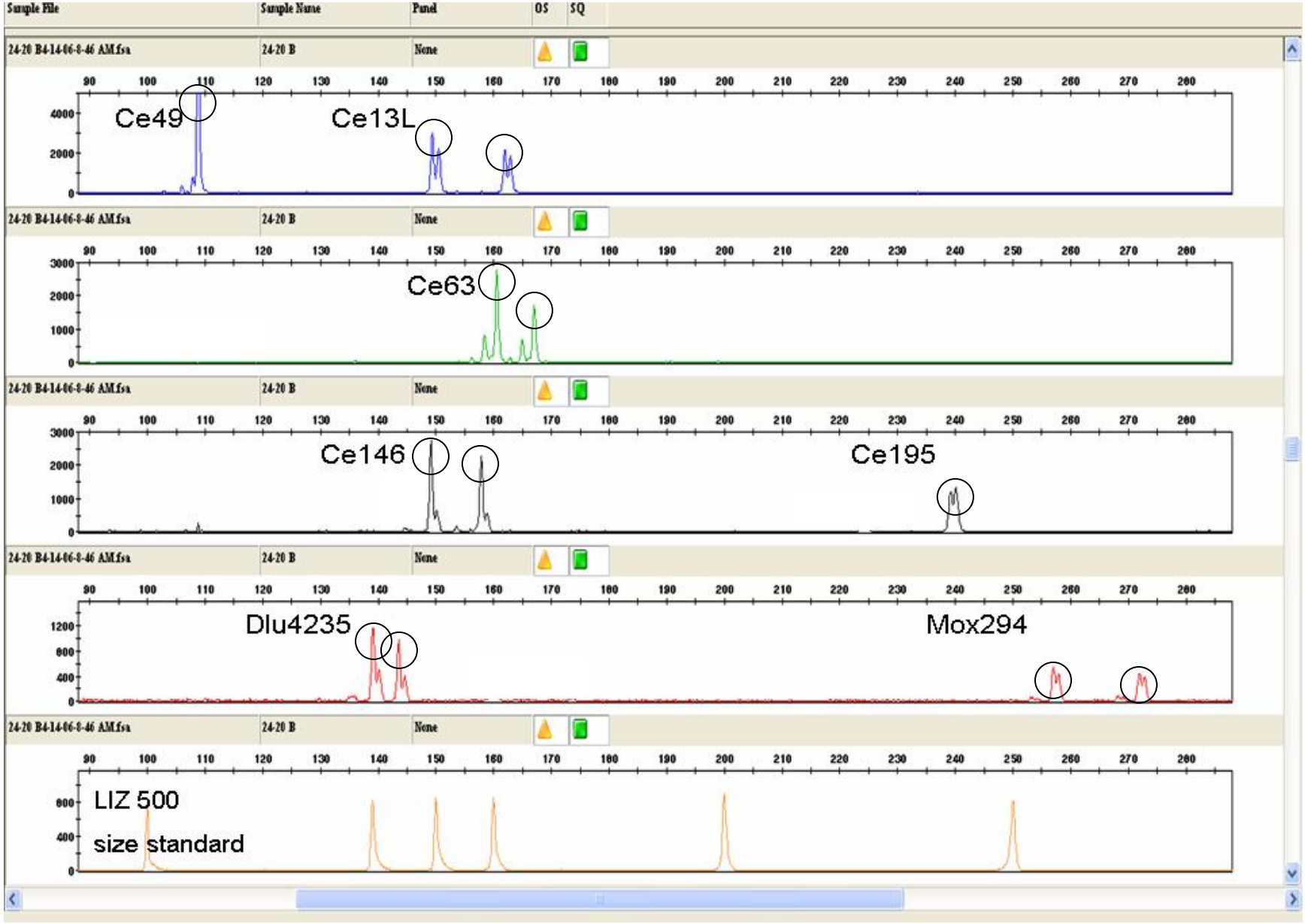


Figure 3.4. Results of Bayesian inference of population structure in the Missouri River drainage as determined by STRUCTURE 2.0 (Falush *et al.* 2003; Pritchard *et al.* 2000); *k* = 2 elicited the highest posterior probability for the number of genetic subgroups. These results represent the outcome when *k* was set at two. Vertical colored bars indicate the fraction of an individual's genome that has ancestry in a given subgroup (in this case, one of two subgroups).

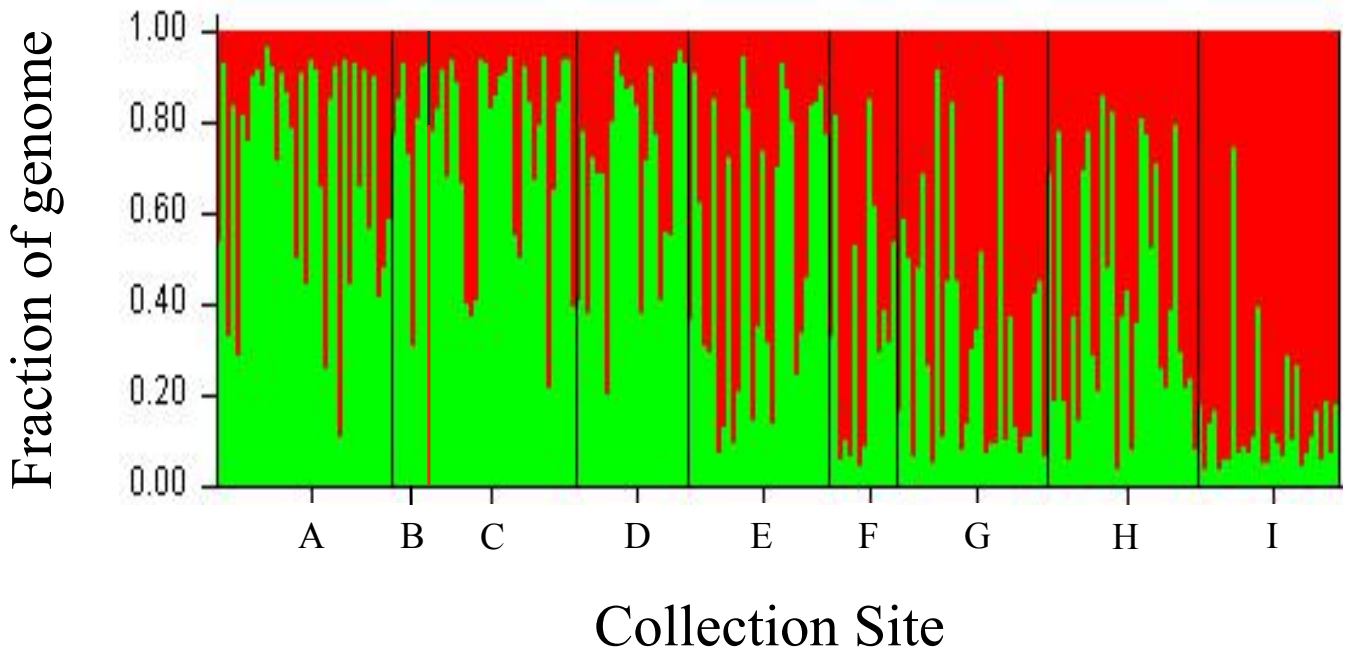


Figure 3.5. Results of Isolation by Distance analysis on Missouri River samples. The graph indicates results of a Mantel test for matrix correlation between log(M) and geographic distance: Z = 44589.1996, r = -0.6186, one-sided p <= 0.9999 from 10000 randomizations (for test of negative correlations, **one-sided p <= 0.0001**)

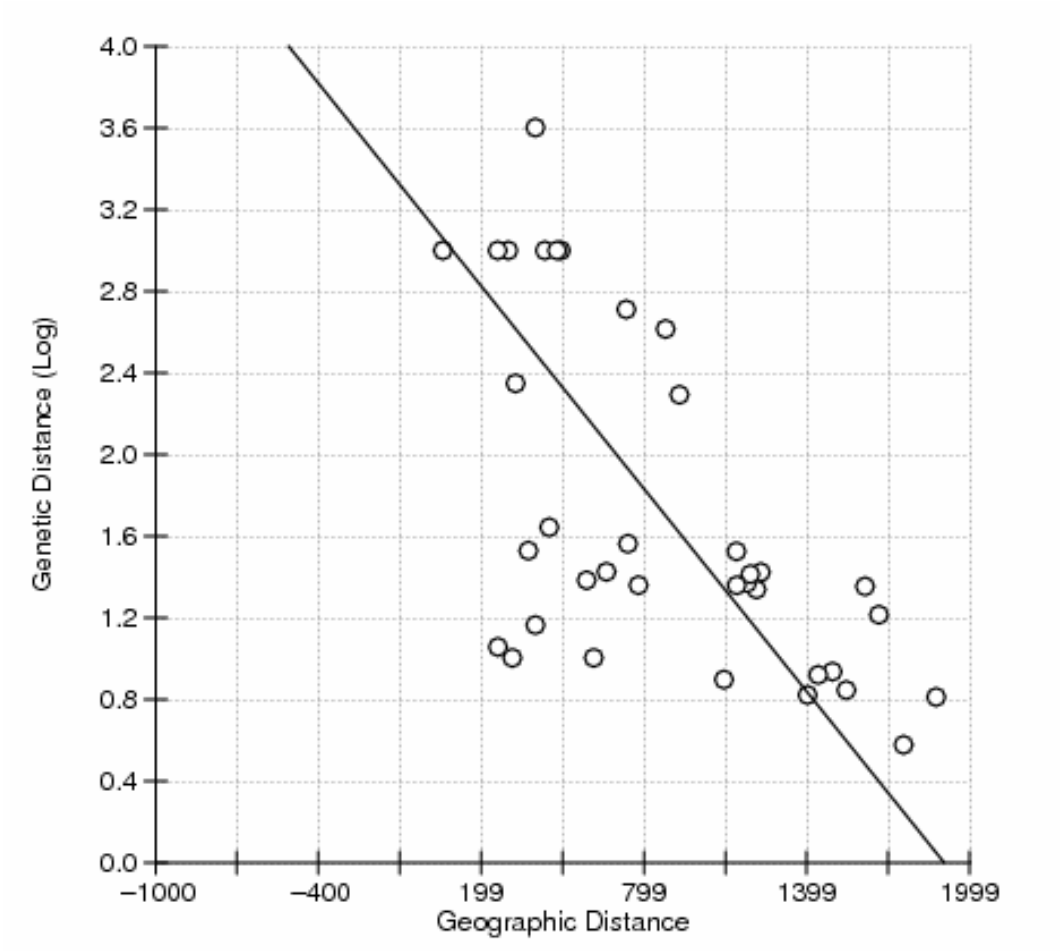
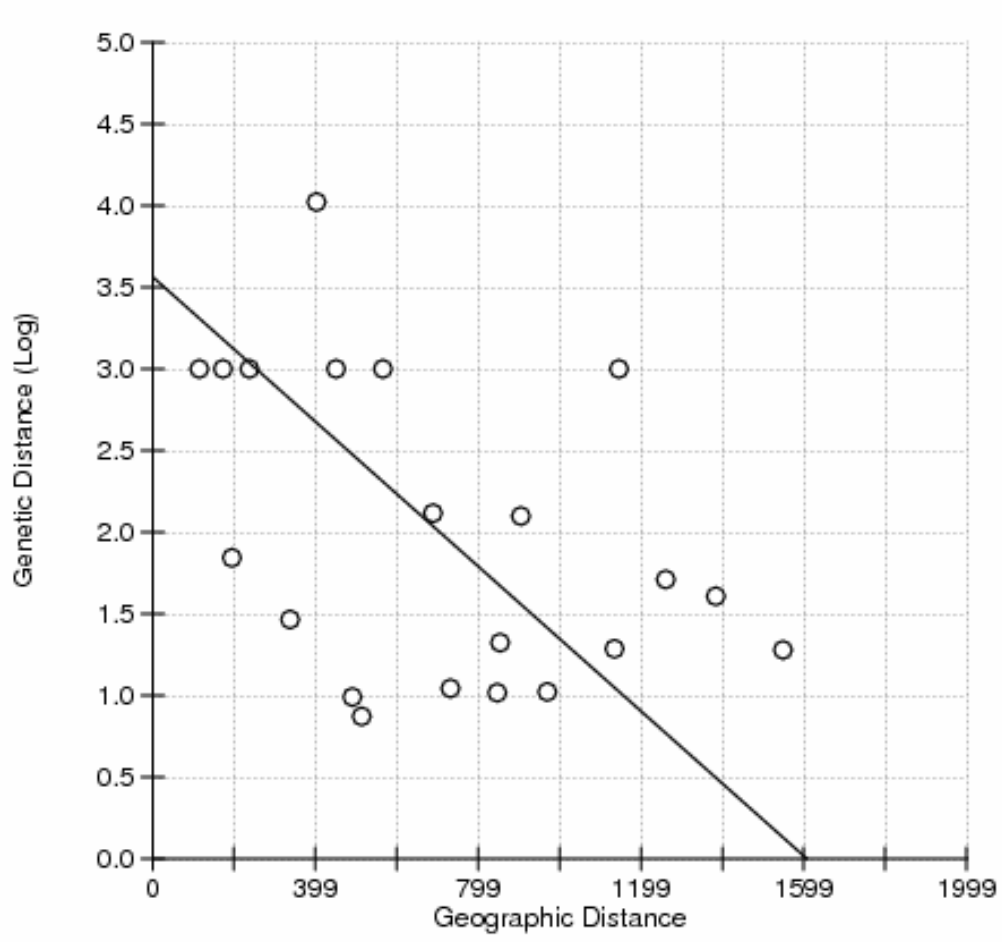


Figure 3.6. Results of Isolation by Distance analysis on Mississippi River samples. The graph shows the results of a Mantel test for matrix correlation between log(M) and geographic distance: Z = 26403.4071, r = -0.4225, one-sided p <= 0.9140 from 1000 randomizations (for test of negative correlations, one-sided p <= 0.0860)



CHAPTER FOUR

Rangewide population structure and intermediate polyphyly in the genus *Cycleptus* (Teleostei: Catostomidae)

Abstract

What are the evolutionary mechanisms responsible for the diversification and extant distribution of large riverine fishes? The attempt to unite observable patterns with evolutionary processes is not a trivial task. Recent advances in molecular and geological data analysis have provided many answers to such questions. Still, our knowledge is far from complete and, owing to wholesale anthropogenic changes, the window of opportunity for investigation is rapidly closing on some systems. Such is the case for large riverine fishes that occupy the Mississippi Basin and Gulf Coastal drainages in North America. The Mississippi Basin is characterized by long-term stability, and, for that reason, harbors many ancient lineages of freshwater fishes (e.g., *Polyodon*, *Scaphirhynchus*, and others). Unfortunately, many have been translocated from one area to another by humans within the past century, thus obscuring the naturally occurring genetic signal. The focal taxon of this research is the freshwater fish genus *Cycleptus*, a highly migratory group of fishes that has not been subject to stocking efforts. These fishes occupy a vast portion of North America and are of prominent conservation concern throughout. Previous studies revealed incomplete lineage sorting in the two described species, *Cycleptus elongatus* and *C. meridionalis*, while the Rio Grande population was reciprocally monophyletic and clearly divergent from the others. The aim of this study

was to characterize inter- and intrabasin population structure throughout the range, to test competing hypotheses regarding the divergence of the two described species, *C. elongatus* and *C. meridionalis*, and to determine whether they are, in fact, genetically isolated. A total of 589 specimens were collected from throughout the known range of the genus. Mitochondrial DNA sequence (control region) and nuclear microsatellite genotypic data (15 loci) were acquired from a total of 151 and 589 individuals, respectively. Bayesian analysis of microsatellite genotypic data indicates distinct subpopulations of *C. elongatus* within the Mississippi basin while mitochondrial markers reveal a pattern of intermediate polyphyly with no gene flow between the two described species.

Introduction

In recent times, increasingly sophisticated methods of inference from molecular genetic data have enabled a better understanding of the evolutionary mechanisms responsible for speciation (Avise 2004; Felsenstein 2004). The attempt to unite observable patterns with evolutionary processes is not a trivial task. New advances in molecular and geological data analysis have provided many answers to such questions. The issue becomes particularly challenging when the taxa in question recently diverged, placing the investigator at the interface between questions of phylogeny and population genetics.

Many recent investigations have illuminated the history of North American freshwater fish taxa; yet, most of these occur east of the Great Plains or west of the Rocky Mountains (Berendzen *et al.* 2003; Near and Keck 2005; Wilson *et al.* 1996). Only a handful of studies involve more wide ranging taxa (Kreiser *et al.* 2001) in the intervening and surrounding landscape, and few have dealt explicitly with troublesome groups that exhibit incomplete lineage sorting. The present study is the first to address these issues in the North American freshwater fish genus *Cycleptus*, a group that is distributed over much of the continent, but whose two described species do not sort at mtDNA loci.

