UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

FACULTAD DE BIOLOGÍA

PROGRAMA INSTITUCIONAL DE DOCTORADO EN CIENCIAS BIOLOGICAS
OPOCIÓN: (CONSERVACIÓN Y MANEJO DE RECURSOS NATURALES)

FILOGENIA Y FILOGEOGRAFÍA DEL CHARAL PRIETO *Chirostoma attenuatum* Y DEL GRUPO DE ESPECIES *Chirostoma humboldtianum* (PISCES: ATHERINOPSIDAE)

TESIS QUE PRESENTA

M. C. ISAÍ BETANCOURT RESENDES
PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS BIOLÓGICAS

Director de tesis: Dr. Omar Domínguez Domínguez
Laboratorio de Biología Acuática, Facultad de Biología, UMSNH

Morelia, Michoacán a 16 de abril de 2018

AGRADECIMIENTOS

Este trabajo no se había podido realizar sin la valiosa colaboración de muchas personas a quienes expreso mis más sinceros agradecimientos.

Primeramente darle gracias a Dios por haberme ayudado hasta el día de hoy, colmándome de su gracia, ciencia y sabiduría para la conclusión de mis estudios.

Al Dr. Omar Domínguez Domínguez director de este trabajo que redundo en mi beneficio y desarrollo profesional. Por la oportunidad de colaborar en este trabajo, su valiosa guía, consejos y sugerencias.

Al posgrado Institucional de Doctorado en Ciencias Biológicas por permitirme formar parte del programa así como por los apoyos otorgados para la realización de estancias, congresos, cursos entre otros.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca otorgada para realizar mis estudios de doctorado (Febrero 2014-2018).

A los proyectos de investigación a cargo del Dr. Omar Domínguez Domínguez: Al Ministerio de Economía y competitividad de España (CGL-2013 41375-P) y a la coordinación de la investigación científica –UMSNH, México.

Al laboratorio de Biología acuática por la disponibilidad de sus instalaciones para realizar este trabajo.

A los integrantes de mi comité tutorial: A la Dra. Sabina Irene Lara Cabrera, a la Dra. Irene de los Ángeles Barriga Sosa, al Dr. Miguel Martínez Trujillo, al Dr. Kyle Piller y al Dr. Ignacio Doadrio Villarejo, por sus valiosas aportaciones, consejos y apoyo para enriquecer la calidad del trabajo.

Al Dr. Rodolfo Pérez Rodríguez por su apoyo, instrucción y consejos que fueron parte importante para enriquecer el trabajo de tesis.

A mi esposa, Sorahida Esquivel Estrada por su apoyo incondicional en cada momento durante mi trabajo de tesis.

A mi hija Amisadaí Betancourt por su comprensión, cariño y motivación-

A mis padres José Luis Betancourt y Nelva Resendes por su gran apoyo incondicional, consejos y ánimos para terminar mi trabajo de tesis.

A mis hermanos Jocabed, Esther, Isaac y Rud Betsabé por mostrarme su cariño y respeto.

A mis compañeros y amigos del laboratorio, Gabriela Beltrán, Berenice García, Paola Palmerin, Eloisa Torres, Yareli Arroyo, Mayra Gómez, Areli Ramírez, Karla Nayarit Ballesteros, Fernando Mar, Moisés Emmanuel, Francisco Loera, Quetzal Torres, Victor Piñeros, Francisco Martínez, Alejandro Lievanos, Luis Fernando, Fidel Anguiano, Diego Montejo, por compartir conmigo sus conocimientos, tiempo, amistad, compañerismo y ayuda para la realización de este trabajo.

ÍNDICE DE CONTENIDO

Página.

Resumen

Se llevó a cabo un estudio en las especies de *Chirostoma* adaptadas exclusivamente a hábitats lacustres, conformado por dos clados hermanos. Un clado incluye al charal prieto *Chirostoma attenuatum* endémico de los lagos de Pátzcuaro y Zirahuén y el otro clado al "grupo *Chirostoma humboldtianum*" conformado por nueve especies. La evolución de los pescados blancos y charales del género *Chirostoma* en el centro de México ha sido poco estudiada y sus relaciones filogenéticas e historia evolutiva son inciertas

Esta tesis aborda dos aspectos redactados en dos capítulos: El primero trata de un estudio filogeográfico en las poblaciones de *C. attenuatum* usando un marcador mitocondrial (*citocromo b*) y un nuclear (primer intrón de la *proteína ribosomal S7*) para: 1) dilucidar la evolución de *C. attenuatum attenuatum* del Lago de Pátzcuaro y *C. attenuatum zirahuen* del lago de Zirahuen si pueden ser identificadas como linajes evolutivos independientes y especies validas; 2) Revelar los procesos biogeográficos implicados en la historia evolutiva de *C. attenuatum*; y 3) dilucidar la historia demográfica y su asociación con eventos climáticos.

Los resultados obtenidos en este estudio muestran un escenario evolutivo en dos etapas: primero, un aislamiento alopátrico temprano en la historia evolutiva (fechado hace 601,000 años) donde las poblaciones aisladas acumularon divergencias genéticas detectadas con ambos marcadores (*cytb*=1.1%; *S7*=0.4%). La segunda, con un contacto secundario al existir una conexión entre ambas cuencas (hace 8,000 a 30,000 años) con bajas tasas de migración y cada una mantuvo su propia trayectoria evolutiva. Los resultados a través de análisis filogeográficos y la historia demográfica de *C. attenuatum* indican un proceso de especiación con dos linajes bien diferenciados con historias evolutivas independientes. *C. zirahuen* del lago de Zirahuén y *C. attenuatum* del lago de Pátzcuaro representan una diversidad genética única e irremplazable que debe ser conservada.

El segundo capítulo aborda un estudio con metodologías de filogenia y filogeografía en el grupo de especies *Chirostoma humboldtianum* usando dos marcador mitocondrial (*citocromo b* y el *D-loop*) y un nuclear (primer intrón de la *proteína ribosomal S7*) para: 1) dilucidar las relaciones filogenéticas entre las especies reconocidas; 2) entender su historia evolutiva y su asociación con los eventos climáticos y geológicos ocurridos en el centro de México; y 3) describir como la vicarianza y fragmentación de hábitats han sido un factor importante en su configuración genética.

