Hidrobiológica 2021, 31 (2): 153-162

UNIVERSIDAD AUTÓNOMA METROPOLITANA
Casa abierta al tiempo
Unidad Iztapalapa

HIDROBIOLÓGICA
http:/hidrobiologica.izt.uam.mx
ISSN: 2448-7333

OPEN ACCESS
Research Article
August,, 2021

Morphometric differentiation of the genera *Poblana*, *Chirostoma* and *Menidia* (Osteichthyes: Atherinopsidae)

Diferenciación morfométrica de los géneros *Poblana*, *Chirostoma* y *Menidia* (Osteichthyes: Atherinopsidae)

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Recibido: 02 de abril de 2021. ***Aceptado:*** 02 de agosto de 2021. ***Publicado:*** agosto de 2021.

ABSTRACT

Background: The recent origin of the genera *Chirostoma* and *Poblana*, and the scarce morphological differentiation between them, have made it difficult to define their taxonomic validity, in addition to the fact that both genera share a common ancestor, *Menidia*. Many taxonomic studies have recognized *Chirostoma* and *Poblana* as well-defined genera. Genetic analyses, however, indicate that *Poblana* is nested within *Chirostoma*, while other authors synonymize all silversides of the Central Plateau of Mexico within *Menidia*. Nevertheless, the differentiation between these genera has not currently been explored through a morphometric analysis. **Goals:** Under this scenario, in which the taxonomic validity of the genus *Menidia* is not in doubt, but the existence of *Poblana* is uncertain given that for some authors it pertains to the genus *Chirostoma*, the morphometric variations among the three genera and their species were analyzed in this study through Geometric Morphometrics. **Methods:** Seventeen landmarks were used on a sample of 393 Mexican specimens from various biological collections (216 of *Chirostoma*, 150 of *Poblana* and 27 of *Menidia*) obtained from several localities. The differences among the genera and species were tested using a Generalized Procrustes Analysis (GPA) and Discriminant Analysis (DA) computing the regression of shape on size-the vector of allometry, then remove this aspect of variation by looking at residual variation. **Results:** The cross-validated analysis showed 98.5% and 84.2% of classification among genera and species respectively, where the misclassifications were among species of *Chirostoma*. **Conclusions:** Morphometric differences were found among *Poblana*, *Chirostoma* and *Menidia*, therefore considered as discrete units.

Keywords: Atherinopsidae, Central Plateau, Generalized Procrustes Analysis, Mexico, Silversides

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To quote as:
López-Valdez, M. L., H. S. Espinosa-Pérez & A. L. báñez. 2021. Morphometric differentiation of the genera *Poblana*, *Chirostoma* and *Menidia* (Osteichthyes: Atherinopsidae). *Hidrobiológica* 31 (2): 153-162.

DOI:10.24275/uam/izt/dcbs/hidro/2021v31n2/Lopez

RESUMEN

Antecedentes: El origen reciente de los géneros *Chirostoma* y *Poblana*, y la escasa diferenciación morfológica entre ellos, han dificultado definir su validez taxonómica, aunado a que ambos géneros comparten un ancestro común, *Menidia*. Varios estudios taxonómicos han reconocido a *Chirostoma* y *Poblana* como géneros bien definidos. Sin embargo, análisis genéticos indican que *Poblana* está contenido en *Chirostoma*, mientras que otros autores agrupan a todos los pejerreyes de la Mesa Central de México dentro de *Menidia*. Sin embargo, la diferenciación entre estos géneros no se ha explorado actualmente a través de un análisis morfométrico. **Objetivos:** Bajo este escenario, en el que la validez taxonómica del género *Menidia* no está en duda, pero la existencia de *Poblana* es incierta dado que para algunos autores pertenece al género *Chirostoma*, la variación morfométrica entre los tres géneros y sus especies se analizaron en este estudio a través de Morfometría Geométrica. **Métodos:** Se utilizaron 17 marcas o “landmarks” para una muestra de 393 individuos de varias colecciones biológicas (216 de *Chirostoma*, 150 de *Poblana* y 27 de *Menidia*) procedentes de diversas localidades de recolecta en México. Las diferencias entre géneros y especies se probaron mediante un Análisis Generalizado de Procrustes (GPA) y Análisis Discriminante (DA) con los residuales de la regresión forma-tamaño, para eliminar la variación asociada al tamaño de los especímenes. **Resultados:** El análisis de validación cruzada mostró 98.5% y 84.2% de clasificación entre géneros y especies respectivamente, las clasificaciones erróneas fueron entre especies de *Chirostoma*. **Conclusiones:** Se encontraron diferencias morfométricas entre *Poblana*, *Chirostoma* y *Menidia*, por lo que se consideraron como unidades discretas.

Palabras clave: Análisis Generalizado de Procrustes, Atherinopsidae, Mesa Central, México, Pejerreyes

INTRODUCTION

Silversides of the Menidiini tribe (Family Atherinopsidae), are separated into four genera: *Chirostoma, Labidesthes*, *Menidia* and *Poblana*. The monophyly of this group has been supported by morphological (Chernoff, 1986; Dyer, 1997; White, 1985) and allozyme analyses (Crabtree, 1987), but there is uncertainty as to the taxonomic validity of the genera and species of the tribe due to a lack of robust morphological diagnostic characters (Echelle & Echelle, 1984), especially between *Poblana* and *Chirostoma*.