Blue suckers (genus *Cycleptus*) are members of the family Catostomidae (suckers), a group of tetraploid fishes that is broadly distributed throughout North America and includes a single species endemic to Asia, *Myxocyprinus asiaticus* (*Catostomus catostomus* occurs on both continents). Most researchers hypothesize that the family originated in Asia, dispersed across Beringia during the Eocene, and

subsequently diversified in North America (Harris and Mayden 2001; Smith 1981; Uyeno and Smith 1972). Although the phylogenetic position of *Cycleptus* within Catostomidae is still debated, most researchers place it as a basal group either sister to *Myxocyprinus* or to the subfamily Catostominae (Ferris and Whitt 1978; Harris and Mayden 2001), which includes all extant suckers except the subfamily Ictiobinae (buffalofishes and carpsuckers). In either case, it is likely that the cycleptid lineage has been in existence since at least the mid-Cenozoic, although supporting fossil evidence has not been found (Cavender 1986; Swift 1968).

Although they occur over such a vast range in North America (Figures 4.1 and 4.2), intrageneric diversity has only been studied on three previous occasions. Following the elevation of *Cycleptus* to genus (Rafinesque 1820), it was considered monotypic as *C. elongatus* for over 175 years until Burr and Mayden (1999) described a sister species, *C. meridionalis*, from a number of disjunct southeastern Gulf Coast drainages based on an array of bimodally-distributed, non-overlapping morphological characters. A subsequent allozyme study based on 23 individuals collected from four disjunct drainages (Buth and Mayden 2001) showed three distinct groups: i) Mississippi Basin, ii) Pascagoula River plus Mobile Basin, and iii) Rio Grande Basin. Most recently, phylogenetic analysis of mtDNA by Bessert and Orti corroborated previous allozyme work and initiated a formal description of the Rio Grande species (see Chapter 1).

Knowledge of *Cycleptus* diversity, both intrageneric and intraspecies, is important for a number of reasons. Awareness of *Cycleptus* population dynamics will enable a better understanding of other large riverine fish taxa in central North America and, in particular, the role of interdrainage exchange in maintaining species boundaries.

Although geological evidence indicates that drainage patterns within the Mississippi basin have evolved significantly in recent (Quaternary) time (Burr and Page 1986; Cross *et al.* 1986), there has been a relatively stable large riverine presence here since the late Cretaceous / early Cenozoic (Briggs 1986). This is evidenced by the persistence of multiple basal Actinopterygian lineages in the system, including Amiiformes (bowfin), Acipenseriformes (sturgeons and paddlefishes), and Semionotiformes (gars), which, along with *Cycleptus*, are often characterized as the "old river ichthyofauna." None of these lineages exhibit a great deal of extant diversity and most, including *Cycleptus*, are of imminent conservation concern throughout their ranges (Becker 1983; Warren *et al.* 2000) (e.g., *Cycleptus*, see Figure 4.1). Unfortunately, multiple translocations have already been performed in a number of these fishes prior to the collection of any genetic data (see Gardner 2004; Rutledge 1989); as such, the natural genetic signals have been obscured. *Cycleptus* is an important exception. To my knowledge, none of these fishes have been stocked from one location to another in any portion of their range; therefore, examination of extant and historic population structure will not be confounded by recent events and may provide new insights into the history of other problematic groups, such as the *Scaphirhynchus* sturgeons (Simons *et al.* 2001).

Assuming a mid-Cenozoic origin and the likelihood that cycleptid predecessors occupied similar large riverine habitats in North America, there are a number of possibilities for vicariance and divergence, especially during Pleistocene time (Briggs 1986; Cross *et al.* 1986). One specific case pertains to the two described species, *C. elongatus* and *C. meridionalis*. The former occurs throughout the Mississippi Basin and extends westward to the disjunct Colorado River of Texas while the latter occurs only in

a handful of disjunct drainages east of the Mississipi River, up to and including the Mobile Basin (Boschung and Mayden 2004). These ranges are nonoverlapping and physically disjunct (Figure 4.2). If the genus was already present in the upper Tennessee at the time, one possible vicariant mechanism contributing to the divergence and extant distribution of these fishes is the breakup of the ancient Appalachian River, which hypothetically connected the upper Tennessee River to the Coosa River (part of the Mobile Basin) during the mid-Miocene, approximately 16.4 to 11.2 million years ago (mya) (Mills and Kaye 2001; Near and Keck 2005). More plausible explanations exist for concordance with the latter breakup of connections between the lower Tennessee and other rivers in the Mobile Basin (e.g., Tombigbee), which occurred in the Pliocene and Pleistocene (Mills and Kaye 2001). Finally, there is the very real possibility of more recent dispersal along the coast via freshets and reduced coastal salinity following Pleistocene glacial melting.

Is there any level of recurrent gene flow among these disjunct populations? Quite possibly there is, given the close proximity of the mouths of these drainages. The mouth of the Mississippi River is less than 200 kilometers from Mobile Bay, and the Pearl and Pascagoula drainages lie in-between. The account of a single *C. meridionalis* specimen captured several kilometers from fresh water at Dauphin island, Alabama (Swingle 1971) suggests these fishes have enough salinity tolerance for limited marine dispersal, possibly accompanying significant freshets. This individual was distressed when captured and its appearance at this locality was, in fact, attributed to a freshet (Burr and Mayden 1999)

As previously-mentioned, understanding intraspecies (indeed, intrabasin) population structure is paramount for appropriate conservation efforts of these fishes.

Both species of *Cycleptus* are known to migrate considerable distances to spawn (Elstad and Werdon 1993; Hand and Jackson 2003b; Peterson *et al.* 1999), but little is known of site fidelity and other aspects of their natural history. If site fidelity is low and dispersal (i.e. gene flow) high, one would expect to see a lack of distinct population structure in a given basin, with the possible exception of groups isolated by impoundments or other barriers (see Chapter 3).

The aims of this study were, therefore, threefold: i) to estimate intraspecies population structure, particularly that of *C. elongatus* in the Mississippi Basin; ii) to test competing hypothesis regarding the *C. elongatus* / *C. meridionalis* split; and iii) to test the hypothesis of recurring gene flow between the two species. In conducting these analyses, we gain a better understanding of the evolution of the genus - and of large riverine fishes in general. Finally, and equally important, the population genetic information gathered here (and in Chapter 3) will facilitate informed decisions regarding conservation of these fishes.

Materials and Methods

Study location and sample collection

Previous microsatellite genotypic and mtDNA sequence data from 231 individuals captured in the Missouri River drainage (Chapter 3: Table 3.2, Figure 3.1) was incorporated into this project. A coordinated effort by more than 150 academic, governmental, and private researchers led to the addition of another 360 individuals from throughout the remaining range, (Table 4.1, Figure 4.2). Fishes were captured with hoop nets, gill nets, and electroshocking devices. A small (1 cm^2 or less) fin clip was removed

from each fish and preserved in 95% EtOH for shipment to the University of Nebraska. Tissues are vouchered in the personal collection of the auther (see Appendix 2) and are available upon written request.

DNA preparation and amplification

DNA was extracted from tissue samples using either a standard phenol-chloroform protocol (Sambrook *et al.* 1989) or DNeasy® Tissue purification kit (Qiagen). DNAs were eluted in either water or EB buffer (Qiagen). A small number of samples were randomly selected and (1-2 µl) electrophoresed through a 1% agarose gel to check quality. All samples were quantified with a GeneQuant II spectrophotometer (Pharmacia Biotech) and a portion diluted to a standard working stock concentration of 100ng/µl. The remainder of each elution was placed in -70º C for long-term storage. The mitochondrial control region and 15 polymorphic microsatellite markers were amplified, purified, and scored according to the procedures outlined in chapter one (p. 17-19) and chapter three (p. 59-61).

Sample preparation and data collection

For microsatellite loci, genotypes were scored with GeneMapper 3.7 (Applied Biosystems) and the data was transformed to GenePop format (Raymond and Rousset 1995) with GMCONVERT 0.32 (Faircloth 2006). Genotypic data were checked for syntax errors, evidence of null alleles (Hardy-Weinberg equilibrium), and adherence to the Stepwise Mutation Model (Ohta and Kimura 1973) with MicroChecker 2.2.3 (VanOosterhout *et al.* 2004) and MSAnalyzer 4.0 (Dieringer and Schlötterer 2002). Additional transformations of the data set for analysis with STRUCTURE 2.0 (Pritchard

et al. 2000) and Arlequin 3.1 (Schneider *et al.* 2000) were performed with CONVERT 1.3.1 (Glaubitz 2004).

For mtDNA control region data, raw sequences were edited with Sequencher 4.2 (Gene Codes) or Contig Express (Invitrogen). Alignment was performed with ClustalX (Thompson *et al.* 1997) and checked by eye for obvious misalignments. For analysis with NETWORK 4.2 (Bandelt *et al.* 1999) and IM (Hey and Nielsen 2004), the alignment was formatted by hand.

Data Analysis – Population structure

In addition to observed and expected heterozygosities for each locality, deviations from Hardy-Weinberg equilibrium and pairwise F_{ST} estimates (Weir and Cockerham 1984) were calculated for each locality using GenePop3.3 (Raymond and Rousset 1995). The program FSTAT 2.9.3.2 (Goudet 1995; Goudet 2002) was also used to calculate the average allelic richness (A) at each locality with 10 or more samples. The distibribution of genetic variation within and among (i) the three species and (ii) within and among basins and disjunct coastal drainages was assayed with a hierarchical analysis of molecular variance (AMOVA – see Table 4.2) (Excoffier *et al.* 1992) performed in Arlequin 3.1 (Excoffier *et al.* 1992; Schneider *et al.* 2000). This test was performed under the standard data model ("different alleles are considered mutationally equidistant from each other").

A second approach that does not require assignment of invidividuals to groups *a priori* (as in AMOVA) is the Bayesian clustering technique implemented in the software STRUCTURE 2.0 (Pritchard *et al.* 2000). Here, the data are used to define the number of subpopulations (*k*). The method assumes HW and linkage equilibrium within

subpopulations and seeks combinations of individuals which maximize these values. To use the method, one performs several runs at *k* values from 1 to n. Assuming quality control in preparation and performance of these runs (e.g., unambiguous data, adequate burn-in, etc.), the value of *k* eliciting the highest mean posterior probability over several runs indicates the true number of genetic subpopulations present. Four simulations were conducted at each value of *k* from one to twelve. A burn-in period of 2.5×10^5 generations was followed by 1.0×10^6 generations of data collection.

Phylogenetic structure was previously estimated in the genus with mitochondrial markers (Chapter 1); yet, I wished to visualize the distribution of haplotypes among the two species with an unrooted haplotype network. This was calculated with the software NETWORK 4.2 (see Bandelt *et al.* 1999; www.fluxusengineering.com) using the MJ algorithm (Figure 4.4). The advantage in doing this is that it allows the investigator to quickly assess the degree of allele sharing across populations (again, across species in this case) and provides an alternate view of phylogenetic structure. Note that specimens from the Rio Grande were excluded from this analysis due to the previously-determined level of divergence (Chapter 1). In essence, they are not pertinent to the question at hand and would have simply appeared as a monophyletic clade separated from the others by a very long branch.

Demography

Population divergence times, historical gene flow, and (female) effective population sizes were estimated using a model of Isolation with Migration as implemented in the software IM (Hey and Nielsen 2004). Briefly, the program applies the IM model (Figure 4.5) to genetic data from a pair of closely related populations (or

species, in this case). It uses a Markov Chain Monte Carlo (MCMC) simulation process to estimate posterior probability densities for each of the parameters included in the model. In this case, the basic 6-parameter model was chosen. This includes q1, q2, and qA, referring to the effective size of population 1, population 2, and the ancestral population, respectively; m1, the migration rate of genes from population 1 to population 2 in the coalescent (or, as time moves forward, from population 2 to population 1) and m2, migration rate in the opposite direction; and *t*, the time since divergence of the populations. A subroutine also allows the user to obtain an estimate of the time to most recent common ancestor (TMRCA) for all alleles and/or genotypes included in the input file.

The model parameters are scaled to the 'per year' mutation rate (μ) for the entire locus; thus, they can be converted to demographic parameter estimates if the user defines the mutation rate (that is, for each locus considered – in this case, just the mtDNA control region) and provides a generation time for the organism in the input file. The generation time (in the IM software, 'u') for both *C. elongatus* and *C. meridionalis* is approximately ten years (Becker 1983; Boschung and Mayden 2004).

A recent phylogenetic study of Otocephalan fishes based on mitogenomic DNA placed an approximate divergence time of 101 mya for *Myxocyprinus asiaticus* and *Carpiodes carpio* (Peng *et al.* 2006), both of which have been hypothesized as sister (or part of a sister clade) to *Cycleptus* (Harris and Mayden 2001; Smith 1992). A comparison of *M. asiaticus* and *C. carpio* mtDNA sequences from GenBank (Accession numbers; *Carpiodes carpio*: AY366087, NC_005257, AB126083, AF454867*; Myxocyprinus asiaticus*: AF036176, AY526869, NC_006401, AB223007, AY986503)

yields a cytochrome *b* divergence of 17%, which equates to a substitution rate of approximately 0.17 % / MY. Considering the total length of cyt *b*, 1140 base pairs, this amounts to 9.59×10^{-7} mutations per year for the locus ('μ' in IM). Since the mean control region sequence divergence between *C. elongatus* and *C. meridionalis* is approximately 2.75 times greater than that for cyt *b* (see Chapter 1, Table 1.1), a control region rate of 2.64×10^{-6} mutations per year (or 2.64×10^{-5} mutations per generation given an estimated 10 year generation for *Cycleptus* species) was estimated (e.g., Omland *et al.* 2006).

In order to obtain reliable results from this analysis, it is necessary to perform three separate runs (simulations). The first simulation allows the user to determine appropriate burn-in lengths, uninformative prior distributions, and a number of other settings that can be advantageous when collecting real data. An initial run was performed with unrealistic prior settings as follows: $t = 300$; q1, q2, and qA all = 500, and m1 and m2 = 100. All of these priors were adjusted substantially for the final two runs, which are identical to one another except that each starts from a different 'seed' point. Priors for the final two runs were set to: $t = 10$; q1 = 100, q2 = 300, qA = 300, and m1 and m2 both = 5. A burn-in of 1.0×10^6 generations was used for the final two simulations and was followed by another 3.0×10^6 generations of data collection to ensure that the ESS (effective sample size) for each parameter was 50 or higher (Pritchard and Wen 2004). Seed numbers used for the two runs were 416 and 3119, respectively. As anticipated, these two runs converged to similar estimates for all six model parameters. These were converted to more meaningful demographic parameter estimates (see the IM

documentation) and plots of posterior distributions were prepared with Microsoft Excel (Figure 4.6, A-F).

Results

Summary Statistics and Population structure

The number of alleles (*na*) detected per sample ranged from a low of 40 in nine samples from the Sabine River to a high of 134 among 38 samples from the Wabash River (Table 4.1). Within the Mississippi Basin, gene diversity was relatively high: 0.66 or higher when the sample size was greater than 10 (Table 4.1). In contrast, disjunct drainages, including the Rio Grande, Colorado River, Sabine River, and Alabama River (site numbers 1,4,5, and 6, respectively, in Figure 4.2), showed reduced levels. The measure of allelic richness (A), in which all values are scaled to the smallest sample size, was also reduced in those drainages. Finally, the upper Missouri River showed significantly less allelic richness than the rest of the Mississippi Basin (Table 4.1; also see Chapter 3, p. 65-66).

The first AMOVA, which assessed the distribution of molecular variance within and among the three cycleptid species, indicated that roughly 12% of the variance occurred among species with 4% among populations (sample sites) within species and 84% within populations (Table 4.2A). These numbers changed slightly when the samples were grouped by disjunct drainage (Alabama, Sabine, Colorado, Rio Grande, and Mississippi Basin). When this was done, more than 15% of the variance occurred between drainages while that between populations within drainages dropped to approximately 1% and that within populations remained stable at approximately 84% (Table 4.2B).

The second measure of population structure, Bayesian analysis with STRUCTURE 2.0 (Pritchard *et al.* 2000), was run four times at each value of *k* from one to twelve. The setting of $k = 9$ best explained the data as it yielded the highest mean posterior probability over the four simulations. The method clearly recovered the disjunct Rio Grande, Colorado, Sabine, and Mobile basins as distinct subpopulations with very little admixture in the genomes of any individuals from those drainages (Figure 4.3). The other five clusters fall within the Mississippi Basin, as follows: 1) one large Tennessee / Ohio / upper Mississippi cluster that includes the lowest sample site from the Missouri River (site number 20 in Figure 4.2); 2) two intergraded clusters in the Missouri River, in a pattern nearly identical to that indicated by exclusive analysis of this drainage (Chapter 3); and 3) two distinct clusters in the lower Mississippi. Note that cluster 6 in Figure 4.3 is actually a sample of 33 individuals from the Red River in the southeast corner of Oklahoma.