Los resultados obtenidos muestran inconsistencias taxonómicas y los datos genéticos no soportan la monofilia de las especies, evidenciando una mezcla genética entre las especies. Sin embargo, existe una segregación genética acorde con la geografía, reconociéndose cinco linajes genéticos cercanamente relacionados. El escenario cladogenético entre los cinco linajes encontrados, muestra una diversificación en el pleistoceno fechado en menos de un millón de años. La evolución del grupo en etapas tempranas está asociada a la vicarianza debido al aislamiento de cuencas hidrográficas. El segundo escenario evolutivo tiene que ver con una diferenciación intralacustre, que pueden estar asociada con la especiación simpátrica. Aunque, los marcadores utilizados presentan poca resolución para explicar una especiación simpátrica en los lagos de Chapala y Pátzcuaro, no se descarta que esté ocurriendo. Pero, es necesario abordarla con marcadores de mayor resolución como son los SNPs. En el grupo *Chirostoma humboldtianum* los análisis genéticos contrastan con la hipótesis de la actual clasificación, y han surgido nuevas implicaciones biogeográficas y evolutivas derivas de la presente investigación. Las discrepancias encontradas entre los datos genéticos y morfológicos pueden ser atribuidas a la alta plasticidad fenotípica dentro del grupo, relacionada con las presiones del hábitat y a las rápidas divergencias adaptativas.

En general los resultados de los procesos que rigen la evolución de las especies de *Chirostoma* adaptadas exclusivamente a hábitats lacustres, están asociados a eventos de vicarianza como resultado de la fragmentación del hábitat,

influidos por eventos geológicos y climáticos así como mecanismos únicos de especiación intra lacustre en los lagos del centro de México.

Palabras clave: Especiación, diversificación, delimitación de especies, plasticidad fenotípica, polimorfismos.

Abstract

We carried one study of the silversides of *Chirostoma* genus inhabiting in lacustrine ecosystem. This group is composed by two sister clades. One clade include to slender silverside *Chirostoma attenuatum* endemic to Patzcuaro and Zirahuen Lakes and the second clade is comprised by nine currently recognize species called "*Chirostoma humboldtianum* species group". The evolution of de "pescados blancos and charales" of *Chirostoma* in Central Mexico have been poorly understood and their phylogenetic relationships and evolutionary history are unclear.

This thesis approach two works that are included in two chapter: First, is a Phylogeographic studies in population of *Chirostoma attenuatum* using one mitochondrial gene (*cytochrome b*) and one nuclear intron (first intron of *S7 ribosomal protein* gene) to 1) test the independence of lineage evolution of the population and determine whether *C. a. attenuatum* from Patzcuaro and *C. a. zirahuen* from Zirahuen could be identified as independent evolutionary significant units and valid species; 2) reveal the biogeographic processes implicated in the evolutionary history of *C. attenuatum*; and 3) elucidate the demographic history and its link with climate events.

The results showed two speciation stage: First, an early cladogenetic events occurred in evolutionary history (dated ca. 601 000 years ago). When the isolated population accumulated unique adaptation detected with both molecular markers as genetic divergences (*cytb*=1.1%; *S7*=0.4%). The second, with low migration rate and the maintenance of the evolutionary trajectory. The Phylogeographic findings and the demography history of *C. attenuatum* indicated that the well-differentiated lineages underwent independent evolutionary histories in respective lakes. *Chirostoma zirahuen* from Zirahuen and *C. attenuatum* from Patzcuaro represent unique and irreplaceable genetic diversity that must be conserved

The second chapter approach to study using combined methodologies of phylogeny and phylogeography in *Chirostoma humboldtianum* species group using two mitochondrial markers (*cytochrome b* and *D-loop*) and one intron nuclear (first intron of *S7 ribosomal protein* gene) to: 1) elucidate the phylogenetic relationship between the recognized species, 2) understand their evolutionary history, and the link with the geological and climate history in Central México, and 3) described how the vicariants events and habitats fragmentation occurred on Central Mexico has been important factor in their genetic configuration.

The results showed several taxonomic inaccuracies and our genetic data did not support the species monophyly, proving a genetic mixture between recognized species. Therefore, there are a genetic segregation according to geography recognizing five major genetic lineages closely relationships. The cladogenetic scenario between five lineages, showed that diversification occurred in Pleistocene less to 1 M.a. The evolution of *Chirostoma humboldtianum* species group in early stages was associated with ancient hydrological history. The second evolutionary stage showed a recent intra lacustrine genetic differentiation that could be associated to sympatric or ecological speciation. Although, these molecular marker have to low resolution for explicate sympatric speciation that have been occurred in Chapala and Patzcuaro lakes. However, is needs use molecular marker more powerful as SNPs. The genetic data did not support the current classification in *Chirostoma humboldtianum* species group and new biogeography and evolutionary insight have arisen. The discrepancies found between genetic and morphological data can be attributed to the high phenotypic plasticity within the group, related to the pressures of the habitat and to fast adaptive divergences.

In general the results of the process that govern the evolution of the phylogenetically most derivate *Chirostoma* species inhabiting in lacustrine ecosystem, have been associated with vicariant events as resulted of habitat fragmentation influenced by geological and climate events occurred in Central Mexico as well as the unique mechanism of sympatric speciation.

Keywords: Speciation, diversification, species boundaries, phenotypic plasticity, polymorphisms.

1. Introducción

La cladogénesis y radiación adaptativa de las especies de peces del centro de México han sido ampliamente estudiados (Dominguez-Dominguez et al., 2006 y 2008; Perez-Rodriguez et al., 2009; García-Martínez et al., 2015; Corona-Santiago et al., 2015; Beltrán-Lopéz en prensa), y en la mayoría de ellos mencionan a los eventos climáticos y geológicos como el motor de dicha radiación. Estos procesos han modelado la alta diversidad específica en familias como Atherinopsidae, Cyprinidae y Goodeidae (Miller et al., 2005). Los eventos climáticos ocurridos durante el Pleistoceno, se caracterizaron por presentar ciclos glaciares e interglaciares, (Clark et al., 2009). Estos ciclos afectaron de manera importante los procesos limnológicos de los lagos del centro de México (Bradbury, 2000; Israde-Alcantara y Garduño-Monroy, 1999; Ferrari et al.2011), teniendo evidencia de sus efectos en la historia paleo climática del centro de México reflejando cambios demográficos en las especies de peces dulceacuícolas (Bradbury, 2000; Miller et al., 2005; Domínguez et al., 2008; Caballero et al., 2010; Metcalfe et al., 2010; Ortega et al., 2010; Vásquez et al., 2010; Torres-Rodríguez et al., 2012; Bloom et al., 2013; Corona-Santiago et al., 2015; García-Martínez et al., 2015).