The taxonomy of *Chirostoma* is quite complex (Barbour, 1973) and it has, on several occasions, been separated into two or three genera (Álvarez, 1970; Jordan & Evermann, 1896). Meek (1904) recognized a single genus and set its species in three subgenera, though Jordan & Hubbs (1919) did not find enough evidence to recognize the subgenera. De Buen (1945) classified three genera and six subgenera, and Barbour (1973, 1974) established a single genus with 18 species contained in two groups: *jordani* and *arge*. Echelle & Echelle (1984) later discussed an unpublished study of Barbour where he mentioned that *Poblana* was a synonym of *Chirostoma* and, based on an allozyme analysis, they suggested that *Chirostoma* and *Poblana* should be subsumed under the name *Menidia*, and they also highlight that the atherinids on the Mexican Central Plateau comprise, with *Menidia peninsulae* Goode & Bean 1879, a monophyletic group that excludes all other species of *Menidia*.

Coyote-Hidalgo (2000) analyzed the taxonomic relationship between *Poblana* and *Chirostoma* using RAPD markers and observed at intergeneric level that 0.87% of these were exclusive for both genera and since the molecular space of variability presented a strong overlap, concluded that a taxonomic review is necessary to determinate its actual status. Later, Miller *et al.* (2005) grouped all the silversides of the Central Plateau under the name *Menidia*.

Bloom *et al.* (2009) assessed the monophyly of the tribe Menidiini and phylogenetic relationships among their genera and species, using the mitochondrial ND2 gene, in this study the monophyly of the tribe was supported. *Menidia* and *Chirostoma* were not recognized as monophyletic, and a central Mexican clade inclusive of *Chirostoma* and *Poblana* was recovered as monophyletic. The genus *Poblana* formed a monophyletic group, within a larger clade that included *Chirostoma arge* Jordan & Snyder 1899, *C. contrerasi* Barbour 2002 and *C. riojai* Solórzano & López 1965 to the exclusion of other species of *Chirostoma*. This study rejected the hypothesis of Barbour (1973) about of a diphyletic origin of *Chirostoma* (*jordani* and *arge* groups). The authors mention that the close relationship of *Menidia* to Central Plateau silversides (*Chirostoma* and *Poblana*) seem to support a recent origin such as a connection between the Central Plateau and the Rio Grande (= Rio Bravo).

Bloom *et al.* (2012) used mitochondrial and nuclear sequence data to generate a phylogeny for seven of the eight families of Atheriniformes, including the family Atherinopsidae which they did not recognize as monophyletic. However, in the phylogeny it was observed that a species of *Poblana* was nested within *Chirostoma.*

Campanella *et al.* (2015) supported the monophyly of the tribe Menidiini, but at the generic level they do not support the monophyly of *Chirostoma*, *Poblana* and *Menidia*, suggesting also necessary revisions to the taxonomy. In addition, the phylogenetic relationships obtained in this study propose a new classification of families of Atheriniformes and subfamilies, tribes and genera of Atherinopsidae, such is the case of *Chirostoma* and *Poblana* considered as synonyms of *Menidia*.

Morphometrical studies have addressed the morphological variation of the members of the "*humboldtianum* group" (Barriga-Sosa *et al.,* 2002; Alarcón-Durán *et al.,* 2017) and *Chirostoma grandocule* Steindachner 1894 (Barriga-Sosa *et al.,* 2004) but, to our knowledge, there is no information on morphological variation between different genera of the silversides. Thus, the purpose in the present study was to examine the taxonomic validity of *Chirostoma, Poblana* and *Menidia* using geometric morphometric data. Specifically, we tested if body shape data could distinguish *Poblana* from *Chirostoma* and *Menidia.* Finally, we assessed the degree of morphological variation between *Poblana* and *Chirostoma*. So the differences between the three genera and their species were tested using geometric morphometrics analysis.

MATERIALS AND METHODS

Fish collection. Specimens of *Chirostoma* were obtained from the Colección Nacional de Peces del Instituto de Biología de la Universidad Nacional Autónoma de México (CNPE-IBUNAM). Specimens of *Poblana* and *Chirostoma jordani* Woolman 1894 (Villa del Carbon, Mexico State) were obtained from the Colección de peces de la Facultad de Estudios Superiores Zaragoza de la Universidad Nacional Autónoma de México (FES-Z). Specimens of *Menidia* were provided by Manuel Castillo from the Colección del Laboratorio de Peces de la Universidad Autónoma Metropolitana, Iztapalapa (UAM-I).

We examined 393 specimens that had been previously identified at the institutions mentioned above, of which 216 belonged to the genus *Chirostoma*, 150 to *Poblana* and 27 to *Menidia*. We obtained a total of 12 species (1 species of *Menidia,* 3 of *Poblana* and 8 of *Chirostoma*). The localities from which species were collected could be seen in Table 1 and Figure 1. For our study only well-preserved samples with no damage or deformations were selected, as well as those collected over a short period to avoid variations generated by the passing of time. For this reason, only the species described in Table 1 were analyzed, being aware that there are other representative species for the three genera.

The specimens were stained with methylene blue to better observe every anatomical structure. The digitalization of each specimen was carried out using a 14 mexapixel Pentax camera. Seventeen landmarks were located over the fish anatomy (Fig. 2a, b). The anatomical landmarks were assigned following a homology criterion, that is, the landmarks were the same for all specimens (homology in geometric morphometrics *sensu stricto*) by which these structures are homologous for the three genera, apart from being easily identifiable and similar to those used by Barbour (1973), Rodríguez-Ruíz & Granado-Lorencio (1988), Alaye-Rahy (1993), Soria-Barreto & Paulo-Maya (2005) and Crichigno *et al.* (2013).