The minimum spanning haplotype network (Figure 4.4) reflects the polyphyly of *C. elongatus* and *C. meridionalis*: three distinct, monophyletic *C. meridionalis* clades are nested within the *C. elongatus* network at different points. Note that no haplotypes are shared between the two species (i.e. no multicolored pie diagrams appear in the figure).

Demography

The second and third IM simulations converged to similar parameter estimates and the longer of the two runs is presented here. Posterior distribution peaks for each parameter in the IM model yielded were: q1 = 22.2, q2 = 117.5, qA = 4.65, $t = 4.65$, m1 = 0.005, and m2 = 0.005. The 95% L/H (low and high point; confidence interval) for each were q1 {13.64, 46.52}, q2 {79.95, 184.65}, qA {1.35, 79.05}, t {3.13, 7.21}, m1

{0.005, 0.245}, and m2 {0.005, 0.085}. Conversions to demographic parameter estimates are plotted in Figure 4.5. In summary, θ_{Cm}, the effective number of female *C. meridionalis*, is approximately 210,000; θ_{Ce} (female *C. elongatus*) = 1,109,000; and θ_{Anc} (female ancestral) = 44,000. The time of population divergence (*t*), is estimated at 1.76 mya, and occurred long after the time to most recent common ancestor (TMRCA = 2.43 mya) for all haplotypes in the analysis. Finally, migration rates (rate at which genes come into a population) in either direction converted to negligible rates of 1.32×10^{-8} per year or 1.32×10^{-7} per generation.

Discussion

What mechanisms underlie the extant distribution of any given organism and why is it important to know this? As anthropenic change envelopes the global landscape, it is important that we seize opportunities to understand natural population dynamics while windows for study remain open. The results of these kinds of investigations not only allow us to make predictions about sustainability of focal taxa, but may also provide us with interesting glimpses into the past. Outcomes may ultimately provide for better-informed mitigation efforts (if necessary), or at worst, satisfy a natural curiousity regarding the evolution of the groups in question.

Here, rangewide population structure was investigated in the fish genus *Cycleptus*, a group of fishes that inhabits a vast, yet diminishing range in North America. Given the rangewide conservation status of these fishes, it is important to understand population structure, and, since the only genetic investigations of these fishes appear in earlier chapters here, the opportunity was taken to include all three species in the same analysis. Since the natural genetic signal in cycleptids has not been obscured by

translocations (i.e. 'stocking,') as it has in other 'old river' ichthyofauna, this study may also lend comparative insights regarding the evolution of those groups.

Analyses of molecular variance indicated that there is distinct structure within the genus, and that much of the structure is associated with geography. If the three defined species (two previously defined plus the third in Chapter 1) satisfy the biological species concept of reproductive isolation, we would expect at least a moderate portion of the molecular variance would to occur between them. This proved to be the case as 12% of the variance was attributed to among-species effects. Interestingly, though, geographic isolation increased this effect. When populations were grouped according to disjunct locality rather than current taxonomy, 15% of the variance occurred among them (Table 4.2). This suggests further study of geographically isolated populations and possible classification as distinct management units (MU) following the criteria outlined by Moritz (1994).

Bayesian analysis with STRUCTURE 2.0 (Pritchard *et al.* 2000) corroborated the AMOVA results as all populations inhabiting disjunct drainages were recovered as distinct and separate (see Figure 4.3, clusters 1, 7, 8, and 9) with very little admixture. Within the Mississippi Basin, an additional five distinct clusters were recovered, but with a generally higher degree of admixture (= higher gene flow) as reflected in the individual (vertical) bars in Figure 4.3. The notable exception, as outlined in Chapter 3, is the pattern occurring in the Missouri River, where almost certainly there is an absence of upstream gene flow, but only in very recent time. In terms of ESU or MU designation for subdivisions in the Mississippi basin, these results do not refute a more detailed null hypothesis of exchangeability (Crandall *et al.* 2000; Templeton 1989); however, it is

unclear whether any of the Mississippi Basin subdivisions outlined here possess adaptations to local environmental conditions, as has been seen in other fishes (Cooke and Kassler 2001). If this is the case, the clusters may be rendered ecologically inexchangeable and would further support their designation as distinct units (Crandall *et al.* 2000). Variation in the length of lip papillae among cycleptids in the Mississippi Basin (Burr and Mayden 1999) lends credence to this possibility. The results of this analysis provide a logical starting point for further investigations in this direction.

From a conservation standpoint, a clear understanding of extant population structure is critical; yet, it is equally important to understand the evolutionary history of the taxa in question. For instance, how and when did the *C. elongatus* / *meridionalis* split come about? More precisely, when did a common ancestor come to reside in the coastal drainages now occupied by *C. meridionalis*, and when did the two lineages begin to diverge? Finally, does the timing of the split coincide with any geological events that could facilitate vicariance?

One approach that has gained recent popularity, particularly in its applicability to taxa that exhibit incomplete lineage sorting, is the Bayesian technique implemented in the software IM (Pritchard *et al.* 2000). This method allows the estimation of parameters in a model of isolation with migration between two closely related populations or species (Figure 4.5). Two long simulations (4.0×10^6 generations) converged to similar estimates for all model parameters and these were converted to more meaningful demographic parameter estimates (Figure 4.6A-F).

The posterior distribution peak for population divergence time occurred at 1.76 mya (95% L/H = 1.19-2.73), post-dating TMRCA (2.43) by an estimated 670,000 years

(Figure 4.6D). This suggests that: i) much of the extant genetic diversity among the two species was already present prior to divergence, and ii) divergence occurred in the early to mid-Pleistocene, or, at the earliest, the late Miocene. This contradicts the hypothesis of the mid-Miocene breakup (16.4-11.2 mya) of the ancient Appalachian River (Mills and Kaye 2001; Near and Keck 2005), which hypothetically connected the upper Tennessee River with the Coosa River of the Mobile Basin, as a mechanism for vicariance. However, the estimated time of divergence is consistent with the Pleistocene breakup of other hypothetical connections, such as that between the Hatchie River (current tributary of the lower Mississippi River) and the Pearl and/or Pascagoula drainages in present-day northern Mississippi. Given the reduced effective size of the common ancestral population relative to extant populations of both species (Figure 4.6A-C), it is also reasonable to hypothesize expansion from a reduced Pleistocene refugium in the lower Mississippi. Unfortunately, it is difficult to ascertain the potential impact of marine dispersal in forming the extant distribution of these fishes; however, a more intense effort focused only on those drainages harboring *C. meridionalis* populations would yield valuable information. Future efforts are planned in this direction.

Another salient features of the IM analysis is the estimated rate of gene flow in each direction (m1 and m2) of 0.005 (Figure 4.6E-F). This equates to 1.32×10^{-8} migrants per generation, which effectively means long-term reproductive isolation since the 'populations' diverged. This, in combination with the pattern of monophyletic subclades in the haplotype network (Figure 4.4), indicates that the two species are in the latter stages of intermediate polyphyly, or, in the recently proposed terminology by Omland *et al.* (2006), they would be classified as allophyletic. This combination of

allophyly, reproductive isolation, and morphological differences lends strong support to the species designation of *C. meridionalis* by Burr and Mayden (1999).

Finally, the estimates of effective population size (Figure 4.6A-B) speak to the current conservation status of both species (Figure 4.1). Cycleptid fishes and other round-bodied suckers are considered bioindicators of stream quality (EPA 1989), and their susceptibility to pollutants is a probable cause for (apparent) decline in many portions of the range. IM analysis indicates robust (female) effective population sizes in both species (θ_{Cm} = 210,000; θ_{Ce} = 1,109,000); thus, implying no imminent concern for the persistence of either. While these results are encouraging for the immediate future, caution is suggested in interpreting them as a panacea. Continued monitoring is encouraged throughout, especially given the intrabasin structure implied here – and the possibility of locally adapted populations.

In conclusion, this study sheds important light on evolutionary history, population structure, and demography in the genus *Cycleptus*. Other 'old ichthyofaunal taxa' (Robison 1986) also show ambiguous relationships at mitochondrial markers, yet, are clearly distinguishable morphologically (Simons *et al.* 2001). Future comparative studies using multiple approaches among these taxa may lead to a better understanding of the process of speciation and maintenance of species boundaries in these groups. Less directly, such studies will also serve to unravel the mysteries of drainage evolution in the major basins and coastal drainages of central North America.

Acknowledgements

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Table 4.1. Sampling localities and numbers for microsatellite genotypic analyses. Site numbers correspond to those in Figure 4.2; *n*, number of individuals; *na*, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; A, allelic richness.

Site number	River/drainage	State/Country	*n*	*na*	Gene diversity (H_O)	H_E	A
1	Alabama	AL	30	85	0.592	0.661	5.532
2/3	Pearl / Pascagoula	MS	6	51	0.536	0.635	-
4	Sabine	TX / LA	9	40	0.389	0.469	-
5	Colorado	TX	30	72	0.519	0.520	4.521
6	Pecos / Black	NM	5	52	0.715	0.833	-
7	Rio Grande / Conchos	TX / Mexico	21	86	0.627	0.706	5.916
8	Lower Mississippi	LA	32	127	0.679	0.700	7.510
9	Red	AR	5	48	0.680	0.827	-
10	Red	OK	33	113	0.669	0.681	6.718
11	Hatchie	TN	19	101	0.751	0.759	7.088
12	TN / Cumberland	TN	3	36	0.950	0.917	-
13	Upper Tennessee	TN	9	78	0.758	0.779	-
14	Ohio	KY / OH	30	124	0.697	0.720	7.468
15	Wabash	IN	38	134	0.700	0.706	7.638
16	Middle Mississippi	IA	11	92	0.727	0.767	7.667
17	Wisconsin	WI	32	123	0.713	0.746	7.226
18	Chippewa / Red Cedar	WI	30	121	0.730	0.765	7.302
19	MN / Upper Miss.	MN	18	101	0.751	0.754	7.149
20	Missouri	MO	30	121	0.678	0.696	7.340
21	Kansas	KS	6	51	0.576	0.729	-
22	Missouri	NE	38	125	0.755	0.764	7.290
23	Platte	NE	23	111	0.663	0.693	7.311
24	Missouri / Niobrara	NE / SD	29	121	0.681	0.705	7.271
25	Missouri	ND	14	83	0.688	0.738	6.343
26	Missouri	ND / MT	30	112	0.733	0.748	6.629
27	Yellowstone	MT	31	117	0.733	0.745	6.902
28	Missouri	MT	29	104	0.683	0.678	6.591
	Totals		591	202	0.68	0.72	**6.870**

Table 4.2. AMOVA summary under the standard model when (A) populations are grouped by present species designation (3 groups); and (B) by disjunct location (5 groups).

A)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among species	2	144.613	0.55988 Va	11.95
Among populations within species	24	276.431	0.17446 Vb	3.72
Within populations	1153	4557.026	3.95232 Vc	84.33
Total	1179	4978.070	4.68666	

Fixation Indices
FST : 0.15669
FSC : 0.04228
FCT : 0.11946

B)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among disjunct areas	4	277.779	0.72133 Va	15.24
Among populations within disjunct areas	22	143.266	0.05852 Vb	1.24
Within populations	1153	4557.026	3.95232 Vc	83.52
Total	1179	4978.070	4.73218	

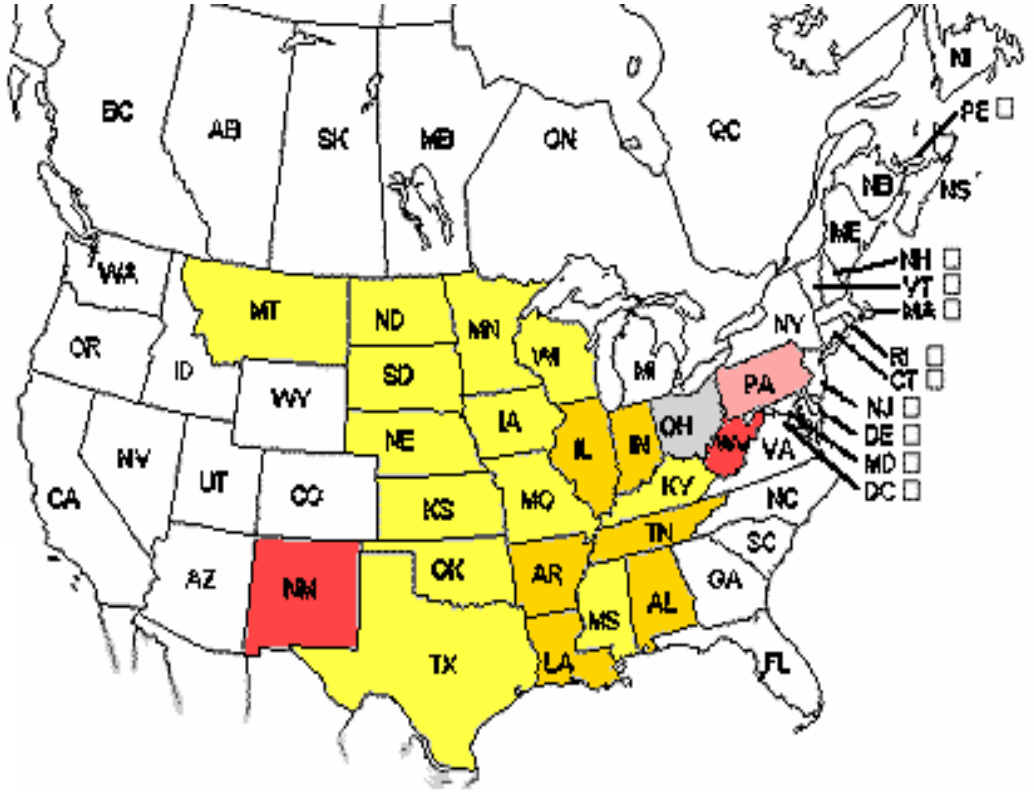
Fixation Indices
FST : 0.16480
FSC : 0.01459
FCT : 0.15243

Figure 4.1. State conservation status for *Cycleptus elongatus* as determined by the Nature Conservancy. For rank definitions, see Master (1997) and Stein (2002).

State/Province Conservation Status Rank

- Presumed Extirpated
- Possibly Extirpated
- Critically Imperiled
- Imperiled
- Vulnerable
- Apparently Secure
- Secure

- Reported
- Migratory Transient
- Exotic
- Unranked
- Under Review
- Unrankable



NatureServe.org 2006

Figure 4.2. Sampling locality map. Circles indicate sampling localities. Locality numbers correspond to those appearing in Table 4.1. Shaded areas represent the range of the three known *Cycleptus* species: red = *C. meridionalis*, blue = *C. elongatus*, and green = the undescribed species from the Rio Grande basin. Map, courtesy of NatureServe (2005), may be accessed at: http://www.natureserve.org/explorer/servlet/NatureServe.