En el Centro de México destaca la presencia de la familia de peces Atherinopsidae. Esta familia está representada por los géneros *Poblana* Bonaparte y *Chirostoma* swaison (Barbour, 1973b; Miller et al., 2005) y constituyen parte fundamental de la ictiofauna dulceacuícola mexicana, además presentan una importancia tanto biológica como cultural y económica en los lagos del centro de México (Barbour, 1973b; Miller et al., 2005; Moncayo-Estrada et al., 2011). Estas especies han sido parte de los recursos pesqueros desde épocas prehispánicas para diversas Etnias y se consideran una de las pesquerías más antiguas y de mayor tradición (Paulo-Maya et al., 2000; Soria -Barreto y Paulo-Maya 2005).

Las especies de *Chirostoma* adaptadas exclusivamente a hábitats lacustres incluye dos grupos monofiléticos (Bloom et al., 2009, 2013), en un clado el charal prieto *Chirostoma attenuatum* Meek 1902, y sus dos subespecies reconocidas: *Chirostoma attenuatum attenuatum* Meek 1902 y *Chirostoma attenuatum zirahuén*

Meek 1902, endémicas a los lagos de Pátzcuaro y Zirahuén respectivamente. El segundo clado denominado “grupo *Chirostoma humboldtianum*”, originalmente fue propuesto por Barbour, (1973b), con evidencia en morfología, osteología (Barbour y Chernoff, 1984), enzimática (Echelle y Echelle, 1984), morfométrica y genética (Barriga-Sosa et al., 2002; 2004; 2005; Bloom et al., 2009; 2012; 2013). El “grupo *Chirostoma humboldtianum*” comprende nueve especies distribuidas en los lagos del centro de México, las cuales han sido referidas como “especies flock”, proponiendo a eventos de especiación simpátrica como el motor de la radiación adaptativa ocurrida en este grupo (Barbour, 1973b; Barbour y Chernoff, 1984; Echelle y Echelle). Sin embargo, a pesar de los numerosos estudios realizados dentro del grupo, sus relaciones filogenéticas e historia evolutiva no han sido resueltas (Bloom et al., 2009, 2012, 2013; Campanella et al., 2015).

Esta tesis contiene dos capítulos, cada uno representa un manuscrito que será sometido a una revista científica internacional para su publicación. Esta investigación utiliza aspectos combinados de filogenia y filogeografía con el objetivo de determinar los procesos evolutivos ocurridos en las especies de *Chirostoma* adaptados exclusivamente a los lagos del centro de México. El trabajo del capítulo uno ha sido publicado en la revista Journal of Zoological Systematic and Evolutionary Research. Explica la historia evolutiva en el charal prieto *Chirostoma attenuatum,* donde ocurre un proceso de especiación temprano mediado por aislamiento alopátrico, esto como resultado de eventos vicariantes asociados a eventos geológicos. Posteriormente, las poblaciones aisladas acumularon adaptaciones únicas que durante un contacto secundario ambas entidades mantuvieron su propia trayectoria evolutiva. El capítulo 2 pretende contrastar la hipótesis morfológica dentro del grupo de especies *“Chirostoma humboltianum*”, la cual asume la existencia de nueve especies, consideradas como especies flock. Además, con datos genéticos se pretende dilucidar si los altos polimorfismos morfológicos en las especies son una respuesta a factores ambientales, y si la variación morfológica es el resultado de la plasticidad fenotípica. Esta investigación también encuentra que el grupo de especies *Chirostoma humboldtianum* es un buen modelo para el estudio de los mecanismos que rigen la diferenciación genética a nivel poblacional intra-lacustre, para dilucidar los procesos evolutivos en las especies adaptadas exclusivamente a hábitats lacustres con divergencias recientes.

1.1. El centro de México

El centro de México (*sensu* Domínguez-Domínguez y Pérez Ponce de León, 2009) es una región que se localiza en el extremo sur del Altiplano mexicano y se caracteriza por tener una historia geológica compleja (Miller y Smith, 1986). Debido a esos procesos ha sufrido una serie de fragmentaciones hidrográficas, permitiendo el surgimiento de grandes lagos y ríos de bajos gradientes, siendo el sistema Lerma-Santiago la mayor cuenca hidrológica (Miller et al., 2005). El río Lerma drena desde el lago de Chapala, mientras que su salida al Océano Pacífico da origen al Río Grande de Santiago, el cual nace al Este del lago. El centro de México también alberga un gran número de cuencas interiores, en estas se incluyen al valle de México, los llanos de Puebla que se orientan al Este, mientras que al Oeste están representados los lagos de Santa María y Juanacatlán, el valle de Tocumbo cercano de la cuenca de Chapala, además de los lagos de Cuitzeo, Pátzcuaro y Zirahuén que son una serie de lagos ubicados en la porción occidental de Michoacán (De Buen, 1943; Álvarez-Del Villar, 1972; Echelle y Echelle, 1984). Debido a la actual distribución e historia evolutiva de diversos grupos de peces en el centro de México, se han evidenciado conexiones antiguas de estos sistemas endorreicos con el sistema Lerma-Santiago, probablemente en el Pleistoceno tardío, además estas conexiones y desconexiones ocurrieron de manera asincrónica en diferentes tiempos y entre las diferentes cuencas que componen el centro de México, generando una compleja historia evolutiva para el componente íctico de la región (Meek, 1904; Barbour 1973a; Schönhuth y Doadrio, 2003; Domínguez et al., 2006, 2008; Pérez-Rodríguez et al., 2009; Corona-Santiago et al., 2015; Beltrán-Lopéz et al., en prensa). Esta región es de gran importancia biogeográfica, ya que algunos autores mencionan que el centro de México representa una zona limítrofe entre las regiones biogeográficas Neártica y Neotropical, las cuales convergen en esta región (Marshall y Liebherr, 2000; Domínguez-Domínguez y Pérez-Ponce de León, 2009). El centro de México ha sido campo de estudio de los componentes bióticos dulceacuícolas y su relación con las cuencas hidrológicas. Diversas contribuciones han referenciado al centro de México, principalmente, en estudios de biogeografía de peces dulceacuícolas (Barbour, 1973b; Barbour y Miller, 1978; Echelle y Echelle, 1984; Miller y Smith, 1986, Barbour y Miller, 1994; Domínguez-Domínguez et al., 2006, 2008, 2010, Pérez-Rodríguez et al., 2009, Bloom et al., 2009).

