# Systematics and Biogeography of the Silverside Tribe Menidiini (Teleostomi: Atherinopsidae) Based on the Mitochondrial ND2 Gene

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**The silverside fish tribe Menidiini (Teleostei: Atherinopsidae) consists of four genera, *Menidia, Labidesthes, Poblana,* and *Chirostoma,* that are distributed along the Atlantic coast of North America, throughout the Gulf of México, insular United States, and the Mesa Central of México. It has been suggested that *Chirostoma, Poblana,* and *Menidia* should be recognized as a single genus under the nominal *Menidia*. To test this hypothesis, phylogenetic relationships within the tribe Menidiini were assessed using the mitochondrially encoded ND2 gene. Monophyly of the Menidiini tribe was supported. Results also failed to support monophyly for the genera *Menidia* and *Chirostoma* as currently recognized. A central Mexican clade, inclusive of *Chirostoma* and *Poblana,* was recovered as monophyletic and strongly supported. Relationships within the Mesa Central clade support a previously recognized ''*humboldtianum*'' clade and the paraphyly of *Chirostoma* with respect to *Poblana*.**

THE New World silverside tribe Menidiini (*sensu* Chernoff, 1986a) consists of approximately 32 species and four genera, *Labidesthes*, *Menidia*, *Poblana*, and *Chirostoma*. The monophyly of the tribe has been supported by morphological (White, 1985; Chernoff, 1986a; Dyer, 1997) and allozymic studies (Crabtree, 1987), based on limited taxon sampling. However, a thorough investigation of relationships within the tribe has not been presented, and there is uncertainty regarding the taxonomic validity and the phylogenetic relationships of the genera and species within Menidiini (Dyer, 1998). This is in part because of highly variable meristics within and among taxa (Chernoff et al., 1981; Chernoff, 1982, 1986b; Duggins et al., 1986; Barriga-Sosa et al., 2002), coupled with an overall lack of diagnostic morphological characters in some groups (Dyer and Chernoff, 1996; Dyer, 1998).

*Labidesthes* is a monotypic genus found in freshwater throughout the entire Mississippi River and Great Lakes Basins, as well as along the Gulf coastal plain from Texas to South Carolina (Lee, 1980; Fig. 1). The genus *Menidia* includes seven or eight species that occur along the Atlantic and Gulf coasts from Maine to Veracruz, Mexico, the Mississippi drainage as far north as Missouri, and also into the Florida Keys (Gilbert and Lee, 1980; Fig. 1). Species of *Menidia* are generally estuarine and marine fishes, however some taxa have entirely freshwater populations. *Poblana* is endemic to the crater lakes in the eastern central state of Puebla, Mexico. Four species/subspecies of *Poblana* have been described, including *P. alchichica*, *P. letholepis*, *P. ferdebueni*, and *P. alchichica squamata*, and each taxon occurs in a separate lake (Miller et al., 2005). The silverside genus *Chirostoma* is the most diverse genus in the tribe and has been referred to as a species flock endemic to the Mesa Central (Barbour, 1973a; Barbour and Chernoff, 1984; Echelle and Echelle, 1984). It is comprised of 18–20 recognized species and includes several subspecies (Barbour, 1973b, 2002). *Chirostoma* is essentially confined to the Mesa Central in Mexico, although three taxa, *C. jordani*, *C. mezquital*, and *C. humboldtianum*, extend beyond this region (Barbour, 1973b; Miller et al., 2005; Fig. 1).

Previous studies have indicated the necessity for a comprehensive phylogenetic analysis of silverside tribe Menidiini. These studies were either conducted prior to the advent of modern cladistic analyses (Barbour, 1973b; Johnson, 1974) or they indicate that some groups within Menidiini may not be monophyletic (Gosline, 1948; Johnson, 1975; Echelle and Echelle, 1984), or, alternatively, were broader in scope, investigating higher level relationships and did not include adequate taxon sampling of Menidiini to address the species and generic level relationships within Menidiini (Chernoff, 1986a; Dyer, 1998). Additionally, as stated earlier, there is some question regarding the validity of genera within the tribe Menidiini (Miller et al., 2005; Nelson, 2006). Therefore, our objectives were threefold: use mitochondrial DNA (mtDNA) sequence data to assess the monophyly of the tribe Menidiini, assess phylogenetic relationships among the genera and species within Menidiini, and discuss the resulting biogeographical implications.