Morphometric analysis. The configurations of the landmark coordinates for the 393 specimens were scaled, translated and rotated using Generalized Procrustes Analysis (GPA). To eliminate the variation associated with the size of the specimens it was computed the multivariate allometric regression and a Principal Components Analysis (PCA) of residuals, later we visualize the Principal Components scores (PCs) and use them as the input to a Discriminant Analysis (DA). The latter was computed using PAST (PAleontological STatistics Version 4.06). The principal component (PC) scores were labeled for the genera and species in order to describe the distribution of the specimens. The extremes of each PC were then used to reconstruct the expected shapes of the landmark configurations with those particular scores. The reconstruction was made by adding the products of these PC scores (PCs) and the eigenvectors for those PCs to the mean tangent coordinates before projecting back from the tangent to the configuration space (O'Higgins *et al.,* 2001). The differences in shape between the mean and the shapes represented by the extremes of the PCs of interest were visualized using deformation grids (Bookstein, 1989; Marcus *et al.,* 1996; Dryden & Mardia, 1998) and computed using MORPHOLOGIKA2 (O'Higgins & Jones, 2006).

The scores of the specimens on all the non-zero PCs were submitted to a Discriminant Analysis (DA) (SPSS v.18.0.0) to examine the potential that differences in shape may have in classifying unknown specimens. Generalized 'Mahalanobis' distances and discriminant functions were then computed to assess the efficacy of the discriminant analysis in the classification. The discriminant analysis was carried out using a cross-validation approach in which multiple repeated analyses were carried out leaving out one individual in the construction of the discriminant function before classifying this individual according to the function. The exclusion of an individual reduces the likelihood of overestimating the efficacy of the discriminant functions by using them to classify specimens employed in their construction (Ibáñez *et al.,* 2009). This approach was applied to each genus and species, and the percentages of correct classification rates were recorded.

The configurations of the landmark coordinates for each genus were scaled, translated and rotated using a GPA to obtain the consensus configuration (a single set of landmarks which represents the central tendency of an observed sample; i.e., each genus). The morphometric distance matrix of Procrustes distances (the square root of the sum of squared differences between the positions of the landmarks in two optimally superimposed configurations at centroid size) among consensus configurations was then calculated by genus. In addition, the *Chirostoma jordani* Procrustes distances were compared with the genera *Poblana* and *Menidia.*

RESULTS

The standard length (SL) values of the *Poblana, Menidia* and *Chirostoma jordani* populations were similar, varying from 3.8 to 6.1 cm. All other species of *Chirostoma* were larger in size, *C. consocium* Jordan & Hubbs 1919, *C. promelas* Jordan & Snyder 1899 and *C. chapalae* Jordan & Snyder 1899 from 5.7 to 9.2, 6.8 to 9.1 and 5.7 to 9.8 cm respectively. Larger specimens were represented by *C. labarcae* Meek 1902 (range: 5.6-11.5 cm); *C. humboldtianum* Valenciennes 1835 (range: 5.6-12.8 cm); *C. estor* Jordan 1879 (range: 8.3-11.1 cm) and especially *C. lucius* Boulenger 1900 for which the largest size of 15.1-17.9 cm was recorded (Table 1).

Genus Level Classification. The PCA among genera (Fig. 3) indicated that the first two components explained 82.4% of the total variance with the first component explaining 55.6% and the second 26.8%. A clear separation of the genera *Poblana and Menidia* with some overlap with specimens of *Chirostoma* was observed for the two first principal components (Fig. 3) in particular specimens from *Poblana* overlaps mainly with *C. jordani*.

Table 1. Sample characteristics, sample size (N), range, mean and Std. of standard length. Superscripts correspond to the sampling sites in Chapala Lake: 1= (PCC) Petatan and Cojumatlan; 2= (PLC) La Palma.