1.2 Diversidad de peces del centro de México

México es considerado de los países con mayor biodiversidad del mundo. Con relación al medio acuático, el territorio mexicano cuenta con una gran variedad de ecosistemas marinos, estuarinos y dulceacuícolas, destacando estos últimos por la presencia de 70 cuencas fluviales y aproximadamente 70 lagos de gran tamaño (Miller et al. 2005). El centro de México es considerado por el World Conservation Monitoring Center como una de las regiones de mayor importancia para la conservación de los peces de agua dulce en el mundo. Tan sólo para el grupo de los peces, en México se tienen registradas más de 500 especies, representando el 65% de todas las especies descritas en los Estados Unidos y Canadá juntos, teniendo solo la quinta parte de la extensión territorial de estos países (Miller et al., 2005). La composición ictiofaunistica de México está marcada por su alto nivel de endemismo, distribuida en las diferentes regiones. Entre estas regiones destaca el centro de México, con *ca.* 78 especies de peces representado nueve familias (Atherinopsidae, Catostomidae, Characidae, Cichlidae, Cyprinidae, Goodeidae, Ictaluridae, Petromyzontidae, y Poeciliidae) (Miller et al., 2005), de las cuales *ca.* 70% son endémicas (Domínguez-Domínguez et al., 2007).

1.3 Origen y evolución de los Aterinópsidos del centro de México

Parte de la ictiofauna dulceacuícola mexicana tiene un origen marino, atribuido a la invasión de diversos grupos provenientes del océano, que por la inestabilidad geológica del centro de México quedaron aislados, dando origen a un gran número de elementos endémicos (Miller, 1966) como es el caso de la familia Atherinopsidae. La hipótesis más aceptada (Barbour, 1973a; Bloom et al., 2009, 2012, 2013; Echelle y Echelle, 1984; Miller et al., 2005) acerca del origen de los aterinópsidos en el centro de México es la propuesta por Miller, (1966), en base a evidencias geológicas, biogeográficas y paleontológicas, argumenta que entre el plioceno y pleistoceno una especie semejante a *Menidia* penetró al centro de México proveniente del Atlántico por una conexión que existió entre el río bravo y el centro de México, posteriormente, debido a la inestabilidad tanto geológica como climática, las poblaciones quedaron aisladas en cuerpos de agua del centro de México y posteriormente diversificó en los géneros *Poblana* Bonaparte y *Chirostoma* Swaison.

El género *Chirostoma* es el más diverso y con una amplia distribución, ha sido ampliamente estudiado, siendo el trabajo de Barbour, (1973b) uno de los trabajos pioneros acerca de la sistemática del grupo, quien definió que el género está conformado por 18 especies y seis subespecies, además en su trabajo propuso dos morfo-tipos, a los cuales llamó “grupo *Arge*” y “grupo *jordani*”, caracterizados por poseer diferencias en tamaño y en caracteres merísticos, sobre todo en las escamas predorsales y de la línea lateral, y también en relación a su ancestro, por lo que propuso que el género tiene dos centros de origen (Barbour, 1973b).

Por otro lado Echelle y Echelle, (1984), en base a un análisis fenético y cladístico de caracteres electroforéticos, proponen que el género *Poblana* y *Chirostoma* compartían ancestro con *Menidia*, por lo tanto las especies de *Chirostoma* y *Poblana* deberían ser renombrados dentro del género *Menidia.* El trabajo de Chernoff, (1996), no apoya la supuesta relación del grupo “Arge” con *Melaniris crystallina,* debido a que coloca a *Melaniris* en sinonimia con *Atherinella*. Estudios moleculares recientes con genes mitocondriales y nucleares, no soporta la hipótesis de la separación del género en los grupos “*Arge* y *Jordani*” propuestos inicialmente por Barbour, (1973b), y en base a la evidencia molecular multi-locus se ha propuesto incluir a los géneros *Chirostoma* y *Poblana* dentro del género *Menidia* (Bloom et al., 2009, 2012 y 2013; Campanella et al., 2015).

1.4 Secuencias de ADN en estudios de Filogenia y Filogeografía

Las tecnologías de secuenciación del ADN, la cual inició en la década de los 70´S (Sanger et al., 1977), fue una ventana importante para el desarrollo de marcadores moleculares basados en secuencias de genes, revolucionando diversas disciplinas en el campo de la biología. Las secuencias de ADN permitieron la obtención de caracteres homólogos que podían ser comparados y con alto poder de resolución, con lo cual se pudieron poner a prueba diversas hipótesis filogenéticas (Blair y Murphy, 2011; Yang y Rannala, 2012).

El ADN mitocondrial (ADNmt) ha sido ampliamente utilizado en estudios filogenéticos en animales y particularmente en peces para reconstruir las relaciones filogenéticas a distintos niveles taxonómicos (Simon et al., 1994; Avise, 2000). Debido al amplio conocimiento del ADNmt en peces, se han desarrollado cebadores universales para amplificar las regiones codificantes, los genes ribosomales y la región control (Kocher et al., 1989; Meyer et al., 1990; Palumbi, 1996; Simon et al., 1994).

Las secuencias de ADN nuclear (ADNn) también han sido utilizadas para contrastarlas con hipótesis basadas en el ADNmt (Stepien y Kocher, 1997). Las regiones del ADNn como las secuencias que codifican proteínas, las regiones intergénicas e intrones, han sido utilizadas en estudios de sistemática y filogenia (Stepien y Kocher, 1997), debido a que la tasa de sustitución en el ADNn es más lenta respecto al ADNmt. Las discrepancias observadas entre ambos tipos de marcadores, se atribuye a que el ADNmt es haploide y tiene una herencia matrilineal en la mayoría de los animales (Hoech et al., 1991). Por lo tanto en el ADNmt el tamaño efectivo de la población es menor (Hudson y Turnelli, 2003), y la deriva genética actúa más fuerte y los polimorfismos se purga o fijan más rápido porque las tasas de evolución son mayores. (Funk y Omland, 2003), resultando en divergencias más recientes observadas en el ADNmt (Zink y Barrowclough, 2008). Por lo tanto, estas discrepancias entre el ADNmt y ADNn deben ser tomadas en cuenta en la interpretación y discusión de resultados.