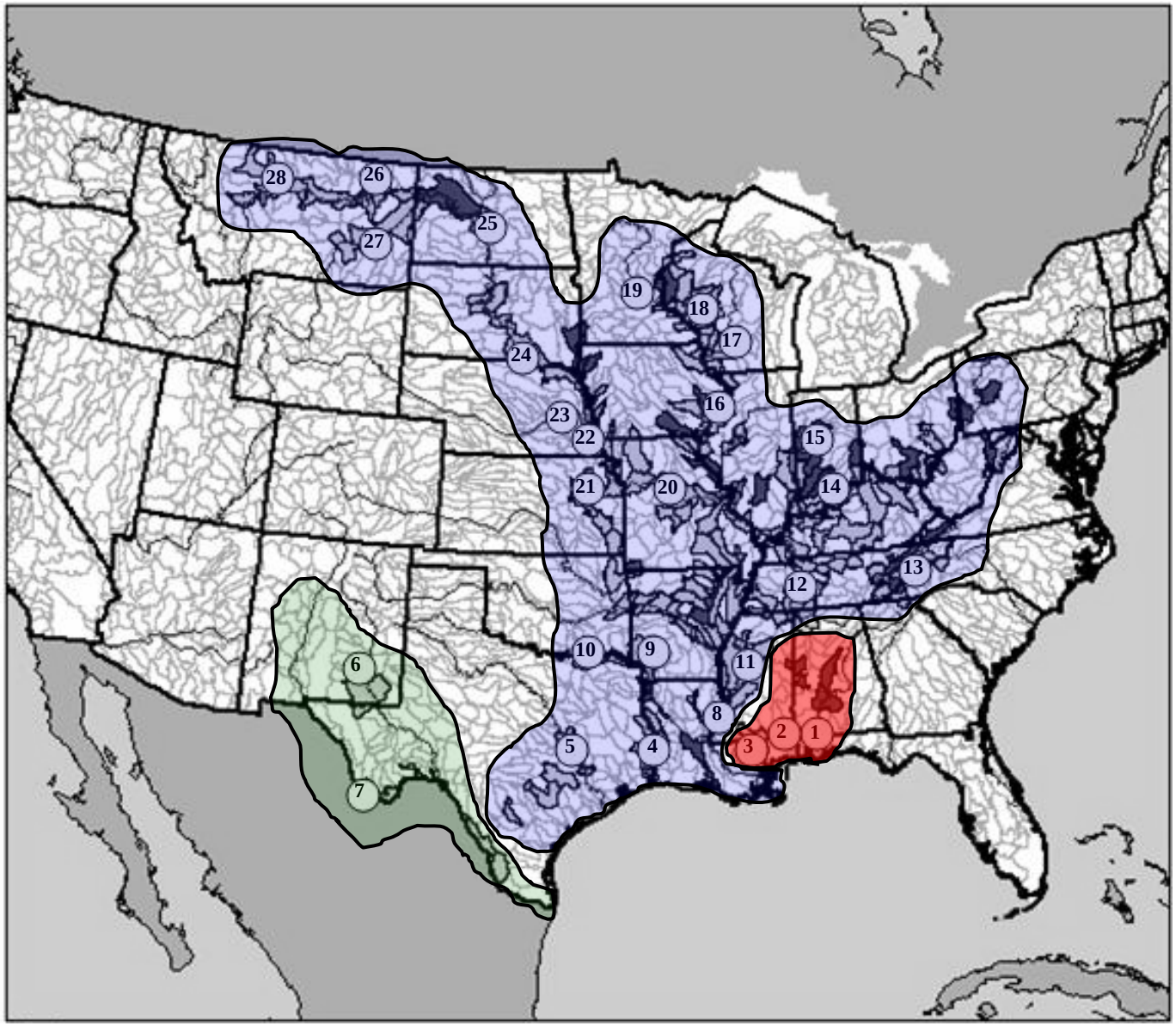
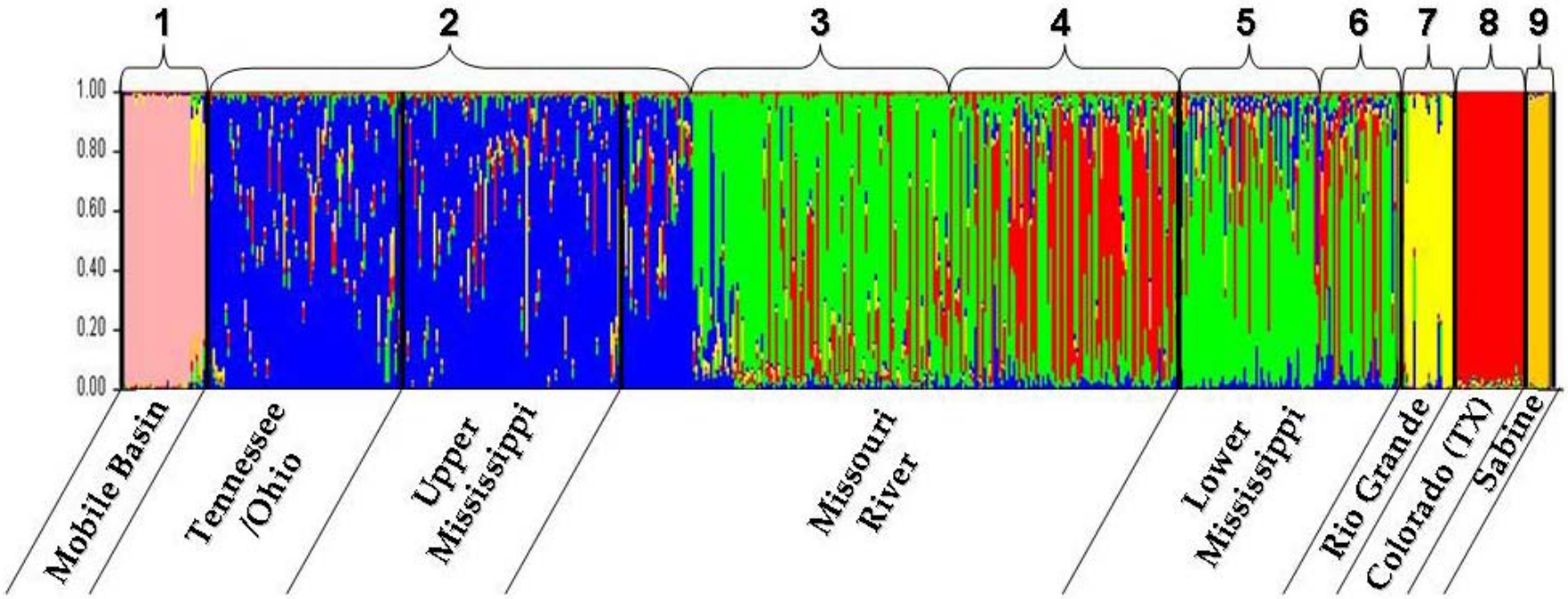


Figure 4.3. Bayesian inference of population structure as determined with STRUCTURE 2.0 (Falush *et al.* 2003; Pritchard *et al.* 2000). The graph represents a range-wide sample of 596 individuals in the genus *Cycleptus*. Four runs were performed with each value of *k* (number of genetic subgroups) from 1 to 12; *k*=9 provided the highest mean posterior probability. The results shown are from the single *k*=9 run with the highest posterior probability. Vertical colored bars indicate the fraction of an individual's genome that has ancestry in a given subgroup. Hydrologic units appear below the x-axis while actual genetic subgroups (*k* = 1-9) appear above. Note the graded structure in the Missouri River drainage as indicated in Chapter 3; the *k1* group (Mobile basin) is *C. meridionalis*; *k7* is the undescribed species from the Rio Grande; all others are *C. elongatus*.



Full range STRUCTURE results: k = 9 (*n*=589)

Figure 4.4. Unrooted haplotype network computed with NETWORK 4.2 (Bandelt *et al.* 1999) based on 125 mtDNA control region sequences from throughout the ranges of *C. elongatus* and *C. meridionalis*. Dark shading represents *C. meridionalis*; non-shaded circles represent *C. elongatus*. The area of each circle corresponds to the number of individuals possessing that haplotype. The text in each circle indicates the sampling locality (state or country) and number of individuals (if >1).

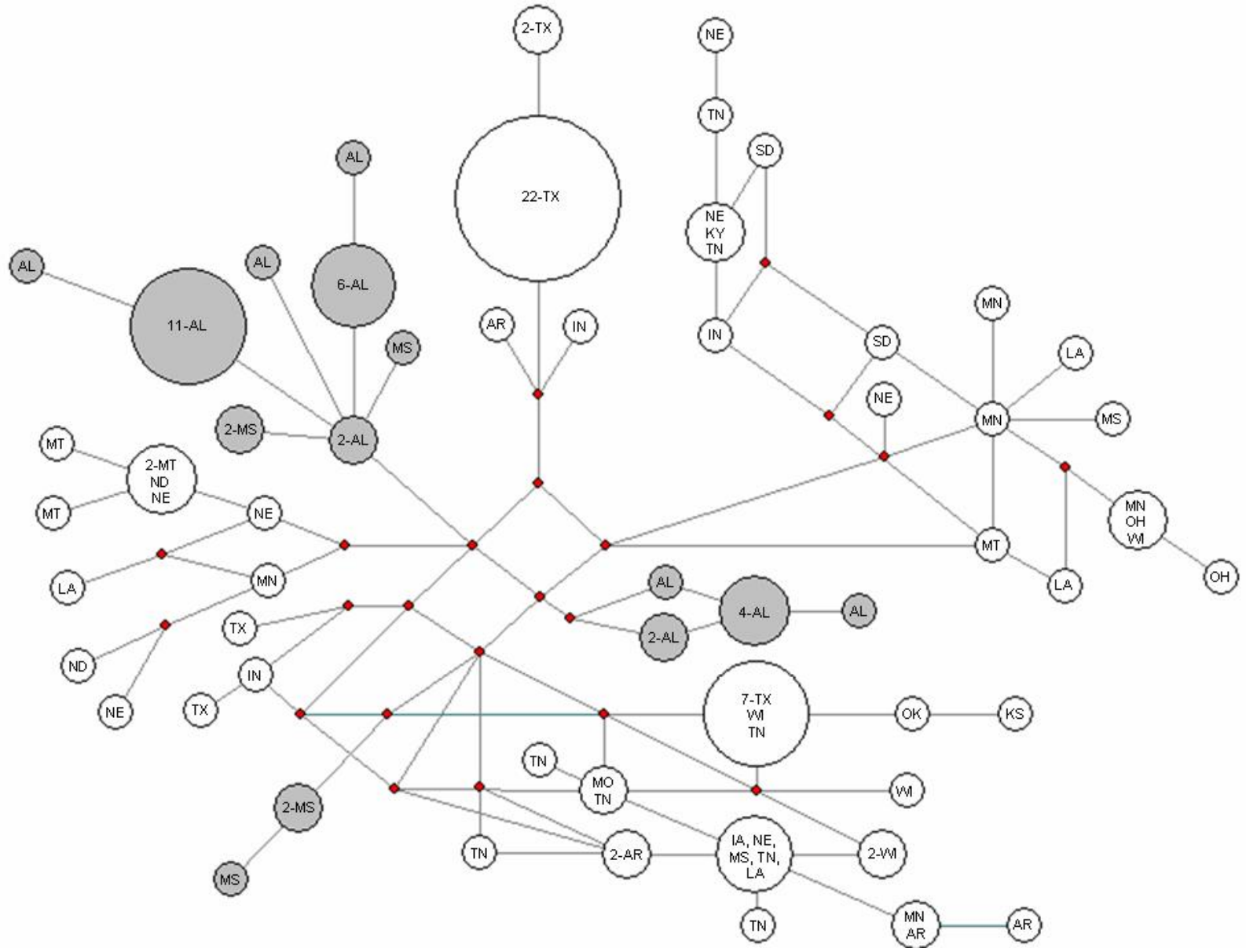


Figure 4.5. The Isolation with Migration model described by Nielsen and Wakeley (2001) and implemented in the program IM (Hey and Nielsen 2004). θ_A, θ_1, and θ_2 indicate effective population sizes of an ancestral population and two descendent populations, respectively; *m1* and *m2* indicate migration rates in the coalescent; *t* is the estimated time of divergence between the descendent populations.

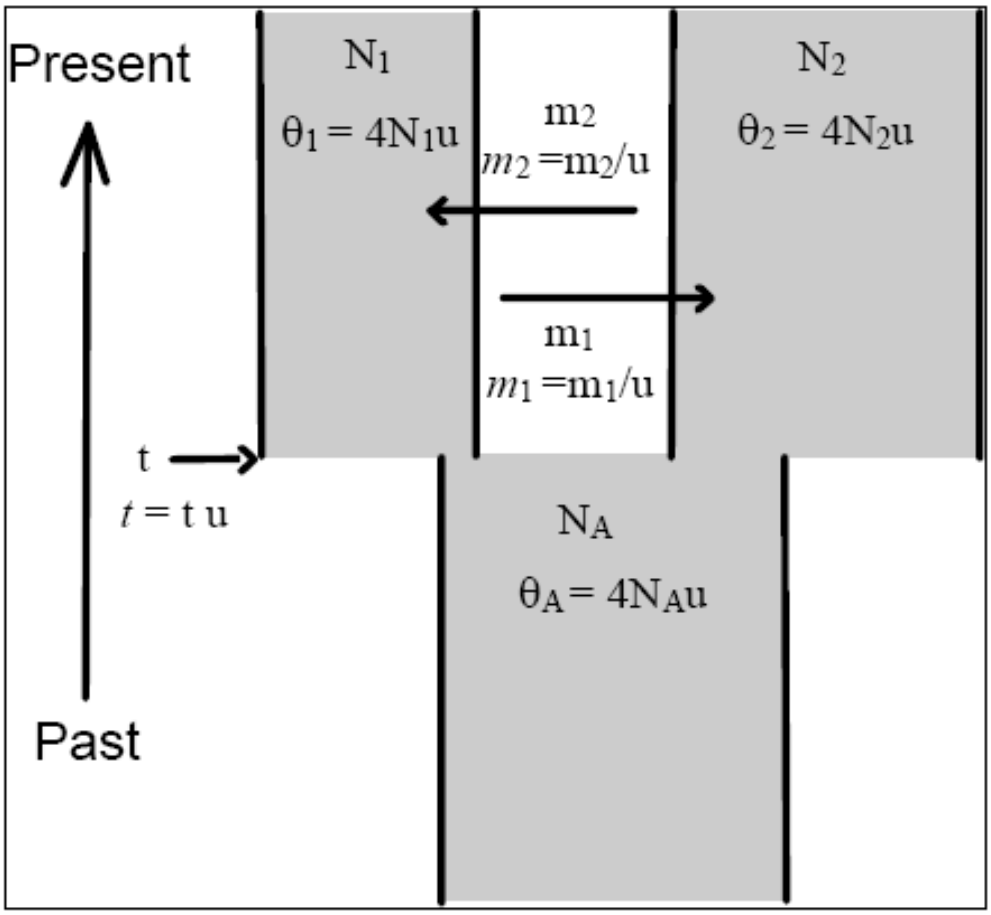
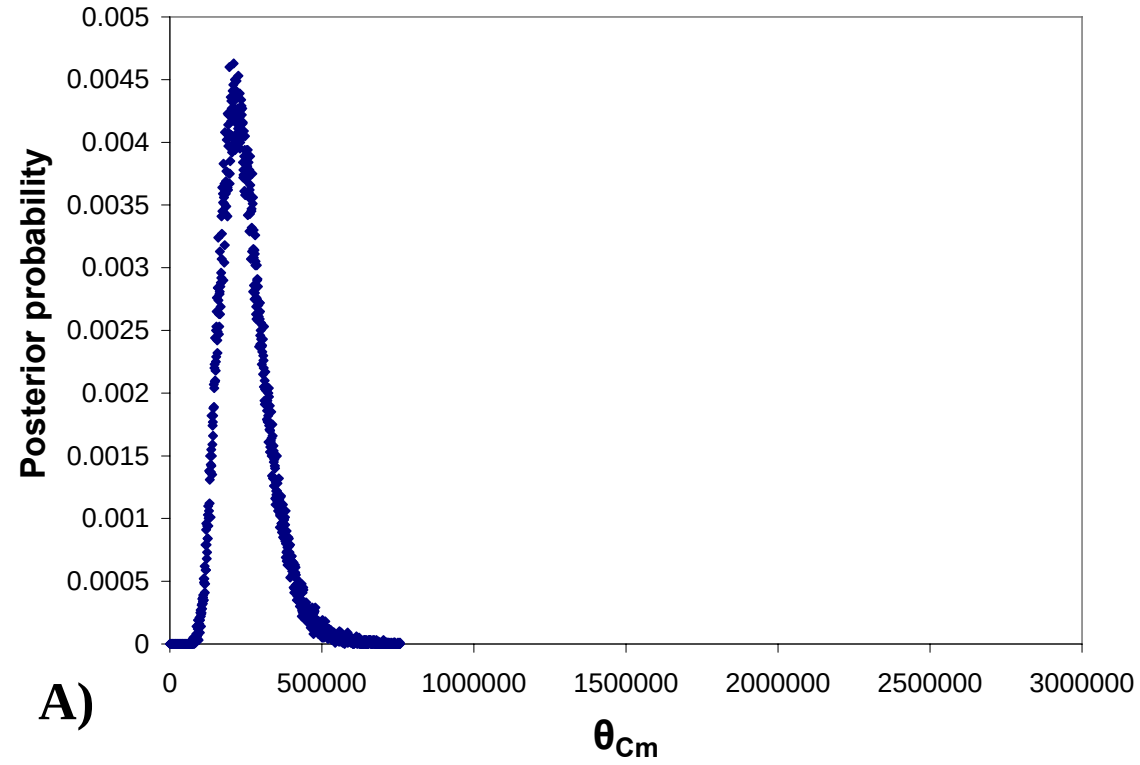
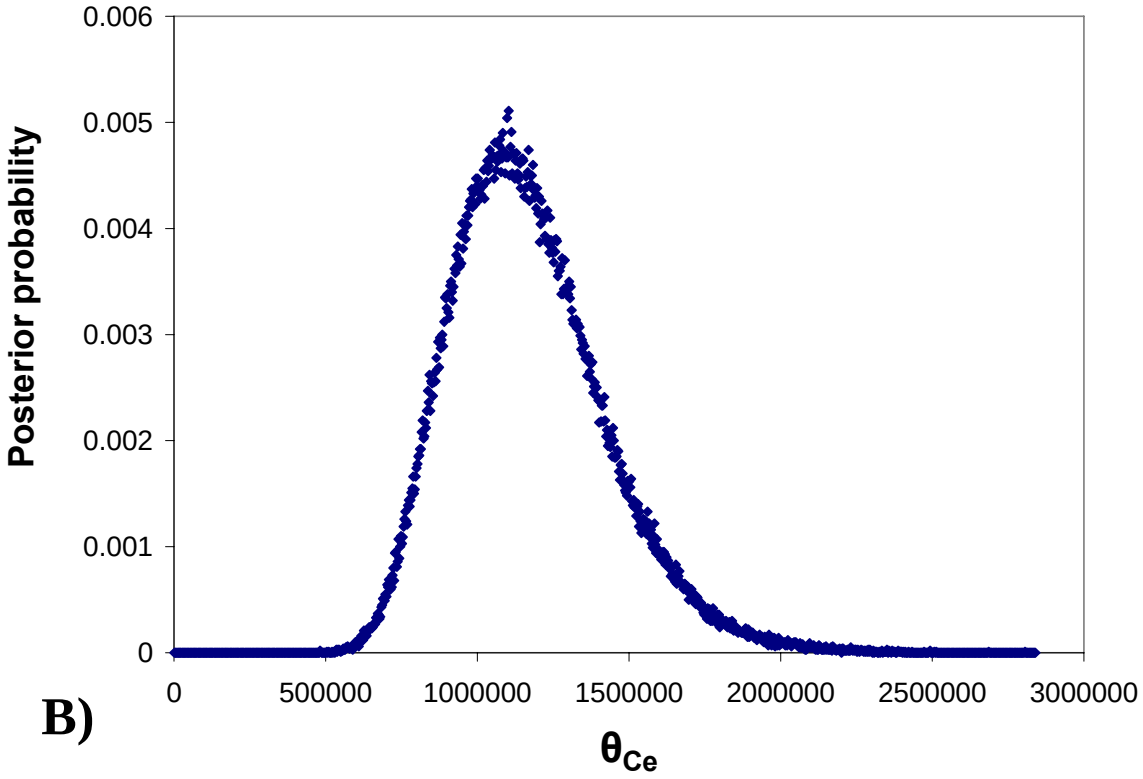