## MATERIALS AND METHODS

***Specimen collection and taxon sampling.***—We used specimens from both subspecies, *L. sicculus sicculus* and *L. sicculus vanhyningi*, for the monotypic *Labidesthes*. Representatives of all species of *Menidia* were included with the exception of *M.* c.f. *audens* and *M. clarkhubbsi*. *Menidia clarkhubbsi* is a gynogenic species from a male *M. beryllina* and female *M. peninsulae*; thus, its mitochondrial genome is identical to *M. peninsulae* (Echelle and Mosier, 1981; Echelle et al., 1983, 1989). All four species of *Poblana* have not been consistently recognized at the species level, as *P. letholepis* and *P. squamata* have been recognized as subspecies of *P. alchichica*

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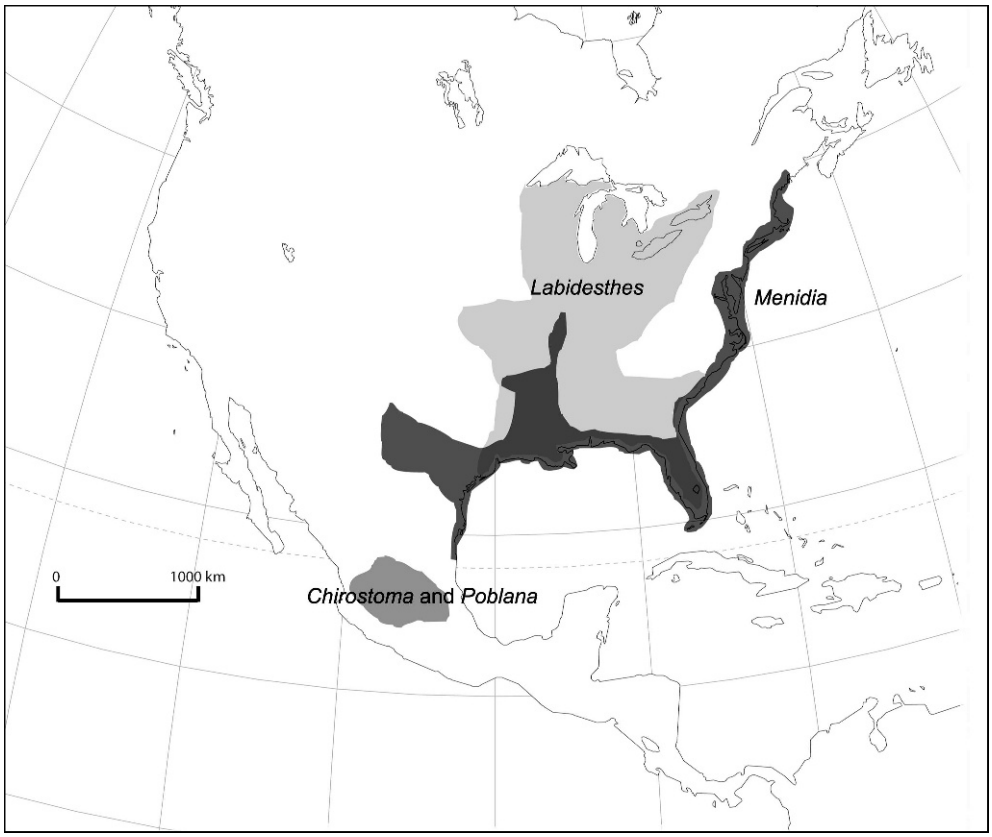


**Fig. 1.** The distribution of the four genera in silverside tribe Menidiini.

(Guerra Magaña, 1986; Miller et al., 2005); however, these taxonomic designations were not based on a published phylogenetic or taxonomic analysis, thus for our study all taxa of *Poblana* were included in the study. All species and subspecies of *Chirostoma* (*sensu* Barbour, 1973b; Miller et al., 2005) were included in this study with the exception of *C. aculeatum*, *C. bartoni*, *C. charari*, *C. melanoccus*, and *C. mezquital* because we were unable to obtain specimens of these species due to their rarity or possible extinctions (Lyons et al., 1998; Bloom et al., 2008).

Specimens were collected from the wild with standard seines, cast nets, electrofishing, purchased from commercial fishermen, or donated by colleagues. Whole specimens or fin clips were placed in 95% ethanol. The 75 individuals used in this study included 26 ingroup and five outgroup species spanning seven genera, three tribes, and both subfamilies of the silverside family Atherinopsidae (Fig. 2). When possible, multiple individuals of the same species were used, and in the case of widespread species, individuals from multiple populations were included (see Material Examined). We included 26 species and 71 individuals from tribe Menidiini, the focus of our study.

***DNA extraction, amplification, and sequencing.***—Whole genomic DNA was extracted from samples using the DNeasy tissue kit (Qiagen, Valencia, CA). Sequence data was generated for the entire mitochondrial encoded NADH dehydrogenase subunit 2 (ND2) gene (1047 bp) using PCR amplification primers GLN and ASN from Kocher et al. (1995). DNA was amplified in 25 µl reactions consisting of 1–4 µl of template DNA, 2.5 µl buffer, 2.5 µl PCR enhancer, 0.25–0.75 µl $MgCl_2$, 1 µl of each primer, 1 µl of dNTPs, and 0.5 µl of Eppendorf (Westbury, NY) *Taq* polymerase and sterilized water for the remaining volume. PCR temperature profile settings were as follows: a touchdown protocol was used consisting of an initial denaturation at 94°C for 2 min followed by 5 cycles each of 94°C for 30 sec, 56, 55, 54°C for