La Filogeografía originalmente se definió como la rama de la biogeografía que estudia los procesos que gobiernan la distribución espacial de los linajes genealógicos dentro de las especies o especies cercanamente relacionadas (Avise, 2000), y se considerada como un puente entre los procesos microevolutivos que ocurren dentro de las poblaciones y los patrones macroevolutivos que ocurren en las especies. La filogeografía pretende describir los patrones espaciales de diversidad genética e inferir los mecanismos subyacentes de esa variación (Buckley, 2009). Además, el estudio de la variación espacial de los linajes genéticos ha permitido entender la variación fenotípica entre las poblaciones o entre especies cercanamente relacionadas (Edwards et al., 2016); pudiendo identificar regímenes selectivos de la variación entre y dentro de las especies, además de determinar zonas hibridas en poblaciones que tuvieron un contacto secundario posterior al aislamiento por alopatría. Es necesario que los estudios filogeográficos aborden la variación fenotípica y ambiental dentro de las especies, contextualice la historia demográfica y los rasgos funcionales, para proponer escenarios de evolución de la diversidad orgánica.

1.5 Delimitación de especies

Ante la necesidad de identificar la diversidad orgánica se han propuesto diversas metodologías para la delimitación de especies. El uso exclusivo de caracteres morfológicos para delimitar especies puede conllevar a subestimar o sobreestimar la diversidad biológica, ya que los caracteres morfológicos pueden estar sujetos a evolución convergente o siendo afectados por distintas presiones selectivas (Yang y Rannala, 2010). Sin embargo, la inclusión de datos genéticos puede proveer información adicional para la identificación de las especies, como son las identidades poblacionales (Pritchard et al., 2000), niveles de flujo genético reciente o antiguo (Edwards y Beerli, 2000; Rannala y Mountain, 1997; Wilson y Rennala, 2003), hibridación (Anderson y Thompson, 2002), y las relaciones filogenéticas entre las especies (Liu et al., 2002). Las secuencias de ADN pueden ser útiles para probar hipótesis de delimitación de especies usando metodologías recientemente desarrolladas a partir de modelos teóricos, las cuales combinan filogenias de especies y genealogía de genes a través de procesos coalescentes. Sin embargo, para las especies cercanamente relacionadas y con divergencia reciente, la delimitación de especies puede presentar una limitante al no estar resueltos los árboles de genes, este conflicto puede representar errores en la inferencia filogenética debido a la introgresión o sorteo incompleto de linajes (Yang, 2002). Sin embargo, los modelos asumen que cada árbol de genes representa una relación basada en genes ortólogos, suponiendo que no hay transferencia horizontal de genes o mezcla genética entre las diferentes especies, por lo tanto asumen que la principal discrepancia entre árboles de genes y árbol de especies es el sorteo incompleto de linajes. Los conflictos de los árboles de genes no resueltos pueden superarse fácilmente en un marco bayesiano, al integrar la incertidumbre en los árboles genéticos e incorporar un modelo explícito de clasificación de linajes a través de un modelo coalescente (Yang y Rannala, 2010).

Capítulo I

Manuscrito publicado en la Journal of Zoological Systematic and Evolutionary Research

https://doi.org/10.1111/jzs.12216

2. Especiación del charal *Chirostoma attenuatum* (Pisces: Atheriniformes) en el centro de México.

Accepted: 21 January 2018
DOI: 10.1111/jzs.12216

ORIGINAL ARTICLE

WILEY JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Speciation of silverside *Chirostoma attenuatum* (Pisces: Atheriniformes) in Central Mexico

Isaí Betancourt-Resendes[1] | **Rodolfo Pérez-Rodríguez**[2] | **Omar Domínguez-Domínguez**[2]

[1]Programa Institucional de Doctorado en Ciencias Biológicas, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia Michoacán, México

[2]Laboratorio de Biología Acuática, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia Michoacán, México

Correspondence
Rodolfo Pérez-Rodríguez
Email: rperezr38@gmail.com

Funding information
Ministerio de Economía y Competitividad, Grant/Award Number: CGL-2013 41375-P; Coordinación de la Investigación Científica-UMSNH

Contributing authors: Isaí Betancourt-Resendes (bq_isai7@yahoo.com.mx); Omar Domínguez-Domínguez (goodeido@yahoo.com.mx)

Abstract

The high speciation rate of *Chirostoma* in Central Mexico has been associated with allopatric speciation events promoted by the emergence of vicariant barriers in freshwater habitats, as well as by sympatric ecological segregation, common in those species inhabiting lacustrine ecosystems. Through nuclear and mitochondrial markers, this study revealed a speciation process within *Chirostoma attenuatum* resulting in two evolutionary independent units that coincide with their morphological differentiation, indicating that *Chirostoma attenuatum* and *Chirostoma zirahuen* may be considered separate species. This process was the result of vicariance associated with geological dynamics of the region. Phylogeographic findings indicated two speciation stages: early allopatric isolation, during which the isolated populations accumulated unique adaptations, and secondary contact with low migration rate and the maintenance of the evolutionary trajectory. Historical demographic analysis indicated that the two well-differentiated lineages underwent independent evolutionary histories in their respective lakes. *Chirostoma zirahuen* from Zirahuen and *C. attenuatum* from Patzcuaro represent unique and irreplaceable genetic diversity that must to be conserved.

KEYWORDS
allopatry, Atherinopsidae, ecological speciation, evolutionary units, reproductive isolation

1 | INTRODUCTION

Atherinopsidae include marine and freshwater lineages. Studies have revealed that the transition from marine to freshwater habitats resulted in their accelerated speciation and extinction rates, yielding a remarkable disparity in species richness between continental and marine lineages (Bloom, Weir, Piller, & Lovejoy, 2013). In Atherinopsidae that inhabit continental habitats, high species richness has been attributed to the frequency of allopatric speciation events resulting from emergence of vicariant barriers, limiting gene flow and promoting genetic divergence (Barbour, 1973; Bloom et al., 2013; Echelle & Echelle, 1984; García-Martínez, Mejia, García -De León, & Barriga-Sosa, 2015; Miller, Minckley & Norris, 2005). For fish species distributed throughout Central Mexico, allopatric events related to geological activity in the Trans-Mexican Volcanic Belt (TMVB), together with the climate history, are regarded as the main source of diversification (Beltrán-López et al., 2017; Domínguez-Domínguez, Doadrio, & Pérez-Ponce de León, 2006; Pérez-Rodríguez, Domínguez-Domínguez, Doadrio, Cuevas-Gacía, & Pérez-Ponce de León, 2015). Diversification of the genus *Chirostoma* Swainson 1839 from Central Mexico has been associated with such allopatric speciation events as well as with sympatric ecological segregation, particularly in the species inhabiting lacustrine ecosystems (Barbour, 1973; Barbour & Chernoff, 1984; Echelle & Echelle, 1984).