Figure 4.6. Bayesian inference of population parameters for *C. elongatus* and *C. meridionalis* using the coalescent approach implemented in the program IM (Hey and Nielsen 2004). Each graph represents the posterior distribution for the parameter indicated, scaled to the estimated 'per year' mutation rate (μ) of 2.64 x 10^{-6} for the control region (see text). (A) θ_{Cm}, (B) θ_{Ce}, and (C) θ_A are the effective number of females for *C. meridionalis, C. elongatus*, and the common ancestral population, respectively; (D) *t* is the time since (population) divergence, TMRCA is the time to the most recent common ancestor of all the haplotypes included in the analysis; (E) m_1 is the migration rate from *C. meridionalis* to *C. elongatus*; (F) *m2* is the migration rate from *C. elongatus* to *C. meridionalis*.



A)



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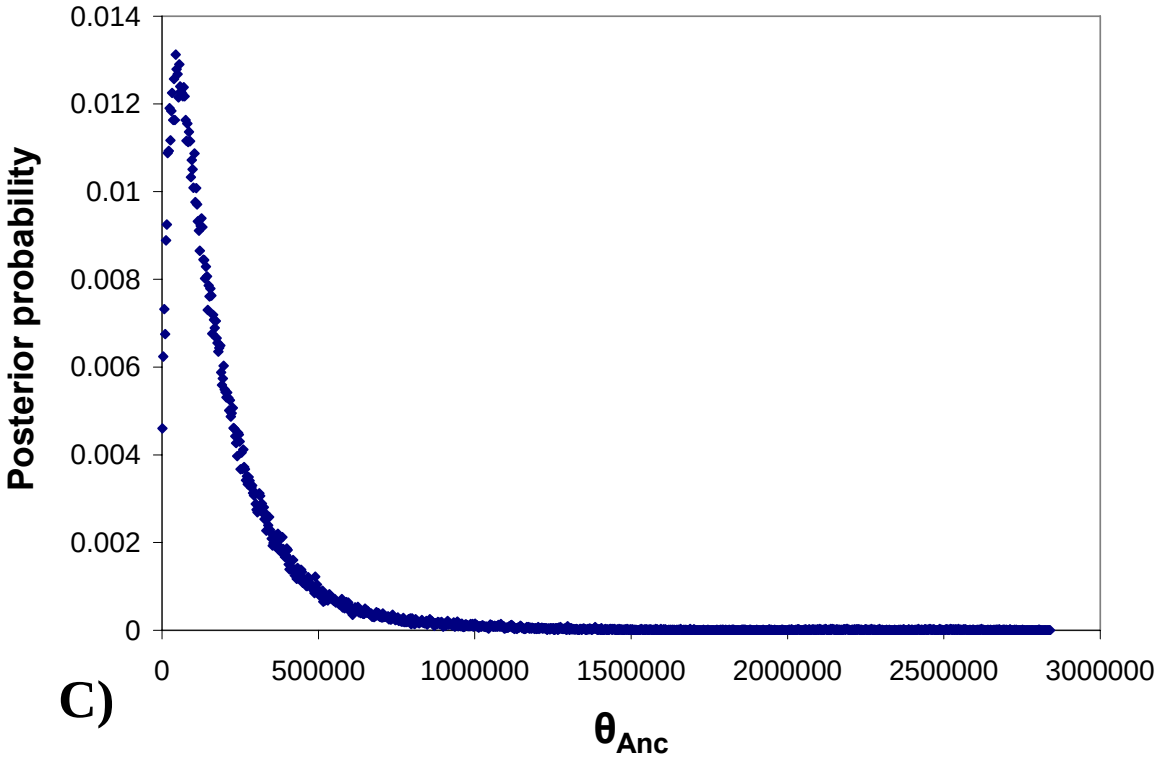

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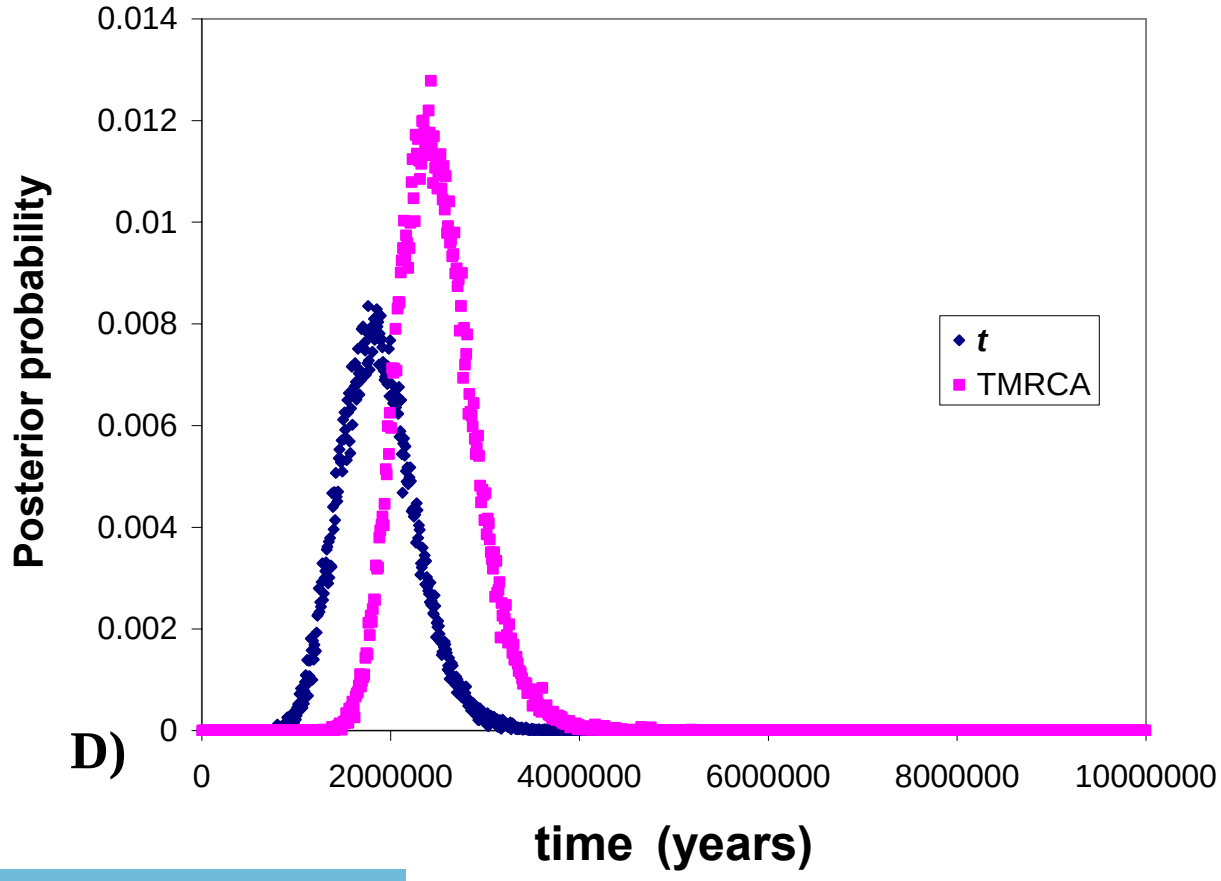

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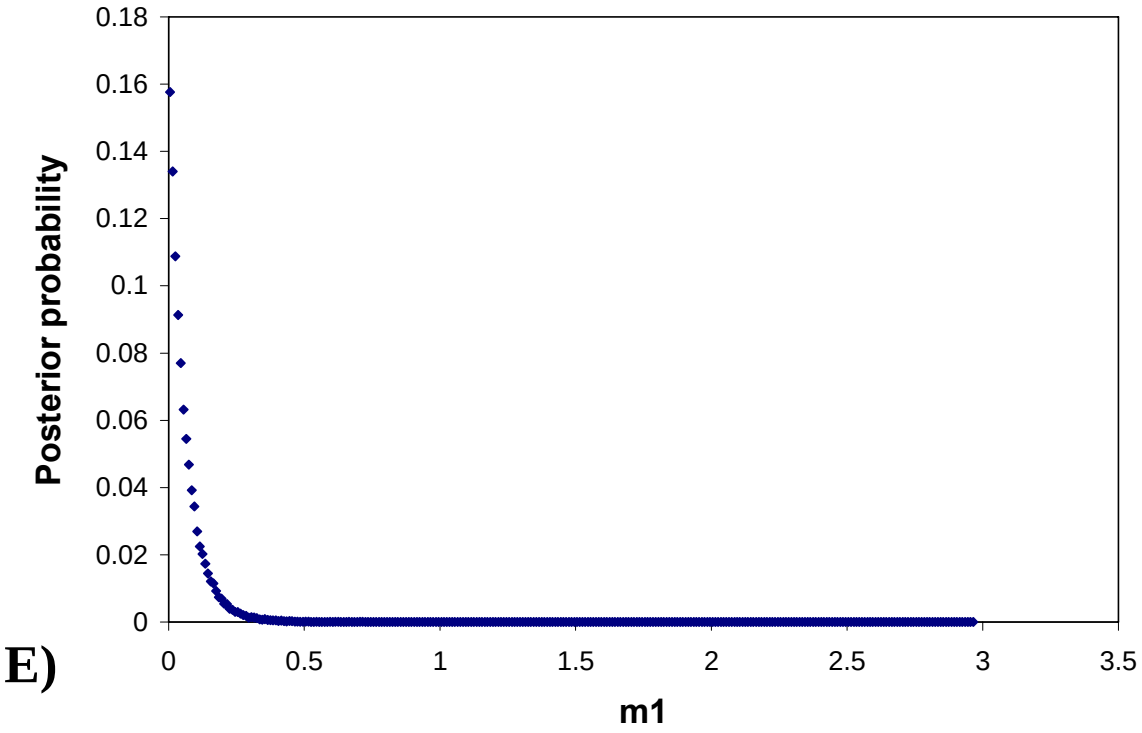
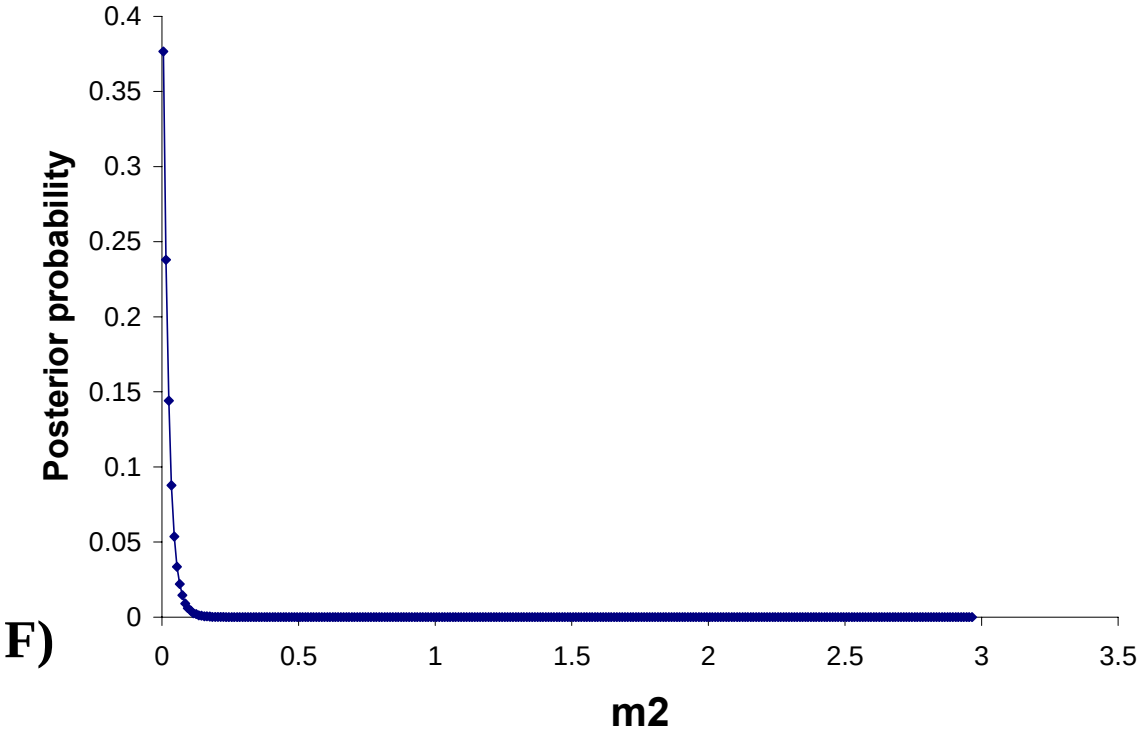


E)



F)

CONCLUSIONS

The overarching theme of this research effort was to investigate hierarchical levels of relatedness in natural populations of the cycleptid fishes (blue suckers), a widespread genus in North America. Formerly, phylogenetic relationships were unknown in the genus. This research has revealed additional biodiversity in the genus (new species, yet to be described, from the Rio Grande Basin) and called into question the existing taxonomy because of incomplete lineage sorting between the two described species at mitochondrial loci.

Other branches of this effort included an examination of rangewide population structure and a more focused assessment of the impacts of dams on population structure in the upper Missouri River. In order to conduct these analyses, it was necessary to develop an array of taxon specific microsatellite markers. In so doing, a useful technique for isolating paralogous loci in a tetraploid genome was discovered. Population screens with isolated paralogs revealed a pattern of chromosomal inheritance that is consistent with an allopolyploid origin.

Highly efficient multiplexed reactions were used to conduct rangewide microsatellite genotypic data. Results of subsequent population structural analyses indicate distinct structure within the Mississippi basin and a distinct pattern of isolation by distance in the Missouri River drainage, a pattern that is not present in the Mississippi River. This is among the first studies to reveal altered genetic patterns resulting from anthropogenic modifications to large river systems. Finally, Bayesian estimates of historical demography and divergence time between the two described species, *C.*

elongatus and *C. meridionalis*, revealed a pattern of longterm genetic isolation. This lends some credence to the current taxonomy.

In carrying out this work, approximately 600 tissue specimens were collected from throughout the range over a one-year time period. This frozen 'point in time' will be maintained by the author and is freely available for use by others upon request. The intent is that this collection will provide a valuable baseline for future repeated measures in tracking the evolution of, and providing appropriate conservation measures for, fishes in the genus *Cycleptus*.

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APPENDIX ONE: Edited clone sequences for each microsatellite locus

Bold, underlined lettering indicates name and primer locations for loci in which primers were designed and optimized for PCR.