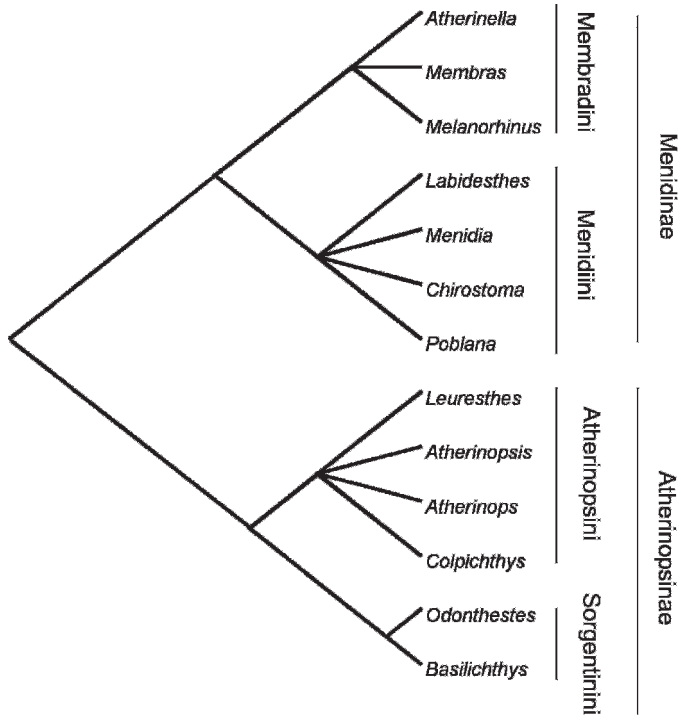


**Fig. 2.** Higher order relationships in New World Silverside family Atherinopsidae redrawn from Dyer (1997).

30 sec, 72°C for 1:15 min, 20 cycles of 94°C for 30 sec, 53°C for 30 sec, 72°C for 1:15 min, and a final extension of 72°C for 10 min. PCR products were purified using spin columns (Qiagen) or ExoSAP-IT exonuclease enzyme (USB, Cleveland, OH). Sequencing reactions (10 µl) were conducted using primers MET and TRP from Kocher et al. (1995) and Bigdye terminator sequencing kit 3.1 (Applied Biosystems, Foster City, CA) according to temperature profiles recommended by the manufacturer. Samples were then cleaned with Edge Biospin columns (EdgeBio, Gaithersburg, MD) and directly sequenced on an ABI 3100 or 3700 DNA sequencer. All sequences have been deposited on GenBank (see Material Examined for accession numbers).

***Phylogenetic analyses.***—The resulting sequences were edited and aligned manually using Sequencher ver. 4.5 (Gene Codes Corp., Ann Arbor, MI). Phylogenetic analyses using maximum parsimony (MP) were conducted using PAUP* (Swofford, 2003). The MP analysis employed heuristic searches with equal weights for all characters, 1,000 random stepwise additions with 100 trees saved at each iteration, and tree bisection and reconnection (TBR) branch swapping. Clade support was provided by nonparametric bootstrapping using 1,000 pseudoreplicates and 100 heuristic replicates and TBR branch swapping with ten trees held at each step.

We used ModelTest 3.06 (Posada and Crandall, 1998) to infer the best model of DNA sequence evolution based on the Akaike Information Criterion (AIC; Posada and Buckley, 2004), with each of the three codon positions treated as a separate data partition. Using this model we then implemented a Bayesian analysis (Huelsenbeck et al., 2001) with ten million Markov chain Monte Carlo generations and trees saved every 100 generations using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Four separate runs were performed, and the log likelihood scores for each run were plotted against generations to determine the point at which stationarity was reached. Trees recovered prior to stationarity were discarded as burn-in. Results from the four separate runs were compared to determine convergence of log-likelihood values and posterior probabilities. Posterior probabilities were considered significant when >0.95.

The integrity of various clades or taxonomic groups of interest was examined quantitatively using two topology tests. The groups tested were the monophyly of *Chirostoma*, *Poblana*, *Menidia* (*sensu stricto*), and the monophyly of *Menidia* (*sensu* Miller et al., 2005). Under maximum parsimony criteria, the monophyly of each group was constrained in separate analyses for comparison of tree length relative to the most parsimonious tree. For the Bayesian inference (BI), the monophyly of each group was constrained and the number of post burn-in trees that fit the constraints was divided by the total number of post burn-in trees from the BI analysis. The hypothesis was statistically rejected if 5% or less of the post burn-in trees recovered a tested relationship.

## RESULTS

Sequence alignment was unambiguous, with no insertions or deletions. Mean nucleotide frequency for all taxa were A = 0.216, C= 0.377, G = 0.161, and T = 0.246, and there was no significant difference in nucleotide composition among taxa ($\chi^2$ = 188.264, $P$ = 0.95). There were 538 variable sites, 483 of which were parsimony informative. The maximum parsimony analysis resulted in 60 equally parsimonious trees with a score of 2093. A strict consensus tree is shown in Figure 3 (CI = 0.42, RI = 0.79, and RC = 0.33).