The continuous activity of the TMVB from the Miocene to the present has been responsible for changes in the physiographic configuration of the region, including the formation and destruction of lake basins in the most important lacustrine region in Central Mexico (Ferrari, Orozco-Esquivel, Maena, & Maena, 2011; Gómez-Tuena, Orozco-Esquivel, & Ferrari, 2005), including the basins of Patzcuaro and Zirahuen lakes. Earlier hypotheses, based on faunistic studies, of connectivity between these lakes proposed the existence of a stream flowing from Zirahuen to Patzcuaro Lake that disappeared approximately 700,000 to 1 million years ago (Álvarez Del Villar, 1972; De Buen, 1943). Geological findings suggest a more recent stream connection between Zirahuen and Patzcuaro lakes *ca.* 30,000 years ago (Israde-Alcántara, Garduño-Monroy, Fisher, Pollard, & Rodríguez-Pascua, 2005). Evidence of a secondary paleoconnection, about 8,000 years ago (Garduño-Monroy et al., 2009; Israde-Alcántara et al., 2005), is supported by phylogeographic findings of the closely related goodeine species *Allotoca meeki* Álvarez, 1959 from Zirahuen and *Allotoca diazi* Meek, 1902 from Patzcuaro that suggest a recent isolation process *ca.* 7,000 years ago (Corona-Santiago, Doadrio, & Domínguez-Domínguez, 2015).

An important aspect in the process of speciation is the potential of migrants to adapt to new habitats and to move through new dispersion routes. Successful colonization of freshwater habitats is often associated with the emergence of key evolutionary innovations, providing the colonizing lineage with the ability to adapt to the new habitat (Seehausen & Wagner, 2014). Among the fish species inhabiting both Patzcuaro and Zirahuen lakes, only the silversides of the genus *Chirostoma* are restricted to lacustrine habitats (Barbour, 1973; Bloom et al., 2013), indicating a lake-adapted natural history of the species. Sympatric ecological radiation has been proposed as the major factor in the radiation of the silversides inhabiting large lacustrine ecosystems in Central Mexico, such as Chapala and Patzcuaro lakes (Barbour & Chernoff, 1984; Echelle & Echelle, 1974).

In the case of slender silverside *Chirostoma attenuatum*, two subspecies have been recognized, *Chirostoma attenuatum attenuatum* Meek 1902 and *Chirostoma attenuatum zirahuen* Meek 1902, endemics from Patzcuaro Lake and Zirahuen Lake, respectively. These subspecies are supported based on biochemical markers, as well as considerable differences in morphological and meristic characteristics (Barbour, 1973; Barriga-Sosa, 2001). Based on it, we present a phylogeographic study of populations of *C. attenuatum* using mitochondrial and nuclear markers to (i) test the independence of lineage evolution of the population and determine whether *C. a. attenuatum* from Patzcuaro and *C. a. zirahuen* from Zirahuen could be identified as independent evolutionary significant units and valid species; (ii) reveal the biogeographic processes implicated in the evolutionary history of *C. attenuatum*; and (iii) elucidate the demographic history and its link with climate events.

2 | MATERIALS AND METHODS

2.1 | Fish sampling

Twenty-five specimens of *C. attenuatum attenuatum* from Patzcuaro and 26 *C. attenuatum zirahuen* from Zirahuen were obtained with the help of local fishermen (Table 1). A fragment of pectoral fin was removed from each specimen and stored in a microtube in 100% ethanol. The fish were fixed in 5% formalin, preserved in 70% ethanol, identified following Miller et al. (2005) and Barbour (1973), and deposited in the ichthyological collection of the Universidad Michoacana de San Nicolas de Hidalgo (CPUM) (Table 1).

2.2 | DNA sequences

DNA extraction was carried out using the proteinase K and phenol–chloroform protocol (Sambrook, Fritsch, & Maniatis, 1989). The mitochondrial *cytochrome b* gene (*cytb*) and the *first intron of S7 ribosomal protein* gene (*S7*) were amplified. The PCR consisted of a 25 μl volume reaction with a final concentration of 50–100 ng DNA, 1× buffer, 1.5 mM $MgCl_2$, 2.5 mM dNTP mix (mM 10), 0.25 μM of each primer, and 1 unit of Taq DNA polymerase (Invitrogen).

For the *cytb* gene (length 1140 bp), the primers were Glud-G (5′-TGACTTGAARAACCAYCGTTG-3′; Palumbi et al., 1991) and H16460 (5′-CGAYCTTCGGATTAACAAGACCG-3′; Perdices, Bermingham, Montilla, & Doadrio, 2002). Amplification was performed with the following conditions: denaturation at 94°C for 45 s, annealing at 48°C for 1 min, extension at 72°C for 90 s, and a final extension at 72°C for 5 min. For the *S7*, the primers were S7RPEX1F (5′-TGGCCTCTTCCTTGGCCGTC-3′) and S7RPEX2R (5′-AACTCGTCTGGCTTTTCGCC-3′) described for Chow and Hazama (1998). The amplification was performed using denaturation at 94°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 2 min, and a final extension at 72°C for 7 min. PCR products were submitted to the sequencing service at the High-throughput Genomics Center, Washington University, Seattle, WA, USA.

2.3 | Sequence analyses

Sequences obtained in both directions were edited using MEGA v5 (Tamura et al., 2011), and the alignment of *cytb* was conducted manually; additionally, two sequences of *C. attenuatum* from Patzcuaro Lake and one sequence of Zirahuen Lake were included (for GenBank accession numbers, see Table 1). For the *S7* sequences, the phase of heterozygous single nucleotide polymorphisms (SNPs) was resolved using DnaSP v. 5.10 (Librado & Rozas, 2009) and performed with the algorithm provided by PHASE 2.0 (Stephens, Smith, & Donnelly, 2001) with default parameters. Posterior sequence alignment was carried out using Clustal_X 1.83 (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) and revised manually. To test the monophyly of *C. attenuatum* samples, the following closely related species (sensu Bloom, Piller, Lyons, Mercado-Silva, & Medina-Nava, 2009) were included as ingroup (Table 1): *Chirostoma estor* Jordan 1897, *Chirostoma grandocule*, Steindeachner 1894, and *Chirostoma patzcuaro* Meek 1902 from Patzcuaro Lake; *Chirostoma humboldtianum* Valenciennes 1835 from Zacapu Lake; and *Chirostoma sphyraena* Boulenger 1900 from Chapala Lake. *Chirostoma jordani* Woolman 1984 from Chapala were used as outgroup in phylogenetic assays (Bloom et al., 2009, 2013). Alignment for each locus was used to estimate and select the substitution model that best fit the datasets using jModelTest v. 1.7 (Posada, 2008) considering the Akaike information criterion (AIC).