Ce13 original clone

GGTTCATCTGAAAGGCTCTGTTCCACTACAGAGTTGACTGCGAGAGACTCGG
ACGCAATTATATTTTCTCTTTTTATTTCTTGTTTGTGTGTTTTATCTTTATATTT
CGGACAGTGGGCTACTAACATGAACAATTGTCAAGTAATTTTATTTTTAGTTT
TGCAAATAGATATTTTTCACTTGAAAAATAATGAT**GTTCTGGGACTTAACAA**
AGGGATTTGTATTATCCGAGGACAGCGGTTTAAAATATTTTTTTATTATCTAT
CTATCTATCTATCTATCTATCTATCTATATCTATCTATCTGTCTATCTATCTATC
TATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCATTTCAGGAA
TGCATGACAAGTTGTTATAACA**TTAGACTACTTGATACCTCATGTTTGT**

***Ce13S allele**

AGTAGTCTAATGTTATAA**CAACTTGTCATGCATTCCTGAA**ATGATAGATAG
ATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATA
GATAGATAGATAGATAGATAATAAAAAAATATTTTAAACCGCTGTCCTCGGA
TAATAC**AAATCCCTTTGTTAAGTCCCAG**AACA

***Ce13L allele**

TACAAACATGAGGTATCAAGTAGTCTAATGTT**GTAACAATTTTTCATGCATT**
CCTGGAAGCCTTAATTGTTAGATATTAGATACATTATTAGATAGAAAGACGG
ACGGACGGACGGACGGACGGATGGATGGATGGATAGATAGATAGATATATA
CATAGATAGATCTTAAAAAATCATT**TAAACCGCTGTCCTCGGC**TAATACAA
ATCCCTTTGTTAAGTCCCAGAACA

Ce35 clone

AC**CTTCACACCCAGCTCAAGTCACAT**AGTGATTGTTGTGAGAGTTTGGGCT
TTGCATTTATGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGTAGTT
TGTGTTTGCTTCA**TAGAGCATTAAGCTTAGGCTGCCA**TTCAGAATAATAGA
GGGGGGTGCATGTGAACGGCTGGTGCTGTCACCATGGATATGGCTCCTCTAT
GATAACAGCGTCACCGGGAACTTCCGACCCAGTTGTGTCCTGCCAAGGCCAG
GTTGCCATTACAATGCTG

Ce49 clone

ACAAACAACTTCATTAAACACACACAGGTAATCATAATTACAGTTTAAAGAG
AGAGAGATGATGCATTT**TTTAAGATTTTCTTCCTTCGACTAA**ACAACAACA
ACAACAACAACAACCTCCAAACACATCTGAATTCAATCCTGACATGTATTCT
GTTTG**TGTTCATGCGCACGGGCACATTC**ATCAACTGAACTGACAAAGATAA
CAGATTATTTAGACAAAACCCGTGTGAGGCCGTCCTGTTTTGGACGATAGTTT
TTGCCCCGAGACTGTCGAATC

Ce52 clone

ATTGTGTGATAAAGGAAA**ATGACAGCATCCATGCACATTTA**GGCTACATAA
CCATCTCAGTATTTGCTCTTGAAACATATCATTGTTACAATCAATCGATCAAT
CATTCAATCAATCAATCACTCAATCAATCAATCATTCAATCAATCAATCAATC
ACTCAATCAATCAATCAATCCTCTACCGGACTGCTTAAATTCAGATTTAAATT
CTTGACAATTCAATT**GGGTAAATTGGTATCCATGGAAAC**

Ce63 clone

ACCCACAACAACTCAAGTGGTTTGTTCTGTTCTAAT**CCAAAAGCGTCTTGAA
ATGTTCA**TGAGCTTATCTCTGTGTGTGTGTGTGTGTGAGAGAGAGAGAGAGA
GAGAGAGAGAGAATCTGGGCTCATATTAGACTTTATATATCTAATACAACCA
GCACCAAAACCCATT**TCCATCTCCTCTCGCCGTCTG**CAACATTGTAACTTTG
TAAACTCTTTGACAAATGCATCCAGTCTGATGTTACTTTGTTTCATGTTTGCGC
AACACAATCACTTTTTTCCCCCTGCCTGT

Ce104 clone

ACTCTGCTGTCTGGACATTAAATGATTGTCATGAGACATGAAGGAGAAA**CAC
ACCCATTACGGCAGGATTA**TCTCTCTCTCTCTCTCTCTCTTTCTCTCTCTCTC
TCTGGATTATGTGCTTTTGGAGCTGTAATCTAGATTTTGGCATGAAGTGGCAC
ATGTTGCGTG**TGTGTTATGAAAGCTCATTGCTGTATC**TTCAAGCACAAGAG
CATGACATCATTATGTCTGATCAAAGCAGTGATGTCACTTTCAGTGTGTTTGT
GTTTCTGCCACTGCTACATACAGTGCAGTTCAAAAGTCTCAGTCTAGTAGAAA
TGCTTATGTTTTAAATTTTTCTAATTGAATTTAAAAGTTTTTTTCATTTAAAAT
TATCTGCAAAAAATGTGTGAAAAGCTGCTTTTCATTAAAAAAAATATGAATTT
ATTAGTGTTTGCAATGT

Ce126 clone

AGTTCACATTTGGTGAATTTGGCACTCATAAAAATCTGTAGACATATTCATGA
ATAACAGCGAACAATGACTGACAGTCACAGATGGGCTTCTATCAGGCGAAAC
GGCATAATACGAATGGAAATGAGTT**TTCGCTCTCCGTCCCTTTCATTCT**TC
CTTCTTTATTACCACTCTTCTCTTTCTTTTTATTTGTCTGTCTCTCTCCCTCTCTC
TCTCTCTCTCACTGTTGGTCACCTCTCTTAACGCACCATTAGCAGAGAAAACC
AAGTGGACTTGC**TGATAATGTCTCTTTTTCGCTCTCCA**TTTTTCCTCTGGCT
AGATTTCGTGTCTCCTGATACCTTGATTAGTTTGTGAGGGCTCCATTTGGGCT
CCATTAGGAGTGCTGCTGTCAGCGTGTGAACCCTCAGACTCTGTTTATGCGGC
TGT

Ce146 clone

ACAGTAGTAGTCAACATAGGTAAAGGCATAGTT**AACCCAAAAATGAAAATT**
GTGTTATCATTTACTCCAAACCCATATGACTTTCATAGATAGATAGATAGAT
AGATAGATAGATAGATAGAATATCTAGTAAGGAATATGCAGAAGAACTATCA
GAAGCC**AATCAGAGTCTCTTAATAGCGAGCA**ATTTATTACAGAGCTGACCT
GGAAAG

Ce195 clone

ACTACAAGCTTGCATTTCTCAGGAATGTTCCTTAGTCCGGAG**ACATTGCGAT**
TAATTGCATTCATTTTTTTAACGCGTTATTTTTAATAAAATGTATCGCACTGA
ATTAACGCGTTAAATCGACAGCCCTAATATATACATATACATATACAGACAC
ATACATACGCATACATACATACATATATACATTCATACATACACACATACAC
ATATGTAGTGCAAAACAAAATACAAATCTGTTATATACAGTGCAAGGG**AATG**
TAATGGCAGAAGAGGATGGATATGTTGGATAAATATAAAAAGACTAGACTG
TGAATTGCACATAATTATTGCTCAGTGGGGCAGTCTTTAACTGTTCATGGAAA
AAAACTGTTCCTGTGCCTGACAGTTCTGGTGCTCAGAGCTCTGAAGTGTCGGT
CAGAAGGCAATAGTTCAAAAAGAAGACAAT

Ce215 clone

ACTTTGTTGATGATATGGCATGCAGTTGCA**TTGTCACACCTTTATGGGATTC**
ATAAAGTGTCTACATTTTGTTTTATTTTTTTATCTGGTTTTATTGAAAGTCTGC
ATAATTAAATCTATCTATGAGCGATCTATCTATCTATCTATCTATCTATCTATC
TATCTATCTATCTATCTATCTATCTATCTATCTATCCTATCTATCTATCTATCTA
TCTATCTATCCATCCATCCATCCATCTATCCATCTATCCATTGCATACTATATA
AAGAACTACATTTCGCTATTGAGAGTGCTGAAAGTCTAGGTGTAGCACGAT
GTGTTGCTGACTCGTTTTGAGAAGCCCGGGTTCAGACATGTGAGAAGCCGCA
GGGTCACAGGCAAATTAATGATGATGT

APPENDIX TWO. Complete locality and collection information for all specimens used in these studies.

Individual	Species	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
1-1	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-2	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-3	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-4	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-5	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-6	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-7	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-8	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-9	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-10	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-11	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-12	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
1-13	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-14	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-15	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/15/2004	S. Mettee	Alabama GS
1-16	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-17	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-18	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-19	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-20	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-21	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-22	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-23	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-24	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-25	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-26	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
1-27	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/16/2004	S. Mettee	Alabama GS
1-28	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/30/2004	S. Mettee	Alabama GS
1-29	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/30/2004	S. Mettee	Alabama GS
1-30	*C. meridionalis*	AL	Alabama	132.5	32.08357	87.40307	3/30/2004	S. Mettee	Alabama GS
1-31	*C. meridionalis*	AL	Alabama					R. Mayden	Univ. of Alabama
1-32	*C. meridionalis*	AL	Alabama					R. Mayden	Univ. of Alabama
1-33	*C. meridionalis*	AL	Alabama					R. Mayden	Univ. of Alabama
2-1	*C. elongatus*	AR	Red		33.61083	93.85639	9/24/2004	W. Layher	Layher Biologics
2-2	*C. elongatus*	AR	Red		33.61083	93.85639	9/24/2004	W. Layher	Layher Biologics
2-3	*C. elongatus*	AR	Red		33.61083	93.85639	9/24/2004	W. Layher	Layher Biologics
4-1	*C. meridionalis*	MS	Leaf		31.2204	89.0285	10/7/2003	T. Slack	MS Mus. Nat. Sci.
4-2	*C. meridionalis*	MS	Leaf		31.2204	89.0285	10/7/2003	T. Slack	MS Mus. Nat. Sci.
4-3	*C. meridionalis*	MS	Leaf		31.19459	89.16099	6/22/2004	B. Krieser	Univ. Southern MS
4-4	*C. meridionalis*	MS	Leaf		31.06121	88.48307	6/22/2004	B. Krieser	Univ. Southern MS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
4-5	*C. meridionalis*	MS	Leaf	0.2			3/16/2004	B. Krieser	Univ. Southern MS
8-21	*C. elongatus*	AR	White	258.7		3945919	3/7/2006	J. Quinn	AR Game and Fish
8-28	*C. elongatus*	AR	White	258.7		3945919	3/7/2006	J. Quinn	AR Game and Fish
9-1	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-2	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-3	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	4/3/2004	T. Kennedy	Purdue University
9-4	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-5	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	4/10/2004	T. Kennedy	Purdue University
9-6	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-7	*C. elongatus*	IN	Wabash	380	40.77552	85.92357	5/19/2004	T. Kennedy	Purdue University
9-8	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	5/6/2004	T. Kennedy	Purdue University
9-9	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	4/10/2004	T. Kennedy	Purdue University
9-10	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	3/27/2004	T. Kennedy	Purdue University
9-11	*C. elongatus*	IN	Wabash	315	40.47564	87.87091	4/3/2004	T. Kennedy	Purdue University

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
9-12	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	3/27/2004	T. Kennedy	Purdue University
9-13	*C. elongatus*	IN	Wabash	315	40.47564	87.87091	4/3/2004	T. Kennedy	Purdue University
9-14	*C. elongatus*	IN	Wabash	300	40.40429	87.06691	6/9/2004	T. Kennedy	Purdue University
9-15	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	5/6/2004	T. Kennedy	Purdue University
9-16	*C. elongatus*	IN	Wabash	315	40.47564	87.87091	4/3/2004	T. Kennedy	Purdue University
9-17	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	5/6/2004	T. Kennedy	Purdue University
9-18	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-19	*C. elongatus*	IN	Wabash	321	40.51317	86.79308	4/21/2004	T. Kennedy	Purdue University
9-20	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	3/27/2004	T. Kennedy	Purdue University
9-21	*C. elongatus*	IN	Wabash	304	40.40764	87.00857	6/9/2004	T. Kennedy	Purdue University
9-22	*C. elongatus*	IN	Wabash	380	40.77552	85.92357	5/19/2004	T. Kennedy	Purdue University
9-23	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	4/10/2004	T. Kennedy	Purdue University
9-24	*C. elongatus*	IN	Wabash	380	40.76648	85.95422	5/19/2004	T. Kennedy	Purdue University
9-25	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	4/3/2004	T. Kennedy	Purdue University