Maximum parsimony and BI methods yielded nearly congruent topologies with the only exceptions corresponding to the placement of *Chirostoma attenuatum* and several minor differences at the tips of the ''*Poblana*'' clade. The monophyly of the silverside tribe Menidiini (*sensu* Chernoff, 1986a) was strongly supported by both maximum parsimony and Bayesian inference. At the generic level, *Poblana* and *Labidesthes* were recovered as monophyletic, whereas *Menidia* and *Chirostoma* were paraphyletic.

*Labidesthes* was sister to the remainder of the tribe, excluding *M. extensa*. Our data set included two specimens of *L. sicculus vanhyningi* from Florida and one individual of *L. s. sicculus* from the Upper Mississippi River basin. Average uncorrected sequence divergence between the two taxa was 14.7% and the specimens of *L. s. vanhyningi* had identical haplotypes.

Several species of *Menidia* formed a monophyletic ''*Menidia*'' clade exclusive of *M. extensa* and *M. menidia* (Figs. 3, 4). *Menidia beryllina* was a monophyletic basal lineage of the ''*Menidia*'' clade, while *M. peninsulae* was paraphyletic with *M. conchorum* nested within, and *M. colei* sister to the *M. peninsulae/conchorum* lineage. However, *Menidia* as recognized traditionally and by Miller et al. (2005) was not recovered as monophyletic (Figs. 3, 4). Topology tests indicated that the traditionally recognized *Menidia* would require 35 additional steps in MP and was not recovered in any of the post burn-in trees (0/80,000).

*Chirostoma* formed a paraphyletic assemblage with *C. riojai*, *C. arge*, and *C. contrerasi* more closely related to *Poblana* than to other members of *Chirostoma*. The remaining species of *Chirostoma* formed a large monophyletic ''*Chirostoma*'' group of two major clades, the ''*humboldtianum*'' clade of Barbour (1973b) and a clade with *C. jordani* sister to *C. larbarcae*. All ten individuals from nine populations of the widespread *C. jordani* were recovered as a monophyletic group. Within the ''*Chirostoma*'' group MP placed *C. attenuatum* as sister to the rest of the ''*Chirostoma*'' group, whereas BI placed *C. attenuatum* as sister to the ''*humboldtianum*'' clade within the larger ''*Chirostoma*'' group. There was a general lack of resolution within the ''*humboldtianum*'' clade, where sequence divergences were low (approx. 1.0%).

The (*C. arge*, *C. contrerasi*)(*C. riojai*, *Poblana*) clade forms a monophyletic group although support values are low, with an MP bootstrap value of 74% and no support from BI. The genus *Chirostoma* was never recovered as monophyletic among the taxon bipartitions (0/80,000) and required 63 additional steps for monophyly.

The genus *Poblana* formed a monophyletic group, within a larger clade that included *C. arge*, *C. contrerasi*, and *C. riojai* to the exclusion of other species of *Chirostoma*. The MP analysis supported monophyly for all of the species of *Poblana*. A clade consisting of *Poblana ferdebueni* was sister to a group comprising *P. alchichica* and an unresolved clade inclusive of *P. letholepis* and *P. squamata*. No species of *Poblana* was recovered as monophyletic in the Bayesian analysis.

## DISCUSSION

Both MP and BI infer a monophyletic Menidiini (*sensu* Chernoff, 1986a; Figs. 3, 4), corroborating conclusions based on morphology (Chernoff, 1986a; Dyer, 1997) and allozymes (Echelle and Echelle, 1984). Sparks and Smith