TABLE 1 Voucher, localities, and GenBank accession numbers for analyzed specimens of the genus *Chirostoma*

Species	Tissue collection	Locality	Basin	GB *cytb*	GB *S7*	Fish collection
C. attenuatum	10623	Uranden	Patzcuaro	MG592205	MG98478	No voucher
C. attenuatum	9720	Ukasanatacua	Patzcuaro	MG592206	MG98476	No voucher
C. attenuatum	34245	Uranden	Patzcuaro	MG592207	MG98480	No voucher
C. attenuatum	34244	Uranden	Patzcuaro	MG592208	MG98479	No voucher
C. attenuatum	10618	Uranden	Patzcuaro	MG592209	–	No voucher
C. attenuatum	10621	Uranden	Patzcuaro	MG592210	MG98477	No voucher
C. attenuatum	35209	Uranden	Patzcuaro	–	MG98481	14104
C. attenuatum	35212	La Pacanda	Patzcuaro	MG592211	MG98482	14104
C. attenuatum	35214	La Pacanda	Patzcuaro	MG592212	MG98483	14104
C. attenuatum	35217	La Pacanda	Patzcuaro	MG592213	–	14104
C. attenuatum	35260	La Pacanda	Patzcuaro	MG592214	–	14104
C. attenuatum	35254	La Pacanda	Patzcuaro	–	MG98484	14104
C. attenuatum	35257	La Pacanda	Patzcuaro	MG592215	MG98485	14104
C. attenuatum	35269	La Pacanda	Patzcuaro	MG592216	MG98486	14104
C. attenuatum	35270	La Pacanda	Patzcuaro	MG592217	–	14104
C. attenuatum	45322	La Pacanda	Patzcuaro	MG592218	–	14104
C. attenuatum	45323	La Pacanda	Patzcuaro	MG592219	–	14104
C. attenuatum	45324	La Pacanda	Patzcuaro	MG592220	–	14104
C. attenuatum	45325	La Pacanda	Patzcuaro	MG592221	–	14104
C. attenuatum	45326	La Pacanda	Patzcuaro	MG592222	–	14104
C. attenuatum	45328	La Pacanda	Patzcuaro	MG592223	–	14104
C. attenuatum	45329	La Pacanda	Patzcuaro	MG592224	–	14104
C. attenuatum	45330	La Pacanda	Patzcuaro	MG592225	–	14104
C. attenuatum	45333	La Pacanda	Patzcuaro	MG592226	–	14104
C. attenuatum	45334	La Pacanda	Patzcuaro	MG592227	–	14104
C. attenuatum	35519	Zirahuen	Zirahuen	MG592228	MG598465	14103
C. attenuatum	35512	Zirahuen	Zirahuen	MG592229	–	14103
C. attenuatum	35514	Zirahuen	Zirahuen	MG592230	MG598463	14103
C. attenuatum	35515	Zirahuen	Zirahuen	MG592231	–	14103
C. attenuatum	35516	Zirahuen	Zirahuen	MG592232	–	14103
C. attenuatum	35517	Zirahuen	Zirahuen	MG592233	MG598464	14103
C. attenuatum	35518	Zirahuen	Zirahuen	MG592234	MG598462	14103
C. attenuatum	35520	Zirahuen	Zirahuen	–	MG598466	14103
C. attenuatum	35523	Zirahuen	Zirahuen	MG592235	–	14103
C. attenuatum	35524	Zirahuen	Zirahuen	MG592236	MG598467	14103
C. attenuatum	35525	Zirahuen	Zirahuen	MG592237	MG598468	14103
C. attenuatum	35527	Zirahuen	Zirahuen	MG592238	–	14103
C. attenuatum	35529	Zirahuen	Zirahuen	MG592239	MG598469	14103
C. attenuatum	35531	Zirahuen	Zirahuen	MG592240	–	14103
C. attenuatum	35532	Zirahuen	Zirahuen	MG592241	MG598470	14103
C. attenuatum	35533	Zirahuen	Zirahuen	MG592242	–	14103
C. attenuatum	35534	Zirahuen	Zirahuen	MG592243	–	14103
C. attenuatum	35535	Zirahuen	Zirahuen	MG592244	MG598471	14103
C. attenuatum	35536	Zirahuen	Zirahuen	MG592245	–	14103
C. attenuatum	35537	Zirahuen	Zirahuen	MG592246	–	14103
C. attenuatum	35538	Zirahuen	Zirahuen	MG592247	–	14103

(Continues)

TABLE 1 (Continued)

Species	Tissue collection	Locality	Basin	GB *cytb*	GB *S7*	Fish collection
C. attenuatum	35539	Zirahuen	Zirahuen	MG592248	MG598472	14103
C. attenuatum	35540	Zirahuen	Zirahuen	MG592249	MG598473	14103
C. attenuatum	35541	Zirahuen	Zirahuen	MG592250	–	14103
C. attenuatum	35543	Zirahuen	Zirahuen	MG592251	MG598474	14103
C. attenuatum	35546	Zirahuen	Zirahuen	–	MG598475	14103
C. attenuatum	nd	Patzcuaro	Patzcuaro	KM400699	–	SLU nd
C. attenuatum	nd	Patzcuaro	Patzcuaro	KC736405	–	SLU 5036[a]
C. attenuatum	nd	Zirahuen	Zirahuen	KC736404	–	SLU 5036[a]
C. estor	3320	Patzcuaro	Patzcuaro	–	MG747647	No voucher
C. estor	nd	Patzcuaro	Patzcuaro	KC736403	–	SLU 5114[a]
C. grandocule	nd	Patzcuaro	Patzcuaro	KC736369	–	SLU 5118[a]
C. patzcuaro	nd	Patzcuaro	Patzcuaro	JQ282029	–	SLU 5117[a]
C. humboldtianum	nd	Zacapu	Zacapu	JQ282026	–	SLU 5117[a]
C. sphyraena	nd	Chapala	Chapala	KC736400	–	SLU 5025[a]
C. jordani	5090	Cajititlán	Chapala	–	MG747648	No voucher
C. jordani	nd	Chapala	Chapala	KC736407	–	SLU 5033[a]

nd, no data (sequence obtained of GenBank database); GB, GenBank accession number; –, no sequence; SLU, code voucher of each sequence retrieved from GenBank (see Bloom et al., 2013).