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
9-26	*C. elongatus*	IN	Wabash	304	40.40764	87.00857	6/9/2004	T. Kennedy	Purdue University
9-27	*C. elongatus*	IN	Wabash	380	40.77552	85.92357	5/19/2004	T. Kennedy	Purdue University
9-28	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	4/10/2004	T. Kennedy	Purdue University
9-29	*C. elongatus*	IN	Wabash	315	40.47564	86.87091	3/27/2004	T. Kennedy	Purdue University
9-30	*C. elongatus*	IN	Wabash	300	40.40429	86.06691	4/10/2004	T. Kennedy	Purdue University
10-1	*C. elongatus*	IA	Mississippi	532.9			8/5/2004	J. Pitlo	IA DNR
10-2	*C. elongatus*	IA	Mississippi	543.4			8/5/2004	J. Pitlo	IA DNR
10-3	*C. elongatus*	IA	Mississippi	532.9			8/9/2004	D. Weiss	IA DNR
10-4	*C. elongatus*	IA	Mississippi	549.9			8/11/2004	D. Weiss	IA DNR
10-5	*C. elongatus*	IA	Mississippi	549.9			8/11/2004	D. Weiss	IA DNR
10-6	*C. elongatus*	IA	Mississippi	549.9			8/11/2004	D. Weiss	IA DNR
10-7	*C. elongatus*	IA	Mississippi	549.7			8/11/2004	D. Weiss	IA DNR
10-8	*C. elongatus*	IA	Mississippi	540.5			8/11/2004	D. Weiss	IA DNR
10-9	*C. elongatus*	IA	Mississippi	548			8/26/2004	D. Weiss	IA DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
10-10	*C. elongatus*	IA	Mississippi	536.9			9/9/2004	D. Weiss	IA DNR
10-11	*C. elongatus*	IA	Mississippi	556.7			10/13/2004	J. Pitlo	IA DNR
12-9	*C. elongatus*	MN	Minnesota	217.1	44.57181	95.08796	8/12/2004	?	MN DNR
12-10	*C. elongatus*	MN	Minnesota	209.5	44.54417	95.00043	8/13/2004	?	MN DNR
12-11	*C. elongatus*	MN	Minnesota	111.9	44.16522	94.03383	8/17/2004	?	MN DNR
12-12	*C. elongatus*	MN	Minnesota	111.9	44.16522	94.03383	8/17/2004	?	MN DNR
12-13	*C. elongatus*	MN	Minnesota	74.5	44.47513	93.90654	8/21/2004	?	MN DNR
12-14	*C. elongatus*	MN	Minnesota	74.5	44.47513	93.90654	8/21/2004	?	MN DNR
12-15	*C. elongatus*	MN	Minnesota	74	44.53706	93.9009	9/9/2004	?	MN DNR
12-16	*C. elongatus*	MN	Minnesota	74	44.53706	93.90027	9/9/2004	?	MN DNR
13-2	*C. elongatus*	MN	Mississippi	761.4	?	?	7/15/2004	S. DeLain	MN DNR
13-3	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR
13-4	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR
13-5	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
13-6	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR
13-7	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR
13-8	*C. elongatus*	MN	Mississippi	795.6	44.60281	92.59176	7/27/2004	S. DeLain	MN DNR
17-1	*C. elongatus*	MO	Missouri	92.4	38.70524	91.34109	4/19/2004	Mauldin	USFWS
17-2	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-3	*C. elongatus*	MO	Missouri	213.8	38	90	4/12/2004	Mauldin	USFWS
17-4	*C. elongatus*	MO	Missouri	92.4	38.70524	91.34109	4/19/2004	Mauldin	USFWS
17-5	*C. elongatus*	MO	Missouri	92.3	38.69938	91.3388	4/19/2004	Mauldin	USFWS
17-6	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-7	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-8	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-9	*C. elongatus*	MO	Missouri	124	38.61108	91.92516	4/29/2004	W. Doyle	USFWS
17-10	*C. elongatus*	MO	Missouri	40.7	38.68257	90.61002	3/24/2004	A. Starostka	USFWS
17-11	*C. elongatus*	MO	Missouri	16	38.87243	90.34079	5/10/2004	W. Doyle	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
17-12	*C. elongatus*	MO	Missouri	40	38.68127	90.56851	3/22/2004	A. Starostka	USFWS
17-13	*C. elongatus*	MO	Missouri	128	38.61208	91.92825	4/29/2004	W. Doyle	USFWS
17-14	*C. elongatus*	MO	Missouri	0.2	38.80816	90.12071	5/11/2004	W. Doyle	USFWS
17-15	*C. elongatus*	MO	Missouri	42	38.68481	90.66957	4/21/2004	Mauldin	USFWS
17-16	*C. elongatus*	MO	Missouri	42	38.63297	90.63062	4/21/2004	Mauldin	USFWS
17-17	*C. elongatus*	MO	Missouri	92.4	38.70524	91.34109	4/19/2004	Mauldin	USFWS
17-18	*C. elongatus*	MO	Missouri	188.1	38.96808	92.59406	3/19/2004	W. Doyle	USFWS
17-19	*C. elongatus*	MO	Missouri	202	38.97929	92.85339	4/2/2004	A. Starostka	USFWS
17-20	*C. elongatus*	MO	Missouri	92.4	38.70524	91.34109	4/19/2004	Mauldin	USFWS
17-21	*C. elongatus*	MO	Missouri	0.2	38.80816	90.12071	5/11/2004	W. Doyle	USFWS
17-22	*C. elongatus*	MO	Missouri	128	38.61462	91.92518	4/29/2004	W. Doyle	USFWS
17-23	*C. elongatus*	MO	Missouri	92.3	38.69938	91.3388	4/19/2004	Mauldin	USFWS
17-24	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-25	*C. elongatus*	MO	Missouri	202	38.97929	92.85339	4/2/2004	A. Starostka	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
17-26	*C. elongatus*	MO	Missouri	128	38.62086	91.91595	4/29/2004	W. Doyle	USFWS
17-27	*C. elongatus*	MO	Missouri	202	38.97929	92.85339	4/2/2004	A. Starostka	USFWS
17-28	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
17-29	*C. elongatus*	MO	Missouri	92.4	38.70524	91.34109	4/19/2004	Mauldin	USFWS
17-30	*C. elongatus*	MO	Missouri	2	38.82946	90.14062	5/11/2004	W. Doyle	USFWS
18B-1	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-2	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-3	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-4	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-5	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-6	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-7	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-8	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-9	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
18B-10	*C. elongatus*	MT	Missouri	2047.3	47.928	110.49	5/17/2004	R. Rodencal	Montana FWP
18B-11	*C. elongatus*	MT	Missouri	1980.8	47.7471	109.5815	5/20/2004	Wente/McCord	Montana FWP
18B-12	*C. elongatus*	MT	Missouri	1982.5	47.7374	109.6226	5/26/2004	Rodencal	Montana FWP
18B-13	*C. elongatus*	MT	Missouri	1982.5	47.7374	109.6226	5/26/2004	Rodencal	Montana FWP
18B-14	*C. elongatus*	MT	Missouri	1987.5	47.7192	109.696	5/26/2004	Rodencal	Montana FWP
18B-15	*C. elongatus*	MT	Missouri	1982.8	47.7371	109.6183	6/29/2004	Rodencal	Montana FWP
18B-16	*C. elongatus*	MT	Missouri	2030.4	48.03	110.221	6/24/2004	Wente	Montana FWP
18B-17	*C. elongatus*	MT	Missouri	2030.4	48.03	110.221	6/24/2004	Wente	Montana FWP
18B-18	*C. elongatus*	MT	Missouri	1983.6	47.7395	109.6306	6/29/2004	Rodencal	Montana FWP
18B-19	*C. elongatus*	MT	Missouri	2068.9	47.50489	110.3543	7/15/2004	McCord	Montana FWP
18B-20	*C. elongatus*	MT	Missouri	1909	47.589	108.472	7/9/2004	Gerrity	Montana FWP
18B-21	*C. elongatus*	MT	Missouri	1884.8	47.5872	108.1302	8/3/2004	Rodencal	Montana FWP
18B-22	*C. elongatus*	MT	Missouri	1883.3	47.7331	109.6034	9/8/2004	Rodencal	Montana FWP
18B-23	*C. elongatus*	MT	Missouri	1883.3	47.7333	109.6034	9/8/2004	Rodencal	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
18B-24	*C. elongatus*	MT	Missouri	2032.8	48.0004	110.2539	9/9/2004	Rodencal	Montana FWP
18B-25	*C. elongatus*	MT	Missouri	2034.1	48.0176	110.2837	9/9/2004	Rodencal	Montana FWP
18B-26	*C. elongatus*	MT	Missouri	2029.5	48.03328	110.2062	9/9/2004	Rodencal	Montana FWP
18B-27	*C. elongatus*	MT	Missouri	2034.8	48.00864	110.266	9/9/2004	Rodencal	Montana FWP
18B-28	*C. elongatus*	MT	Missouri	2034.8	48.00864	110.266	9/9/2004	Rodencal	Montana FWP
18B-29	*C. elongatus*	MT	Missouri	2034.8	48.00864	110.266	9/9/2004	Rodencal	Montana FWP
18B-30	*C. elongatus*	MT	Missouri	2031.4	48.0007	110.2479	9/10/2004	Rodencal	Montana FWP
19-1	*C. elongatus*	MT	Missouri	1763.5	48.0269	106.2149	7/29/2004	Dix	Montana FWP
19-2	*C. elongatus*	MT	Missouri	?	?	?		Dix	Montana FWP
19-3	*C. elongatus*	MT	Missouri	?	?	?		Dix	Montana FWP
19B-3	*C. elongatus*	MT	Missouri		48.07592	105.653	10/4/2004	Baxter	Montana FWP
19-4	*C. elongatus*	MT	Missouri	?	?	?		Dix	Montana FWP
19-5	*C. elongatus*	MT	Missouri	1672	48.04629	105.0741	10/27/2004	Dix	Montana FWP
19-6	*C. elongatus*	MT	Missouri	1711.5	48.029	105.3865	11/5/2004	Dix	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
19B-6	*C. elongatus*	MT	Missouri		48.10859	104.5912	10/5/2004	Baxter	Montana FWP
19-7	*C. elongatus*	MT	Missouri	1702	48.04198	105.3303	10/19/2004	Dix	Montana FWP
19B-12	*C. elongatus*	MT	Missouri		48.07592	105.653	10/4/2004	Baxter	Montana FWP
19B-14	*C. elongatus*	MT	Missouri		48.06271	106.3857	9/28/2004	Baxter	Montana FWP
19B-15	*C. elongatus*	MT	Missouri		48.07592	105.653	10/4/2004	Baxter	Montana FWP
19-16	*C. elongatus*	MT	Missouri	1761	48.03421	106.9172	4/27/2004	Baxter	Montana FWP
19-17	*C. elongatus*	MT	Missouri	1761	48.03421	106.9172	4/27/2004	Baxter	Montana FWP
19-18	*C. elongatus*	MT	Missouri	1761	48.02703	106.172	4/27/2004	Baxter	Montana FWP
19-19	*C. elongatus*	MT	Missouri	1684	48.05296	105.1366	6/8/2004	Baxter	Montana FWP
19-20	*C. elongatus*	MT	Missouri	1684	48.04778	105.1317	6/8/2004	Baxter	Montana FWP
19-21	*C. elongatus*	MT	Missouri	1763.5	48.02877	106.2199	6/9/2004	Baxter	Montana FWP
19B-21	*C. elongatus*	MT	Missouri		48.07592	105.653	10/4/2004	Baxter	Montana FWP
19-22	*C. elongatus*	MT	Missouri	1770	48.04172	106.2341	6/10/2004	Baxter	Montana FWP
19-23	*C. elongatus*	MT	Missouri	1770	48.00921	106.2583	6/10/2004	Baxter	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
19B-23	*C. elongatus*	MT	Missouri		48.07592	105.653	10/4/2004	Baxter	Montana FWP
19-24	*C. elongatus*	MT	Missouri	1770	48.00921	106.2583	6/10/2004	Baxter	Montana FWP
19-25	*C. elongatus*	MT	Missouri	1760	48.02499	106.1813	6/15/2004	Dix	Montana FWP
19B-25	*C. elongatus*	MT	Missouri		48.10859	104.5912	10/5/2004	Baxter	Montana FWP
19-26	*C. elongatus*	MT	Missouri	1760	48.02341	106.1788	6/15/2004	Dix	Montana FWP
19-27	*C. elongatus*	MT	Missouri	1760	48.02341	106.1788	6/15/2004	Dix	Montana FWP
19-28	*C. elongatus*	MT	Missouri	1760	48.02484	106.178	6/15/2004	Dix	Montana FWP
19-29	*C. elongatus*	MT	Missouri	1648.5	48.08899	104.5404	6/23/2004	Dix	Montana FWP
19-30	*C. elongatus*	MT	Missouri	1648.5	48.08949	104.5287	6/23/2004	Dix	Montana FWP
19B-30	*C. elongatus*	MT	Missouri		48.02766	106.2356	9/28/2004	Baxter	Montana FWP
20-1	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	6/23/2004	M. Backes	Montana FWP
20B-1	*C. elongatus*	MT	Yellowstone	68	47.30701	104.4632	9/2/2004	M. Backes	Montana FWP
20-2	*C. elongatus*	MT	Yellowstone	187.8	46.38495	105.914	7/27/2004	M. Backes	Montana FWP
20-3	*C. elongatus*	MT	Yellowstone	187.8	46.38495	105.914	7/27/2004	M. Backes	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
20-4	*C. elongatus*	MT	Yellowstone	189	46.3778	105.9269	7/27/2004	M. Backes	Montana FWP
20-5	*C. elongatus*	MT	Yellowstone	189	46.3778	105.9269	7/27/2004	M. Backes	Montana FWP
20-6	*C. elongatus*	MT	Yellowstone	186.4	46.39555	105.8973	7/27/2004	M. Backes	Montana FWP
20-7	*C. elongatus*	MT	Yellowstone	184.5	46.41703	105.8639	7/27/2004	M. Backes	Montana FWP
20-8	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-9	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-10	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-11	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-12	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-13	*C. elongatus*	MT	Yellowstone	87.6	47.15451	104.6687	8/4/2004	M. Backes	Montana FWP
20-14	*C. elongatus*	MT	Yellowstone	71			8/5/2004	M. Backes	Montana FWP
20-15	*C. elongatus*	MT	Yellowstone	71			8/5/2004	M. Backes	Montana FWP
20-16	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-17	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
20-18	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-19	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-20	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-21	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-22	*C. elongatus*	MT	Yellowstone	182	46.43355	105.8204	8/10/2004	M. Backes	Montana FWP
20-23	*C. elongatus*	MT	Yellowstone	237	46.27588	106.6794	8/12/2004	M. Backes	Montana FWP
20-24	*C. elongatus*	MT	Yellowstone	237	46.27588	106.6794	8/12/2004	M. Backes	Montana FWP
20-25	*C. elongatus*	MT	Yellowstone	187	46.40655	105.8701	8/16/2004	M. Backes	Montana FWP
20-26	*C. elongatus*	MT	Yellowstone	184	46.42953	105.8423	8/16/2004	M. Backes	Montana FWP
20-27	*C. elongatus*	MT	Yellowstone	184	46.42953	105.8423	8/16/2004	M. Backes	Montana FWP
20-28	*C. elongatus*	MT	Yellowstone	184	46.42953	105.8423	8/16/2004	M. Backes	Montana FWP
20-29	*C. elongatus*	MT	Yellowstone	184	46.42953	105.8423	8/16/2004	M. Backes	Montana FWP
20-30	*C. elongatus*	MT	Yellowstone	289.9	46.221	107.4206	8/27/2004	M. Backes	Montana FWP
22-1	*Cycleptus sp.*	NM	Black				7/14/2004	B. Larson	NMGF

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
22-31	*Cycleptus sp.*	NM	Pecos				7/14/2004	B. Dorn	Albuquerque Aqm.
22-32	*Cycleptus sp.*	NM	Pecos				7/14/2004	B. Dorn	Albuquerque Aqm.
22-33	*Cycleptus sp.*	NM	Pecos				7/14/2004	B. Dorn	Albuquerque Aqm.
22-34	*Cycleptus sp.*	NM	Black				7/14/2004	B. Dorn	Albuquerque Aqm.
23-1	*C. elongatus*	ND	Missouri	1299			7/12/2004	J. Hendrickson	North Dakota G&F
23-2	*C. elongatus*	ND	Missouri	1299			7/12/2004	J. Hendrickson	North Dakota G&F
23-3	*C. elongatus*	ND	Missouri	1299			7/12/2004	J. Hendrickson	North Dakota G&F
23-4	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-5	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-6	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-7	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-8	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-9	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F
23-10	*C. elongatus*	ND	Missouri	1320			9/7/2004	J. Hendrickson	North Dakota G&F

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
23-11	*C. elongatus*	ND	Missouri	1343			9/8/2004	J. Hendrickson	North Dakota G&F
23-12	*C. elongatus*	ND	Missouri	1371			9/9/2004	J. Hendrickson	North Dakota G&F
23-13	*C. elongatus*	ND	Missouri	1371			9/9/2004	J. Hendrickson	North Dakota G&F
23-14	*C. elongatus*	ND	Missouri	1371			9/9/2004	J. Hendrickson	North Dakota G&F
24-1	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-2	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-3	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-4	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-5	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-6	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-7	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-8	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-9	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-10	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
24-11	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-12	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-13	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-14	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-15	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-16	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-17	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-18	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-19	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-20	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-21	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-22	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-23	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-24	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
24-25	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-26	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-27	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-28	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-29	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
24-30	*C. elongatus*	KY	Ohio	607	38.28565	85.79193	8/27/2004	Crosby, Duvall	Kentucky DFWR
25-1	*C. elongatus*	SD	Missouri		42.77984	98.03719	11/1/2004	D. Schuman	USFWS
25-2	*C. elongatus*	SD	Missouri		42.77386	97.96674	7/20/2004	D. Schuman	USFWS
25-3	*C. elongatus*	SD	Missouri		42.76314	98.02292	3/16/2005	D. Schuman	USFWS
25-4	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-5	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-6	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-7	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-8	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
25-9	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-10	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-11	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-12	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-13	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-14	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-15	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-16	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-18	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-19	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-20	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-21	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-22	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-23	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
25-24	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-25	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-26	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-27	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-28	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-29	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
25-30	*C. elongatus*	SD	Missouri		42.76471	97.98806	5/4/2005	Wanner	USFWS
28-1	*C. elongatus*	TN	Cumberland				3/30/2004	T. St. John	TWRA
28-2	*C. elongatus*	TN	Tennessee				4/21/2004	P. Bettoli	TN Tech Univ.
29-1	*C. elongatus*	TN	Hatchie		35.51197	89.33594	5/24/2005	M. Clark	TWRA
29-2	*C. elongatus*	TN	Hatchie		35.51317	89.32812	5/24/2005	M. Clark	TWRA
29-3	*C. elongatus*	TN	Hatchie		35.51504	89.32738	5/24/2005	M. Clark	TWRA
29-4	*C. elongatus*	TN	Hatchie		35.51378	89.32431	5/24/2005	M. Clark	TWRA
29-5	*C. elongatus*	TN	Hatchie		35.51173	89.32018	5/24/2005	M. Clark	TWRA

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
29-16	*C. elongatus*	TN	Hatchie		35.38293	89.15309	5/24/2005	D. Barber	TWRA
29-17	*C. elongatus*	TN	Hatchie		35.3119	89.1539	5/24/2005	D. Barber	TWRA
29-18	*C. elongatus*	TN	Hatchie		35.31507	89.15386	5/24/2005	D. Barber	TWRA
29-19	*C. elongatus*	TN	Hatchie		35.3177	89.15934	5/24/2005	D. Barber	TWRA
29-20	*C. elongatus*	TN	Hatchie		35.3177	89.15962	5/24/2005	D. Barber	TWRA
29-21	*C. elongatus*	TN	Hatchie		35.31609	89.15971	5/24/2005	D. Barber	TWRA
29-22	*C. elongatus*	TN	Hatchie		35.31526	89.15976	5/24/2005	D. Barber	TWRA
29-23	*C. elongatus*	TN	Hatchie		35.3166	89.16089	5/24/2005	D. Barber	TWRA
29-24	*C. elongatus*	TN	Hatchie		35.31658	89.161	5/24/2005	D. Barber	TWRA
29-25	*C. elongatus*	TN	Hatchie		35.31882	89.16582	5/24/2005	D. Barber	TWRA
29-26	*C. elongatus*	TN	Hatchie		35.36226	89.50298	5/24/2005	D. Barber	TWRA
29-27	*C. elongatus*	TN	Hatchie		35.36263	89.50322	5/24/2005	D. Barber	TWRA
30-1	*C. elongatus*	TN	Nolichucky	44.7	36.03558	82.53038	5/7/2004	R. Bivins	TWRA
30-2	*C. elongatus*	TN	Nolichucky	44.7	36.03558	82.53038	5/7/2004	R. Bivins	TWRA