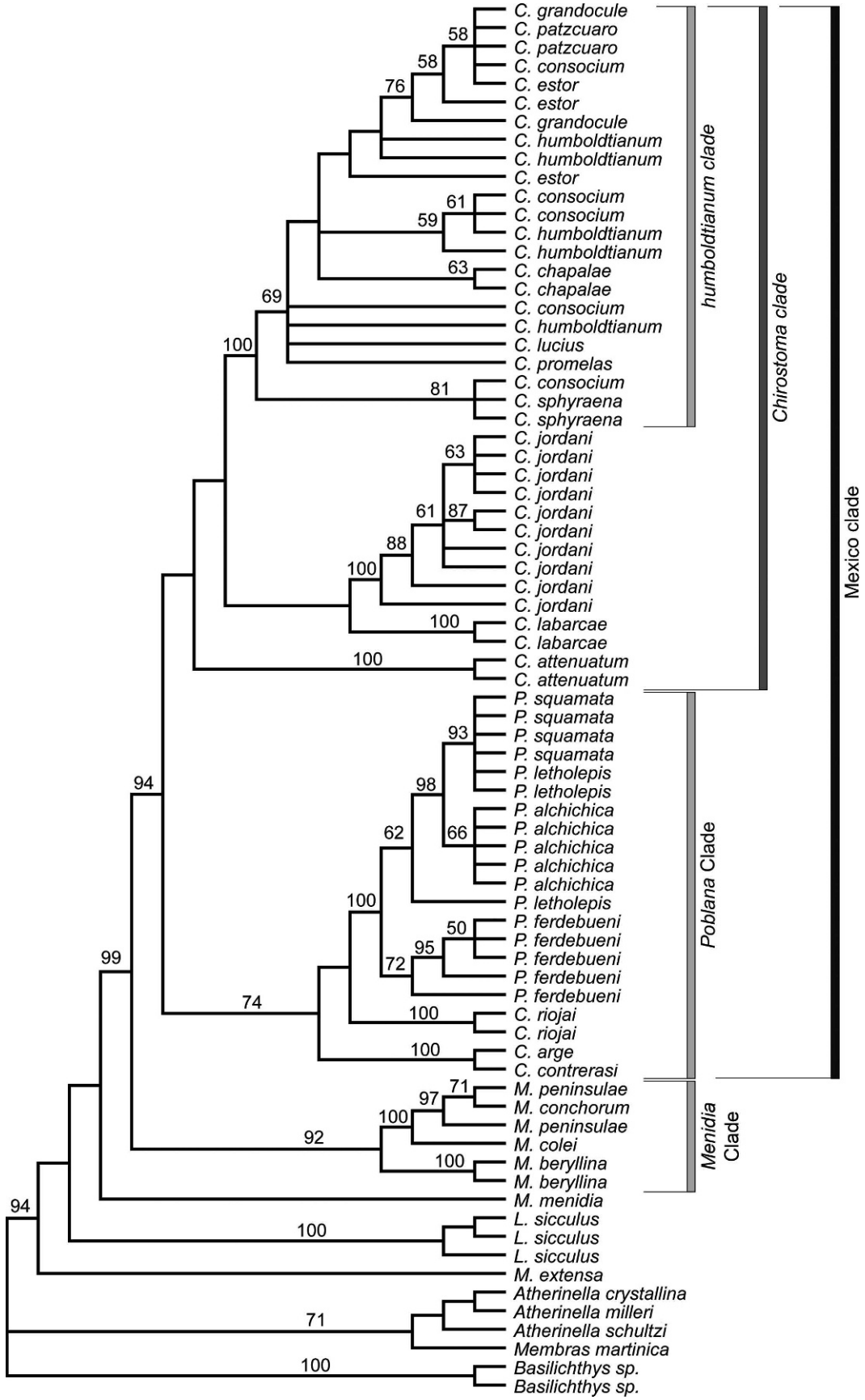


**Fig. 3.** Phylogeny of Menidiini silversides from a strict consensus of 60 equally parsimonious trees resulting following a heuristic search with 1,000 random stepwise additions and 100 trees saved at each iteration. Numbers above branches represent bootstrap values (1,000 pseudoreplicates) shown when ≥50.


**Fig. 4.** Phylogeny resulting from the Bayesian analysis consisting of 10 million generations. Nodes supported by ≥95% posterior probabilities are indicated with an asterisk.

(2004) did not recover Menidiini as monophyletic in a study of Melanotaenioidei, an Australasian group of atheriniform fishes. However, they included only four atherinopsid taxa in a test of monophyly for their ingroup, and thus lacked taxon sampling of atherinopsids to adequately test relationships of this group.

This study supports the hypothesis that *Chirostoma* and *Poblana* are closely related, comprising a monophyletic clade that consists of all of the silverside taxa from the Mesa Central, Mexico. Species of *Menidia* were basal to a clade consisting of entirely Mexican taxa (hereafter ''Mexico'' clade). Species of *Menidia* are mostly estuarine and marine in distribution, whereas *Chirostoma* and *Poblana* are exclusively freshwater species suggesting the Mesa Central taxa likely arose from a single historical transition from saline to freshwater. Barbour's (1973b) hypothesis of a diphyletic origin of *Chirostoma* also is rejected, although this study does support the hypothesis that *Menidia* is the closest relative to the Mesa Central silversides. Echelle and Echelle's (1984) study found that *M. beryllina* was basal to a group containing *M. peninsulae* and all members of *Chirostoma*, and *Poblana* (Echelle and Echelle, 1984). Our results differ in that *M. peninsulae* and *M. beryllina* are part of a monophyletic ''*Menidia*'' clade that is sister to a larger clade including *Chirostoma* and *Poblana*.

*Labidesthes sicculus* was monophyletic and sister to the remainder of the tribe, with the exception of *M. extensa*. Previous studies addressing relationships within Atherinopsidae that have included *Labidesthes* also recovered it as a basal member of the tribe Menidiini (Echelle and Echelle, 1984; White, 1985; Chernoff, 1986a; Dyer, 1997, 2006). Florida populations of *Labidesthes* have been suggested to represent a distinct species (Bean and Reid, 1930; Grier et al., 1990; but see Bailey et al., 1954). Although taxonomic decisions should not be based on sequence divergence alone, the large degree of sequence variation observed between populations of *L. s. vanhyningi* and *L. s. sicculus* in this study suggests a more comprehensive examination of species limits within *Labidesthes* is warranted.