Species	Nomenclature Authors	Locality	Key name	Sampling date	Catalog number	N	Length range (cm)	Mean±std. (cm)
Poblana letholepis	Álvarez 1950	La Preciosa Lake, Puebla	Ple-LCP	2002	No voucher	50	4.1-5.3	4.8±0.31
P. alchichica	de Buen 1945	Alchichica Lake, Puebla	Pal-LCA	2004	No voucher	50	3.8-5.3	4.4±0.38
P. squamata	Álvarez 1950	Quechulac Lake, Puebla	Psq-LCQ	1995	No voucher	50	4.1-5.4	4.8±0.29
Menidia beryllina	Cope 1867	Pueblo Viejo Lagoon, Veracruz	Mbe-PV	1990	No voucher	27	4.5-6.1	5.2±0.41
Chirostoma jordani	Woolman 1894	Villa del Carbon, Mexico State	Cjo-SLP	1980	No voucher	34	3.8-5.1	4.5±0.32
		Chapultepec Lake, Mexico City	Cjo-LVC	1986	3662	4	4.6-5.5	5.1±0.39
		Duero River, Michoacan	Cjo-CRD	1986	10442	18	3.8-5.1	4.4±0.34
		Xochimilco, Mexico City	Cjo-XDF	2012	18171	8	4.4-5.4	4.8±0.28
C. humboldtianum	Valenciennes 1835	Chapala Lake, Michoacan[1]	Chu-PCC	1985	2709	15	5.9-9.6	8.0±1.08
		Chapala Lake, Michoacan[2]	Chu-PLC	1985	7245	9	5.6-7.0	6.3±0.47
		Del Bosque Dam, Michoacan	Chu-PBM	1983	2036	6	9.8-12.8	11.2±1.10
C. consocium	Jordan & Hubbs 1919	Chapala Lake, Michoacan[1]	Cco-PCC	1985	2706	17	5.7-9.2	7.4±0.88
		Chapala Lake, Michoacan[2]	Cco-PLC	1985	2693	4	7.0-7.8	7.3±0.37
C. promelas	Jordan & Snyder 1899	Chapala Lake, Michoacan	Cpr-PCC	1985	2707	6	6.8-9.1	8.0±0.89
C. chapalae	Jordan & Snyder 1899	Chapala Lake, Michoacan[1]	Cch-PCC	1985	2705	50	6.1-8.7	7.4±0.56
		Chapala Lake, Michoacan[2]	Cch-PLC	1985	2698	6	5.7-9.8	7.3±1.45
C. lucius	Boulenger 1900	Chapala Lake, Michoacan	Clu-PLC	1985	2697	3	15.1-17.9	16.6±1.43
C. labarcae	Meek 1902	Chapala Lake, Michoacan	Cla-PLC	1985	2694	15	5.6-6.8	6.3±0.37
		La Boquilla Dam, Chihuahua	Cla-PBC	1967	7227	10	9.3-11.5	10.6±0.66
C. estor	Jordan 1879	Zirahuen Lake, Michoacan	Ces-LZM	1991	10203	11	8.3-11.1	9.5±0.84

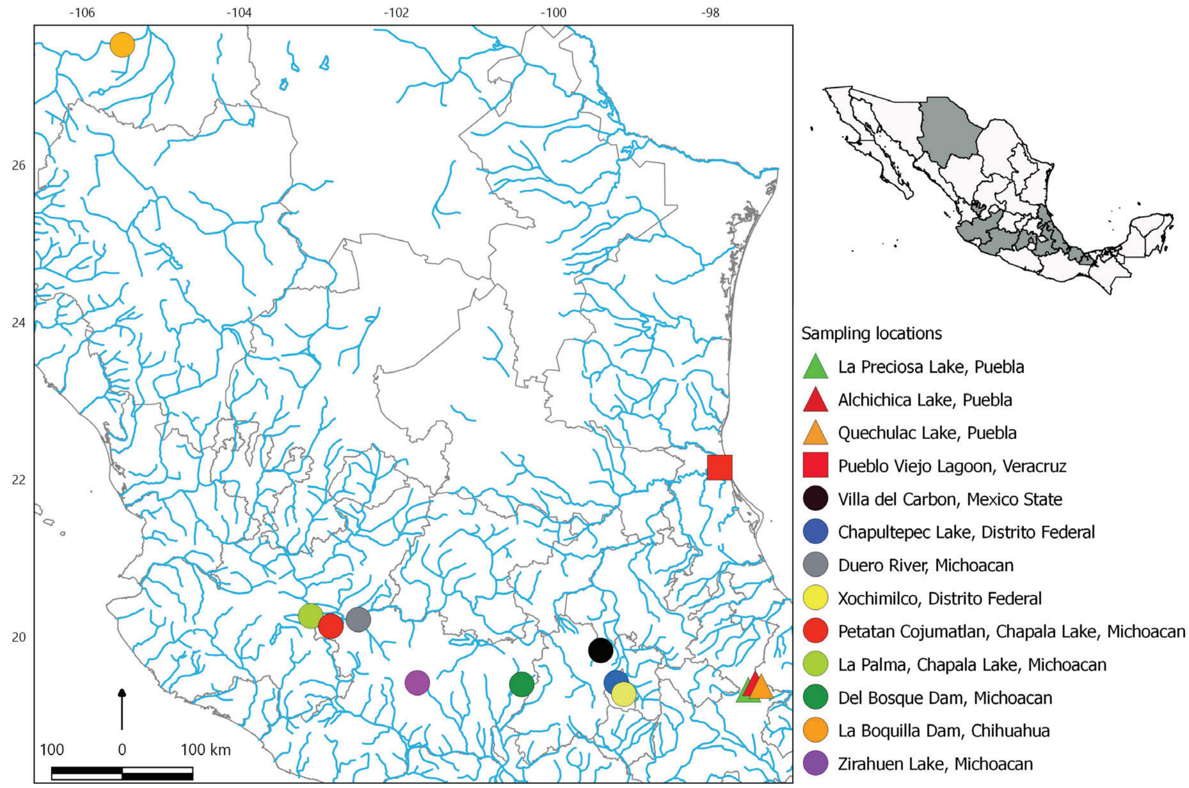

Figure 1. Sampling locations in the high plateau of Central Mexico. Colored symbols represent the localities for each specie described in the Table 1. The shape of the symbol corresponds to each genus: Triangle = *Poblana*; Square = *Menidia*; Circle = *Chirostoma*.