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
30-3	*C. elongatus*	TN	Nolichucky	44.15	36.03363	82.5324	5/7/2004	R. Bivins	TWRA
30-4	*C. elongatus*	TN	Nolichucky	43.7	36.03212	82.53274	5/7/2004	R. Bivins	TWRA
30-5	*C. elongatus*	TN	Nolichucky	43.7	36.03212	82.53274	5/7/2004	R. Bivins	TWRA
30-6	*C. elongatus*	TN	Nolichucky	41.9	36.03488	82.543	5/7/2004	R. Bivins	TWRA
30-7	*C. elongatus*	TN	Nolichucky	35.4	36.0402	82.58315	5/7/2004	R. Bivins	TWRA
30-8	*C. elongatus*	TN	Nolichucky	30.9	36.05336	83.00345	5/11/2004	R. Bivins	TWRA
30-9	*C. elongatus*	TN	French Broad	13.2	35.58021	83.43259	5/18/2004	B. Carter	TWRA
33-1	*C. elongatus*	WI	Wisconsin	30.5	43.1748	90.6877	7/1/2004	J. Lyons	Wisconsin DNR
33-2	*C. elongatus*	WI	Wisconsin	30.5	43.1748	90.6877	7/1/2004	J. Lyons	Wisconsin DNR
33-3	*C. elongatus*	WI	Wisconsin	30.5	43.1748	90.6877	7/1/2004	J. Lyons	Wisconsin DNR
33-4	*C. elongatus*	WI	Wisconsin	30.5	43.1748	90.6877	7/1/2004	J. Lyons	Wisconsin DNR
33-5	*C. elongatus*	WI	Wisconsin	30	43.1702	90.6928	7/1/2004	J. Lyons	Wisconsin DNR
33-6	*C. elongatus*	WI	Wisconsin	30	43.1702	90.6928	7/1/2004	J. Lyons	Wisconsin DNR
33-7	*C. elongatus*	WI	Wisconsin	30	43.1702	90.6928	7/1/2004	J. Lyons	Wisconsin DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
33-8	*C. elongatus*	WI	Wisconsin	30	43.1702	90.6928	7/1/2004	J. Lyons	Wisconsin DNR
33-9	*C. elongatus*	WI	Wisconsin	26	43.1297	90.7478	7/29/2004	J. Lyons	Wisconsin DNR
33-10	*C. elongatus*	WI	Wisconsin	26	43.1297	90.7478	7/29/2004	J. Lyons	Wisconsin DNR
33-11	*C. elongatus*	WI	Wisconsin	26	43.1297	90.7478	7/29/2004	J. Lyons	Wisconsin DNR
33-12	*C. elongatus*	WI	Wisconsin	26	43.1297	90.7478	7/29/2004	J. Lyons	Wisconsin DNR
33-13	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-14	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-15	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-16	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-17	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-18	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-19	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-20	*C. elongatus*	WI	Wisconsin	25.6	43.1231	90.7511	7/29/2004	J. Lyons	Wisconsin DNR
33-21	*C. elongatus*	WI	Wisconsin	20.5	43.0909	90.8305	7/29/2004	J. Lyons	Wisconsin DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
33-22	*C. elongatus*	WI	Wisconsin	20.5	43.0909	90.8305	7/29/2004	J. Lyons	Wisconsin DNR
33-23	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-24	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-25	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-26	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-27	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-28	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-29	*C. elongatus*	WI	Wisconsin	20.3	43.085	90.8311	7/29/2004	J. Lyons	Wisconsin DNR
33-30	*C. elongatus*	WI	Wisconsin	43.1	43.2017	90.4471	9/1/2004	J. Lyons	Wisconsin DNR
34-3	*C. elongatus*	KS	Kansas	140			10/12/2004	C. Paukert	KS State Univ.
34-6	*C. elongatus*	KS	Kansas	140			11/4/2004	C. Paukert	KS State Univ.
34-12	*C. elongatus*	KS	Kansas	140			11/4/2004	C. Paukert	KS State Univ.
34-20	*C. elongatus*	KS	Kansas	140			10/12/2004	C. Paukert	KS State Univ.
34-24	*C. elongatus*	KS	Kansas	140			11/4/2004	C. Paukert	KS State Univ.

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
34-26	*C. elongatus*	KS	Kansas	140			10/12/2004	C. Paukert	KS State Univ.
35-1	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-2	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-3	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-4	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-5	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-6	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-7	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-8	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-9	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-10	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-11	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-12	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-13	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
35-14	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-15	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-16	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-17	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-18	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-19	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-20	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-21	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-22	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-23	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-24	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-25	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-26	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-27	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
35-28	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-29	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-30	*C. elongatus*	OK	Red	725	33.49	96.33	3/16/2005	B. Bristow	USFWS
35-31	*C. elongatus*	OK	Red	123	33.5742.1	95.13.51.1	11/17/2005	B. Bristow	USFWS
35-32	*C. elongatus*	OK	Red	123	33.5742.1	95.13.51.1	11/17/2005	B. Bristow	USFWS
35-33	*C. elongatus*	OK	Red	123	33.5742.1	95.13.51.1	11/17/2005	B. Bristow	USFWS
36-01	*Cycleptus sp.*	TX	Rio Grande	Big Bend NP				T. Bonner	TSU – San Marcos
36-02	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-03	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-04	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-05	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-06	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-07	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-08	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos

36-09	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-10	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-11	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-12	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-13	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-14	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-15	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-16	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-17	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-18	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-19	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
36-20	*Cycleptus sp.*	TX	Rio Grande					T. Bonner	TSU – San Marcos
37-1	*C. elongatus*	WI	Mississippi	672			6/2/2004	P. Short	Wisconsin DNR
37-2	*C. elongatus*	WI	Mississippi	752			8/5/2004	K. VanRuden	Wisconsin DNR
39-1	*C. elongatus*	WI	Chippewa		44.53948	92.04212	8/4/2004	B. Hujik	Wisconsin DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
39-2	*C. elongatus*	WI	Chippewa		44.52868	92.0481	8/4/2004	B. Hujik	Wisconsin DNR
39-3	*C. elongatus*	WI	Chippewa		44.32867	92.04809	8/4/2004	D. Johnson	Wisconsin DNR
39-4	*C. elongatus*	WI	Chippewa		44.50321	92.05177	8/4/2004	D. Johnson	Wisconsin DNR
39-5	*C. elongatus*	WI	Chippewa		44.50321	92.05177	8/4/2004	D. Johnson	Wisconsin DNR
39-6	*C. elongatus*	WI	Chippewa		44.49423	92.05341	8/4/2004	D. Johnson	Wisconsin DNR
39-7	*C. elongatus*	WI	Chippewa		44.49423	92.05341	8/4/2004	A. Lamm	Wisconsin DNR
39-8	*C. elongatus*	WI	Chippewa		44.49423	92.05341	8/4/2004	A. Lamm	Wisconsin DNR
39-9	*C. elongatus*	WI	Chippewa		44.49423	92.05341	8/4/2004	N. Schaff	Wisconsin DNR
39-10	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-11	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-12	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-13	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-14	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-15	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
39-16	*C. elongatus*	WI	Chippewa		44.48598	92.05705	8/4/2004	N. Schaff	Wisconsin DNR
39-17	*C. elongatus*	WI	Chippewa		44.46962	92.06132	8/4/2004	N. Schaff	Wisconsin DNR
39-18	*C. elongatus*	WI	Chippewa		44.46962	92.06132	8/4/2004	N. Schaff	Wisconsin DNR
39-19	*C. elongatus*	WI	Chippewa		44.46962	92.06132	8/4/2004	N. Schaff	Wisconsin DNR
39-20	*C. elongatus*	WI	Chippewa		44.46962	92.06132	8/4/2004	N. Schaff	Wisconsin DNR
39-21	*C. elongatus*	WI	Chippewa		44.46962	92.06132	8/4/2004	N. Schaff	Wisconsin DNR
39-22	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-23	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-24	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-25	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-26	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-27	*C. elongatus*	WI	Chippewa		44.45267	92.06818	8/4/2004	N. Schaff	Wisconsin DNR
39-28	*C. elongatus*	WI	Chippewa		44.43732	92.07194	8/4/2004	N. Schaff	Wisconsin DNR
39-29	*C. elongatus*	WI	Red Cedar		44.78031	91.94038	8/17/2004	D. Johnson	

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
39-30	*C. elongatus*	WI	Red Cedar		44.76468	91.93135	8/17/2004	D. Johnson	Wisconsin DNR
40-1	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	3/19/2004	B. Reed	Lousiana DWF
40-2	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/29/2004	B. Reed	Lousiana DWF
40-3	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-4	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-5	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-6	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-7	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-8	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-9	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-10	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-11	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-12	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-13	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	DWF

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
40-14	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-15	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-16	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-17	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-18	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-19	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-20	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-21	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-22	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-23	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-24	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-25	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-26	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-27	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	DWF

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
40-28	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-29	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
40-30	*C. elongatus*	LA	Mississippi	315	31.0351	91.3537	4/19/2005	B. Reed	Lousiana DWF
44-1	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-3	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-5	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-6	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-7	*C. elongatus*	NE	Platte		41.02162	96.13811	5/14/2004	E. Peters	Univ. of Nebraska
44-8	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-10	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-11	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-13	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-14	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-16	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
44-18	*C. elongatus*	NE	Platte		41.02162	96.13811	5/14/2004	E. Peters	Univ. of Nebraska
44-21	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-22	*C. elongatus*	NE	Platte		41.02299	96.138	5/18/2004	E. Peters	Univ. of Nebraska
44-23	*C. elongatus*	NE	Platte		41.02162	96.13811	5/14/2004	E. Peters	Univ. of Nebraska
44-25	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-26	*C. elongatus*	NE	Platte		41.05132	96.10832	5/14/2004	E. Peters	Univ. of Nebraska
44-27	*C. elongatus*	NE	Platte		41.06734	96.05474	5/14/2004	E. Peters	Univ. of Nebraska
44-28	*C. elongatus*	NE	Platte		41.02162	96.13811	6/15/2004	E. Peters	Univ. of Nebraska
44-30	*C. elongatus*	NE	Platte		41.02162	96.13811	5/14/2004	E. Peters	Univ. of Nebraska
45-1	*C. elongatus*	TX	Colorado		29.42561	96.32302	10/19/2004	J. Webster	Bio-West
45-2	*C. elongatus*	TX	Colorado		29.42561	96.32302	10/19/2004	J. Webster	Bio-West
45-3	*C. elongatus*	TX	Colorado		29.42561	96.32302	10/19/2004	J. Webster	Bio-West
45-4	*C. elongatus*	TX	Colorado		29.42561	96.32302	10/19/2004	J. Webster	Bio-West
45-5	*C. elongatus*	TX	Colorado		29.42561	96.32302	10/19/2004	J. Webster	Bio-West
45-6	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
45-7	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-8	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-9	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-10	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-11	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-12	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-13	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-14	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-15	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-16	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-17	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-18	*C. elongatus*	TX	Colorado		29.33229	96.24072	10/20/2004	J. Webster	Bio-West
45-19	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West
45-20	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West
45-21	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West

45-22	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West
45-23	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West
45-24	*C. elongatus*	TX	Colorado		29.564	90.5415	10/21/2004	J. Webster	Bio-West
45-25	*C. elongatus*	TX	Colorado		30.0054	97.0937	10/22/2004	J. Webster	Bio-West
45-26	*C. elongatus*	TX	Colorado		30.0054	97.0937	10/22/2004	J. Webster	Bio-West
45-27	*C. elongatus*	TX	Colorado		30.08529	97.22236	10/27/2004	M. Robertson	Bio-West
45-28	*C. elongatus*	TX	Colorado		30.08529	97.22236	10/27/2004	M. Robertson	Bio-West
45-29	*C. elongatus*	TX	Colorado		30.08529	97.22236	10/27/2004	M. Robertson	Bio-West
45-30	*C. elongatus*	TX	Colorado		30.08529	97.22236	10/27/2004	M. Robertson	Bio-West
50-1	*C. elongatus*	NE	Missouri	811				M. Bessert	Univ. of Nebraska
50-2	*C. elongatus*	NE	Missouri				11/11/2003	M. Bessert	Univ. of Nebraska
50-3	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-4	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-5	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-6	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
50-7	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-8	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-9	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-10	*C. elongatus*	NE	Missouri	507	40.148	95.433	11/12/2003	M. Bessert	Univ. of Nebraska
50-11	*C. elongatus*	NE	Missouri	507.7	40.15415	95.43599	11/12/2003	K. Steffensen	NE Game and Parks
50-12	*C. elongatus*	NE	Missouri	507.7	40.15415	95.43599	11/12/2003	K. Steffensen	NE Game and Parks
50-13	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-14	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-15	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-16	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-17	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-18	*C. elongatus*	NE	Missouri	507.7	40.15414	95.43598	11/12/2003	K. Steffensen	NE Game and Parks
50-19	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-20	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
50-21	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-22	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-23	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-24	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-25	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-26	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-27	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-28	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-29	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-30	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-31	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-32	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-33	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-34	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
50-35	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-36	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-37	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-38	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-39	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-40	*C. elongatus*	NE	Missouri	574.6	40.8149	95.84428	11/20/2003	K. Steffensen	NE Game and Parks
50-41	*C. elongatus*	NE	Missouri		42.58881	96.69419	4/19/2005	D. Shuman	USFWS
51-1	*C. elongatus*	WI	Wisconsin		43.187	89.902	9/21/1999	H. Bart	Tulane University
51-2	*C. elongatus*	TN	Duck		35.97742	87.82242	6/3/2000	H. Bart	Tulane University
51-3	*C. elongatus*	TX	Sabine		32.32857	94.35413	12/9/2003	H. Bart	Tulane University
51-4	*C. elongatus*	TX	Sabine		32.32857	94.35413	12/9/2003	H. Bart	Tulane University
51-5	*C. elongatus*	LA	Red		32.89277	93.82091	12/9/2003	H. Bart	Tulane University
51-6	*C. elongatus*	LA	Red		32.89277	93.82091	12/9/2003	H. Bart	Tulane University
51-7	*C. meridionalis*	LA	Pearl				3/9/2001	V. Todaro	Tulane University

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
51-8	*C. meridionalis*	LA	Pearl				8/28/1990	D. Jackson	MS State Univ.
51-9	*C. meridionalis*	LA	Pearl				9/19/1990	D. Jackson	MS State Univ.
52-1	*C. elongatus*	MN	Minnesota	35.9	45.1310	92.45	8/16/2004	D. Ellison	MNDNR
52-2	*C. elongatus*	MN	Minnesota	29.7	44.4656	93.35	8/16/2004	D. Ellison	MNDNR
52-3	*C. elongatus*	MN	Minnesota	35.9	45.1310	92.45	8/16/2004	D. Ellison	MNDNR
55-1	*C. elongatus*	IN	Wabash	?	?	?	4/19/2005	T. Stefenavage	IN DNR
55-2	*C. elongatus*	IN	Wabash	?	?	?	5/3/2005	T. Stefenavage	IN DNR
55-3	*C. elongatus*	IN	Wabash	?	?	?	6/1/2005	T. Stefenavage	IN DNR
55-4	*C. elongatus*	IN	Wabash	?	?	?	6/1/2005	T. Stefenavage	IN DNR
55-5	*C. elongatus*	IN	Wabash	?	?	?	6/7/2005	T. Stefenavage	IN DNR
55-6	*C. elongatus*	IN	Wabash	?	?	?	7/19/2005	T. Stefenavage	IN DNR
55-7	*C. elongatus*	IN	Wabash	?	?	?	7/20/2005	T. Stefenavage	IN DNR
55-8	*C. elongatus*	IN	Wabash	?	?	?	8/16/2005	T. Stefenavage	IN DNR
57-1	*C. elongatus*		Conchos, Mexico		29.2620	104.5254	7/23/2005	L. Lozano Vilano	Univ. Autonoma de Neuvo Leon

Individual	*Species*	State	River	River mile	Latitude (N)	Longitude (W)	Date of collection	Collector	Institution or agency
58-1	*C. elongatus*	LA	Sabine		30.52179	93.33378	5/17/2005	B. Reed	LDWF
58-2	*C. elongatus*	LA	Sabine		30.12561	93.40318	8/11/2005	B. Reed	LDWF
58-3	*C. elongatus*	LA	Sabine		30.52034	93.33472	8/18/2005	B. Reed	LDWF
58-4	*C. elongatus*	LA	Sabine		30.52034	93.33472	8/18/2005	B. Reed	LDWF
58-5	*C. elongatus*	LA	Sabine		30.84501	93.56738	3/29/2006	W. DeRidder	Tulane University
58-6	*C. elongatus*	LA	Sabine		30.84501	93.56738	3/29/2006	W. DeRidder	Tulane University
58-7	*C. elongatus*	LA	Sabine		30.84501	93.56738	3/29/2006	W. DeRidder	Tulane University
59-01	*C. elongatus*	MS	Black		32.7073	90.0934	12/12/2005	J.A. Skains	MS Mus. Nat. Sci.
59-02	*C. elongatus*	MS	Black		32.7073	90.0934	12/12/2005	J.A. Skains	MS Mus. Nat. Sci.