The phylogenetic placement of *Menidia extensa* has long been an enigma (Hubbs and Raney, 1946; Gosline, 1948; Johnson, 1975; Echelle et al., 1983). Hubbs and Raney (1946) noted its phylogenetic position was difficult to ascertain as it shared morphological characters with *M. menidia*, and also with *M. beryllina*. In this study, the basal position of *M. extensa* renders *Menidia* a paraphyletic assemblage. The recovery of a paraphyletic *Menidia* brings into question the decision to treat C*hirostoma* and *Poblana* as synonyms of *Menidia* (Miller et al., 2005) Miller et al. (2005) based the decision to use an inclusive *Menidia* (=*Chirostoma* + *Poblana*) on Echelle and Echelle (1984), a study that did not include *M. extensa* or *M. menidia*. However, the results of our topology test as well as those of a decay index (not shown) indicate that with only one additional step *M. extensa* is no longer the basal taxon in the tribe. Therefore, we refrain from revising the status of the genera within the tribe Menidiini until additional individuals and multiple genes can be added to clarify the taxonomic status of these groups.

Within the ''*Menidia*'' clade, *M. beryllina* was recovered as monophyletic, supporting previous hypotheses based on allozymes that it is a distinct lineage (Johnson, 1975; Duggins et al., 1986). However, *M. peninsulae* was not recovered as monophyletic, with one individual being more closely related to *M. conchorum* than to the other specimen of *M. peninsulae*. This lack of reciprocal monophyly supports the conclusion that *M. conchorum* and *M. peninsulae* are conspecific (Duggins et al., 1986). Duggins et al. (1986) and Johnson (1975) both found *M. menidia* to be the basal species of *Menidia*. The topology within the ''*Menidia*'' clade is nearly identical to that of Echelle et al. (1989) with *M. beryllina* as basal to a clade inclusive of *M. colei*, *M. peninsulae*, and *M. clarkhubbsi*.

The ''*Poblana*'' clade from this study is congruent with Echelle and Echelle (1984), in that *C. arge* and *C. riojai*, as well as Barbour's (2002) recently described *C. contrerasi*, are closely related to *Poblana*. Both our study and Echelle and Echelle (1984) suggest that *C. arge* is the basal taxon, followed by *C. riojai*, and that the taxa of *Poblana* (*sensu stricto*) form a monophyletic clade. The species/population interface continues to be of question among taxa in *Poblana*. The parsimony analysis recovered *P. alchichica* and *P. ferdebueni* each as monophyletic lineages, supporting both at the status of species. Meanwhile *P. letholepis* and *P. squamata* were not recovered as monophyletic lineages but rather together formed a single clade to the exclusion of a single specimen of *P. letholepis*, which is generally in agreement with Guerra Magañas's (1986) conclusion that *P. letholepis* is more closely associated to *P. squamata* than to other taxa of *Poblana*. The lack of reciprocal monophyly further questions their appropriate taxonomic assignment, although this may be explained by incomplete lineage sorting (Avise, 2000; but see Frost and Kluge, 1994; Skinner, 2004). In contrast to the MP results, the Bayesian analysis did not recover the same topology, but instead found none of the species of *Poblana* to be monophyletic.

The MP placement of *P. ferbebueni* is of interest in relation to the other taxa of *Poblana* because it has shield-shaped scales as do all *Chirostoma* and most species of *Menidia*, unlike the remaining species of *Poblana*, which have round or oval shaped scales (Clyde Barbour, pers. comm.). The basal placement of *P. ferdebueni* in this study indicates that there was a character state change from shield- to round-shaped scales after divergence of *P. ferdebueni* and all other taxa of *Poblana*.

The species of *Poblana* are restricted to high altitude crater lakes in the state of Puebla. De Cserna and Alvarez (1995) and Guerra Magaña (1986) suggested that a pre-Pleistocene lake covered at least part of the area and receded from the west to the east, resulting in isolated populations. When the topology of the ''*Poblana*'' clade is used to investigate area relationships, a straightforward west to east pattern emerges (Fig. 5). The occurrence of the basal taxa, *C. arge* and *C. contrerasi*, in the Lerma-Santiago basin suggests a former connection to the Valley of México. A number of other fish groups support such a connection, including *C. humboldtianum* and *C. jordani* (Barbour, 1973a) as well as various groups of goodeids (Webb et al., 2004; Gesundheit and Macías Garcia, 2005; Dominguez-Dominguez et al., 2006) and cyprinids (Schönhuth and Doadrio, 2003; Miller et al., 2005). If the hypothesis of a pre-Pleistocene lake is correct, then the east–west trend from basal to derived taxa suggests that the dry lake receded from west to east. However, the timing of the Lerma–Santiago connection needs further investigation to determine whether in fact the ancestor of ''*Poblana*'' had access to the México basin prior to the pre-Pleistocene lake.