The general pattern of morphological differences described by these first two PCs was explored using transformation grids (Fig. 3). Lm 1 moves more to dorsal zone in *Menidia*, as well the Lm 2-4 forms a more pronounced arc in *Chirostoma* than in the other two genera. In *Menidia* specimens there is a relative displacement of the Lm 7 towards the ventral area (Fig. 3a, c). Transformation grids (Fig. 3a, c) show the mean shape of *Chirostoma* (Fig. 3a), *Poblana* (Fig. 3b) and *Menidia* (Fig. 3c) specimens. The genus *Chirostoma* presented a greater variation in landmarks 2 and 3 (space of the predorsal fins). In *Chirostoma*, the pectoral fin had a dorsally leaned position near landmark 2. Pectoral fin shape in *Menidia* and *Poblana* was very similar to the mean shape: almost at the same height as landmark 1, although in every case the end of the pectoral fin (landmark 7) occurred before the pelvic fin (landmark 9). Landmarks 10 and 11 (anal fin base) also presented variations: in *Poblana* it was thinner and getting closer to the caudal fin from landmark 11, while in *Chirostoma* it was thicker and in *Menidia* it remained almost like the mean shape.

Procrustes distances among genera showed that *Menidia* and *Chirostoma* are more similar to each other (0.0432 radians), followed by *Poblana* and *Menidia* (0.0492 radians) and lastly *Poblana* and *Chirostoma* (0.0519 radians). On the other hand, the Procrustes distances matrix among *Poblana*, *Menidia* and *Chirostoma jordani* showed that *Poblana* and *Menidia* to be more similar to each other (0.0492 radians), followed by *Menidia* and *C. jordani* (0.0549 radians), while that *Poblana* and *C. jordani* are less similar (0.0625 radians).

Overall discrimination by cross-validated grouped cases was 98.5%. The three genera clearly separate from each other (Wilks' $\lambda = 0.047$, $p < 0.001$) (Fig. 4). The 97.7% of the specimens of *Chirostoma* were correctly classified only with one specimen classified as *Menidia* (0.5%) and four as *Poblana* (1.9%). The 96.3% of the specimens of *Menidia* were correctly classified only with one specimen classified as *Chirostoma* (3.7%) while *Poblana* was 100% classified (Table 2).

Table 2. Classification results for the discriminant analysis with the cross-validation testing procedure (cross-validated) for the 3 genera: *Poblana*, *Menidia* and *Chirostoma*. Total classification success for cross-validated predicted genus variant membership. In bold the diagonal values.

	Predicted Group Membership			
Genus	*Poblana*	*Menidia*	*Chirostoma*	Total
Poblana	**100**	0	0	100
Menidia	0	**96.3**	3.7	100
Chirostoma	1.9	0.5	**97.7**	100

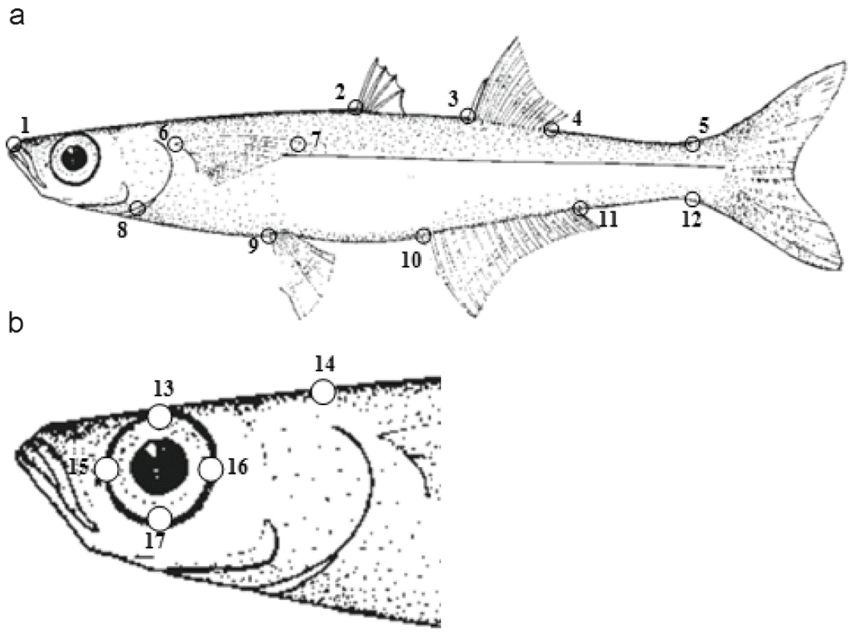

Figure 2. Location of the 17 homologous landmarks recorded on each individual for the analysis of geometric morphometry a) Landmark definitions used in the body of each individual; b) Landmark definitions used in the anterior region.