The ''*Chirostoma*'' clade is a monophyletic group comprising nearly all of species of *Chirostoma*. This study supports

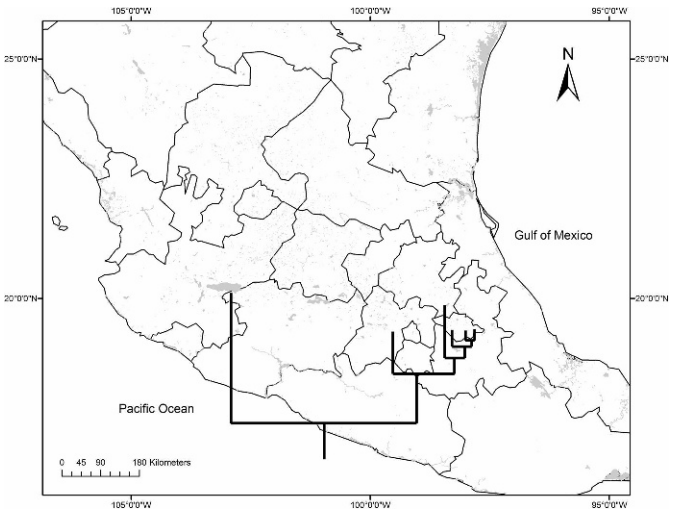


**Fig. 5.** Cladogram of area relationships within the ''*Poblana*'' clade imposed on a map of the Mesa Central, Mèxico.

Echelle and Echelle's (1984) ''*humboldtianum*'' clade, which was a monophyletic group of eight species, all of which were part of Barbour's (1973b) ''*jordani*'' group to the exclusion of *C. jordani*. *Chirostoma grandocule* and *C. patzcuaro*, which were not included in the Echelle and Echelle study (1984), were also found to be members of the ''*humboldtianum*'' group. Our study discovers a novel placement for *C. jordani* as sister to *C. labarcae*. *Chirostoma jordani* is the most widespread of all species of *Chirostoma* and along with *C. humboldtianum* and *C. mezquital* is the only species of *Chirostoma* to extend beyond the Mesa Central. Ten individuals of *C. jordani* from nine populations of *C. jordani* were included in this study in order to thoroughly test the monophyly of the species. A monophyletic *C. jordani* was strongly supported by both analyses.

Neither our study nor Echelle and Echelle (1984) support Barbour's (1973b) ''*arge*'' group. The lack of MP and BI agreement in placement of *C. attenuatum* could explain our inability to recover the ''*arge*'' group especially because we were only able to analyze four of the eight species in Barbour's (1973b) ''*arge*'' group. Unfortunately the taxon sampling within this group is unlikely to improve because of the extreme rarity of the missing taxa (Lyons et al., 1998; Bloom et al., 2008).

The ''*humboldtianum*'' clade is composed of nine currently recognized species (*sensu* Barbour, 1973b; and Miller et al., 2005). These species make up the bulk of what has been referred to as a ''species flock,'' although Barbour and Chernoff (1984) argue that only those taxa in which ''intrinsic mechanisms play the major role in the diversification of populations'' should be considered in terms of a species flock. Barbour and Chernoff (1984) recognized the large piscivorous species found in Lake Chapala, *C. lucius*, *C. sphyraena*, and *C. promelas*, locally known as pescados blancos, as a monophyletic clade forming a species flock. Our results did not recover a monophyletic clade of ''pescados blancos.'' However, this might be an artifact of low levels of divergence and resulting general lack of resolution of relationships within the ''*humboldtianum*'' clade. Mitochondrial DNA phylogenies effectively are single-gene trees with limitations in demarcating species in the face of possible hybridization and lineage sorting (Avise, 2000). Thus, our results do not resolve the issue of monophyly for the pescado blanco group but rather emphasize the need for greater taxon sampling and information from nuclear genes.

There are a number of competing hypotheses regarding the derivation of *Chirostoma* and *Poblana*. Barbour (1973a) argued that ancestral forms gained access to the Mesa Central via a Tertiary marine transgression. Alternatively, Miller and Smith (1986) suggested *Chirostoma* was derived from an ancestor of *Menidia* that followed a ''Plateau track'' whereby the Rio Grande (=Rio Bravo) was connected to the Mesa Central. The Plateau track hypothesis is supported by a number of fish genera such as *Ictalurus*, *Moxostoma*, and an extinct *Micropterus* that are primarily found in eastern United States, but are also represented by species on the Mesa Central (Lee et al., 1980; Miller and Smith, 1986; Miller et al., 2005). Miller and Smith (1986) suggested the great diversity in species number of *Chirostoma* indicates an earlier connection than that of other taxa following a similar track. Although Echelle and Echelle (1984) favored the Plateau track hypothesis, they also suggested the connection may have been more recent (Plio-Pleistocene) based on molecular clock estimations. The relatively low level of DNA sequence divergence found in our study also supports this more recent connection. The close relationship of *Menidia* to Mesa Central silversides (*Chirostoma* and *Poblana*) does not rule out an origin dating to a marine transgression, but the low levels of genetic divergence observed in both this study and that of Echelle and Echelle (1984) seems to support a more recent origin such as a connection between the Mesa Central and the Rio Grande. The oldest fossil record of *Chirostoma* is thought to be Plio-Pleistocene in age (Miller et al., 2005), further supporting a more recent connection. Future studies including a molecular clock calibrated using fossil data may prove informative in investigating the origin of Mesa Central silversides.

## MATERIAL EXAMINED

Institutional abbreviations follow Leviton et al. (1985). The GenBank accession numbers for ND2 are included for each specimen.

*Atherinella crystallina*: México, Jalisco, El Tecuan Lagoon, SLU 5105, EF602045.