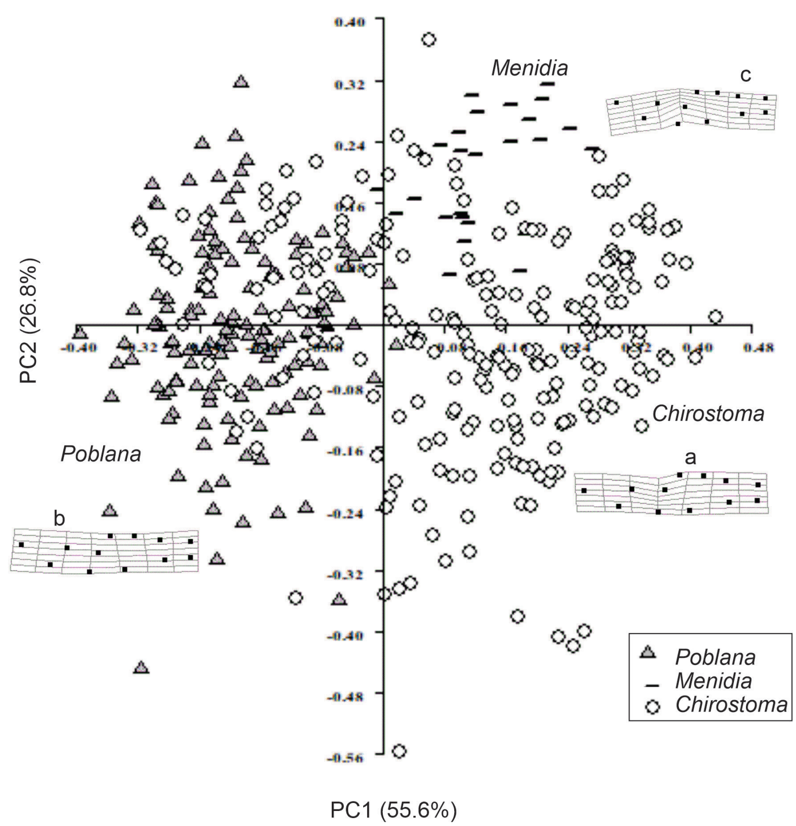

Figure 3. First two principal components (PCs) of fish shape labelled by genus. Thin plate spline deformation grids for the extreme points of each PC are shown; these are superimposed on the shapes predicted when the average landmark configuration of all specimens is deformed into that of a hypothetical specimen positioned at the extreme of the point of interest: a= *Chirostoma.* b= *Poblana.* c= *Menidia.*

Species Level Classification. The cross-validated analysis showed 84.2% of classification by species (Table 3). Misclassifications occurred within each genus (Fig. 5; Table 3). Individuals of *Poblana letholepis* Álvarez 1950, *P. alchichica* de Buen 1945 and *P. squamata* Álvarez 1950 formed a group apart from the other species. *Menidia beryllina* Cope 1867, *Chirostoma jordani* and *C. labarcae* were better classified with 100% of the cross-validation, *C. lucius* and *C. chapalae* grouped with 90.9% and 76.0% respectively. The species with lower percentages of discrimination were *Chirostoma estor* with 73.2%, *C. humboldtianum* with 63.3% (20% similar to *C. chapalae*), *C. promelas* with 50.0% (with 33.3% and 16.70% of misclassification with each *C. humboldtianum* and *C. estor*) and *C. consocium* with 47.6% (38.1% similar to *C. estor*). Differences among species were highly significant (Wilks' $\lambda = 4.7 \times 10^{-5}$, $p < 0.001$).

DISCUSSION

The discriminant analysis present evidence that morphometric variation significantly separates the genera *Poblana*, *Chirostoma* and *Menidia*. In common with the results obtained, this separation indicates the differences in the shape of the three genera and agrees with the genetic analysis of Bloom *et al.* (2009) in which they maintain *Chirostoma* and *Poblana* as independent, highlighting that are closely related, so they support a recent origin such as a connection between the Central Plateau and the Rio Grande. In more recent times, Bloom *et al.* (2012) used genetic analyses to confirm that *Poblana* is nested within *Chirostoma*. Campanella *et al.* (2015) formally included *Poblana* and *Chirostoma* within *Menidia*, confirmed that *Poblana* is nested within *Chirostoma*, and proposed that neither genus is valid as they are both members of *Menidia*, following Miller *et al.* (2005).

Guerra-Magaña (1986) taxonomically analyzed 18 morphological characters in species of *Poblana* and populations of *Chirostoma jordani* and found a clear differentiation of the two groups at the genus level. Our study agrees with that differentiation and it is, at the time, the first approximation of geometric morphometrics that discriminates *Poblana*, *Chirostoma* and *Menidia*. Nevertheless, our results statistically discriminate between the shapes of the *Poblana* species, for which reason we do not concur in considering them as subspecies of the *Poblana* genus, as Guerra-Magaña (1986) mentioned.

Phenotypic traits are essential for identifying discrete phenotypic entities. Our results show that *Poblana* and *Chirostoma jordani* are statistically different morphs, i.e., each one keeps its own identity, though *C. jordani* grouped preferentially with *Poblana*, in contrast with the other species of *Chirostoma*.

The Central Plateau is a region that has been subdivided several times according to its geographical, hydrological and ichthyological features (Díaz-Pardo *et al.,* 1993). It is generally established that these subdivisions favored the fragmentation of different populations of fish that followed their own evolutionary history after they were isolated. Thus, the genera *Chirostoma* and *Poblana* are examples of these endemic monophyletic groups. There are other examples of this fragmentation in different populations, i.e., in goodeids (Domínguez-Domínguez *et al.,* 2008), catostomids (Pérez-Rodríguez *et al.,* 2016), poecilids (Beltrán-Lopéz *et al.,* 2018), *Chirostoma attenuatum* (Betancourt-Resendes *et al.,* 2018) and the "*humboldtianum*" clade (Betancourt-Resendes *et al.,* 2019) between others. Several authors have proposed hypotheses that try to explain the origin of both *Poblana* and *Chirostoma*. Smith & Miller (1986) suggested that a species resembling *Menidia* penetrated Mexican continental waters from the Atlantic coast through the Rio Bravo in the Pliocene-Pleistocene. Back then, the Rio Bravo was connected with the Central Plateau. Afterwards, this communication was interrupted, and populations were isolated, diverged, dispersed and reached a wide distribution throughout the Central Plateau. The isolation of the

Table 3. Classification results for the discriminant analysis with the cross-validation testing procedure (cross-validated) for the 12 species: *Poblana letholepis*= Ple. *Poblana alchichica*= Pal. *Poblana squamata*= Psq. *Menidia beryllina*= Mbe. *Chirostoma jordani*= Cjo. *Chirostoma humboldtianum*= Chu. *Chirostoma consocium*= Cco. *Chirostoma promelas*= Cpr. *Chirostoma chapalae*= Cch. *Chirostoma lucius*= Clu. *Chirostoma labarcae*= Cla. and *Chirostoma estor*= Ces. Total classification success for cross-validated predicted species variant membership. In bold the diagonal values.