*Atherinella milleri*: Honduras, Rio Cangrejal, SLU5104, EF602046.

*Atherinella schultzi*: México, Chiapas, Rio Palenque, SLU 5103, EF602044.

*Basilichthys* sp.: Peru, Rio Santuario ($n$ = 2), ANSP 180736, EF602042–EF602043.

*Chirostoma arge*: México, Guanajuato, Rio Laja, SLU 5110, EF602099.

*Chirostoma attenuatum*: México, Michoacán, Lago Pátzcuaro, SLU 5036, EF602082; México, Michoacán, Lago Zirahuén, SLU 5036, EF602083.

*Chirostoma chapalae*: México, Jalisco, Lago Chapala ($n$ = 2), SLU 5016, EF602075–EF602076.

*Chirostoma consocium*: México, Jalisco, Lago Chapala, SLU 5015, EF602077; México, Jalisco, Lago Chapala, SLU 5023, EF602077; México, Michoacán, Lago San Juanico ($n$ = 3), SLU 5035, EF602079–EF602081.

*Chirostoma contrerasi*: México, Michoacán, Lago Negritas, SLU 5080, EF602098.

*Chirostoma estor*: México, Michoacán, Lake Pátzcuaro ($n$ = 2), SLU 5114, EF602067–EF602068; Mexico, Michoacán, Lake Zirahuén, SLU 5026, EF602067.

*Chirostoma grandocule*: México, Michoacán, Lake Pátzcuaro (*n* = 2), SLU 5118, EF602061–EF602062.

*Chirostoma humboldtianum*: México, Michoacán, Lago Texpuxtepec, SLU 5039, EF602073; México, Michoacán, Lake Zacapu (*n* = 2), SLU 5011, EF602071–EF602072; México, Michoacan, Presa Santa Teresa, SLU 5119, EF602074; México, Nayarit, San Pedro Lagunillas, SLU 5095, EF602070.

*Chirostoma jordani*: México, Jalisco, Lago Chapala (*n* = 2), SLU 5033, EF602086–EF602087; México, Jalisco, Lago Atotonilco, SLU 5046, EF602088; México, Michoacán, Lago Negritas, SLU 5081, EF602089; México, Michoacán, Lake Cuitzeo, SLU 5111, EF602090; México, Michoacán, Presa Alvareina, SLU 5030, EF602091; México, Jalisco, Rio Mazcúa, SLU 5044, EF602092; México, Jalisco, Lago San Pablo de Naszas, SLU 5045, EF602093; México, Guanajuato, Presa Ignacio Allende, SLU 5113, EF602094; México, Guanajuato, Lago Yuriria, SLU 5112, EF602095.

*Chirostoma labarcae*: México, Jalisco, Lago Chapala (*n* = 2), SLU 5017, EF602084–EF602085.

*Chirostoma lucius*: México, Michoacán, Lake Negritas, SLU 5022, EF602059.

*Chirostoma patzcuaro*: México, Michoacán, Lake Pátzcuaro (*n* = 2), SLU 5117, EF602063–EF602064.

*Chirostoma promelas*: Tizapan Hatchery, no voucher, EF602060.

*Chirostoma riojai*: México, México, Lago Guadalupe Victoria (*n* = 2), SLU 5079, EF602096–EF602097.

*Chirostoma sphyraena*: México, Jalisco, Lake Chapala (*n* = 2), SLU 5025, EF602065–EF602066.

*Labidesthes sicculus*: United States, Minnesota, Lake Winona, SLU 5101, EF602056.

*Labidesthes s. vanhyningi*: United States, Florida, Pine Log Creek (*n* = 2), SLU 5106, EF602057–EF602058.

*Membras martinica*: United States, Mississipi, Gulf of Mexico, SLU 5102, EF602047.

*Menidia beryllina*: United States, Texas, Rio Grande at del Rio, SLU 5109, EF602048; United States, Louisiana, Bayou Lacombe, SLU 5108, EF602049.

*Menidia colei*: México, Yucatan Peninsula, no voucher, EF602051.

*Menidia conchorum*: United States, Florida Keys, Grassy Key, no voucher, EF602052.

*Menidia extensa*: United States, North Carolina, Lake Waccamaw, no voucher, EF602055.

*Menidia menidia*: United States, North Carolina, Wrightsville Beach, no voucher, EF602050.

*Menidia peninsulae*: United States, Florida, Panama City, SLU 5107, EF602054; United States, Florida, Wabasso, Indian River, no voucher, EF602053.

*Poblana alchichica*: México, Puebla, Lago Alchichica (*n* = 4), SLU 5034, EF602108–EF602111, EF602115.

*Poblana ferdebueni*: México, Puebla, Lago Chignahuapan (*n* = 5), SLU 5028, EF602100–EF602104.

*Poblana letholepis*: México, Puebla, Lago Preciosa (*n* = 3), SLU 5116, EF602105–EF602107.

*Poblana squamata*: México, Puebla, Lago Quechulac (*n* = 4), SLU 5115, EF6021120–EF602114, EF602116.

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