	Predicted Group Membership												
Species	Ple	Pal	Psq	Mbe	Cjo	Chu	Cco	Cpr	Cch	Clu	Cla	Ces	Total
Ple	**92.0**	4.0	4.0	0	0	0	0	0	0	0	0	0	100
Pal	0	**96.0**	4.0	0	0	0	0	0	0	0	0	0	100
Psq	10.0	8.0	**82.0**	0	0	0	0	0	0	0	0	0	100
Mbe	0	0	0	**100.0**	0	0	0	0	0	0	0	0	100
Cjo	0	0	0	0	**100.0**	0	0	0	0	0	0	0	100
Chu	0	0	0	0	0	**63.3**	13.3	0	20.0	0	0	3.3	100
Cco	0	0	0	0	0	14.3	**47.6**	0	0	0	0	38.1	100
Cpr	0	0	0	0	0	33.3	0	**50.0**	0	0	0	16.70	100
Cch	0	0	0	0	0	8.0	8.0	8.0	**76.0**	0	0	0	100
Clu	0	0	0	0	0	9.1	0	0	0	**90.9**	0	0	100
Cla	0	0	0	0	0	0	0	0	0	0	**100**	0	100
Ces	0	0	0	0	0	7.1	16.1	3.6	0	0	0	**73.2**	100

populations then resulted in the genera *Chirostoma* and *Poblana*. This hypothesis supports the results obtained in this study where *Chirostoma* and *Poblana* have different shapes.

The Procrustes distances matrix among genera determined that *Poblana* and *Chirostoma* are less alike to each other (with a greater distance between them) and *Menidia* and *Chirostoma* are the most similar. The Procrustes distances indicated a lower similarity between *Poblana* and *Chirostoma jordani* and a greater similarity between *Poblana* and *Menidia*. The results also show that the mean shapes of *Poblana-Chirostoma* and *Poblana-C. jordani* are more different than those of the other genera, supporting the idea of *Poblana* and *Chirostoma* being morphometrically different.

Chirostoma estor, *C. humboldtianum*, *C. promelas* and *C. consocium* were species that showed the lowest value of discrimination with greater overlapping with other species, these results could be related to high degree of morphological polymorphism of the atherinopsid's species (Barriga-Sosa *et al.,* 2002; Bloom *et al.,* 2009). Additionally, morphological differences within species have been closely linked to habitat adaptations related with swimming mode where body shapes are associated to lentic and lotic habitats (Fluker *et al.,* 2011; Foster *et al.,* 2015; Alarcón-Durán *et al.,* 2017), these same characteristics could explain the changes in form at the genus level.

We found significant divergent morphometrics in the sampled silversides which were useful in discriminating genera and species despite the strong genetic relationships. Phenotypic variations are a product of genotype and environment interactions. They are thus a complex and important biological phenomenon that is still poorly studied. Although not all species of *Poblana*, *Chirostoma* and *Menidia* were incorporated in this study, significant morphometric differences were found at genus level.

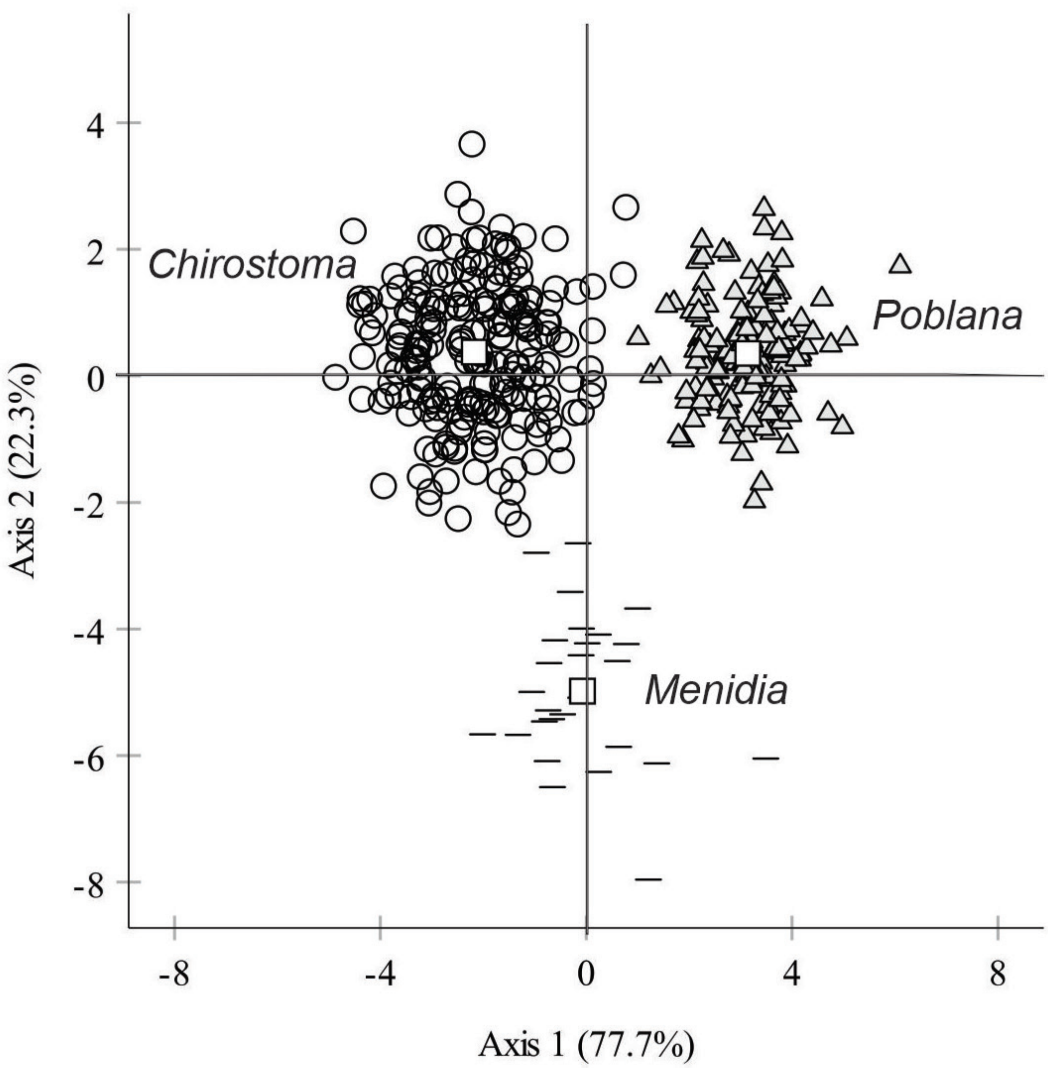

Figure 4. Plot of first and second axis of the discriminant analysis among *Poblana*, *Chirostoma* and *Menidia*. White square are centroids of each genus.

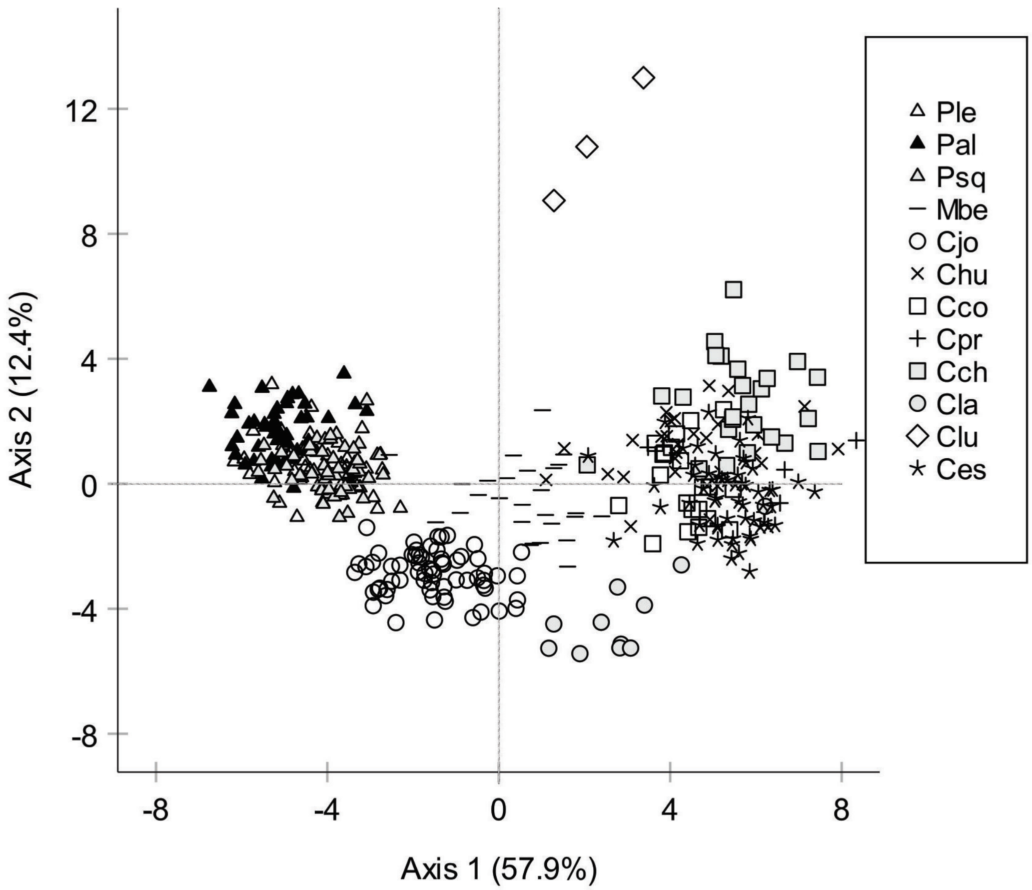

Figure 5. First two axes of the discriminant analysis of fish shape labelled by species. Key names of species as in Table 3.

ACKNOWLEDGEMENTS

The authors would like to thank Ernesto Mendoza (FES-Z) and Manuel Castillo (UAM-I) for providing the samples of *Poblana* and *Chirostoma jordani* (Cjo-SLP), and *Menidia* respectively. Also, to Christian Lambarri for her help on the translation of the manuscript.

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