Collection numbers of tissues and specimens deposited in Tissue bank and/or Fish Collection of the Universidad Michoacana de San Nicolas de Hidalgo (CPUM) are indicated in columns 2 and 7, respectively.

[a] Data were obtained of GenBank database.

2.4 | Phylogenetic, species trees and haplotype network

Phylogenetic trees were constructed for the *cytb* and *S7* genes based on maximum likelihood (ML) and Bayesian inference (BI). The ML was run in RAxML (Silvestro & Michalak, 2012; Stamatakis, Hoover, & Rougemont, 2008). Bayesian analyses were run in MrBayes 3.2.2 (Ronquist et al., 2012). Two independent runs were implemented with four-chain MCMC and 10,000,000 generations, sampling every 100 generations. Chain convergence was verified by suitable effective sample size for all parameters in Tracer 1.5 (Rambaut & Drummond, 2007), discarding 10% of generations as burn-in.

To deal with incomplete lineage sorting, the common source of discrepancy between mtDNA and nDNA (Tows & Brelsford, 2012), especially in recently radiated lineages (Niemiller, Near, & Fitzpatrick, 2012), a species tree under a multispecies coalescent model was created using *BEAST v. 2.0 (Bouckeart *et al.*, 2014; Heled & Drummond, 2010) through the CIPRES Science Gateway v. 3.3 (Miller et al., 2015). Patzcuaro and Zirahuen populations were designated as distinct groups. The best fit models of nucleotide evolution for *cytb* (TN93+G) and the *S7* intron (GTR+G) were used with four gamma categories. Due to the lack of fossil data for *Chirostoma*, a lognormal relaxed clock model was chosen for *cytb*, using the mutation rate of 1% estimated for Atheriniformes (Campanella et al., 2015). For the *S7*, the mutation rate was estimated relative to the mitochondrial mutation rate, choosing a lognormal relaxed clock using a normal prior (0.005 ± 0.01). For species tree and population size models, a Yule model species tree prior and a constant species tree population size were chosen. Two independent MCMC runs were performed for 30,000,000 generations sampling every 1,000 generations. Chain convergence was assessed by visualizing the sampled parameter values in Tracer. Runs were pooled using LogCombiner in the BEAST 2 package, with 10% of the generations discarded as burn-in. The maximum clade credibility species tree was created by TreeAnnotator in the BEAST 2 package.

We constructed a haplotype network to describe the intraspecific relationships of *C. attenuatum* in HaploView v. 4.2 (Barrett, Fry, Maller, & Daly, 2005).

2.5 | Divergence times

To estimate the time of the cladogenetic event separating the Patzcuaro and Zirahuen populations, divergence times of *C. a. attenuatum* and *C. a. zirahuen* based on the *cytb* and *S7* were obtained. The mutation rate of 1% estimated for Atheriniformes (Campanella et al., 2015) was used for *cytb*; for *S7*, the mutation rate was estimated relative to the mitochondrial mutation rate, using the normal prior (0.05 ± 0.01). The best fit models of nucleotide evolution for *cytb* (TN93+G) and the *S7* (GTR+G) were used with four gamma categories. We used a prior coalescent model of Bayesian skyline plot. The assay was performed with BEAST 2 implemented on the webserver CIPRES Science Gateway v. 3.3 (Miller et al., 2015). Two independent runs of 50,000,000 generations, sampling every 500 generations, were implemented with 10% discarded as burn-in.

2.6 | Genetic diversity and structure

A conventional diversity index was obtained for both genes. Haplotype diversity (h), nucleotide diversity (π), and segregating sites (S) were calculated using DnaSP v5. Differences between *C. a. attenuatum* and *C. a. zirahuen* were calculated by the uncorrected genetic distances (D_p) with 1,000 bootstrap replicates, using MEGA v5. To analyze the structure of populations, an analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992) was performed, with significance levels set at $\alpha = .05$ and 10,000 random permutations, implemented in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010). The degree of genetic variation between *C. a. attenuatum* and *C. a. zirahuen* was calculated using the fixation index Φ_{st}. The assay was run in ARLEQUIN with a significance level of $\alpha = .05$ and 10,000 random permutations.

2.7 | Historical demography and gene flow

To detect signatures of demographic changes in the two populations, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997; Fu & Li, 1993) were implemented in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010) with significance levels set at $\alpha = .05$ and 10,000 random permutations. To infer population size through time, we created Bayesian skyline plots (Drummond, Rambaut, Shapiro, & Pybus, 2005) in BEAST 2 for the subspecies based on the *cytb* gene. This coalescent-based method provided a robust framework to infer population dynamics through time and to deduce historical change in populations from DNA sequences. The molecular clock was calibrated with a mutation rate of 1% estimated for atherinopsids (Campanella et al., 2015). The assay was conducted in BEAST, implemented on the webserver CIPRES Science Gateway v. 3.3 (Miller et al., 2015) for 30,000,000 generations, sampling every 1,000 generations. The Bayesian reconstruction of each population was performed in Tracer v. 4.

To estimate the level and direction of historical gene flow between populations from Patzcuaro and Zirahuen lakes, migration rates were computed using MIGRATE-N 3.6 (Berreli, 2009). MCMC simulations were performed as follows: one long chain and 12 short parallel chains with initial temperatures of 1.0, 1.5, 3.0, and 100,000.0 and a static heating scheme. Final MCMC searches used 100,000 steps in 50-step increments, discarding the first 20,000 as burn-in. Initial uniform priors were Θ (0.0–0.1) and M (0.0–25,000.0).

3 | RESULTS

3.1 | Sequence data

Twenty-five sequences of the mitochondrial *cytb* gene (length of alignment 1,121 bp; Dataset S1) of *C. a. attenuatum* and 25 of *C. a. zirahuen*, along with 11 sequences of *S7* (length of alignment of 671 bp; Dataset S2) of *C. a. attenuatum* and 14 of *C. a. zirahuen*, were included in the analyses (Table 1). For *S7*, all heterozygous sites were successfully resolved for SNP variation (phase threshold value >85%), resulting in 50 genotypes, 22 for *C. a. attenuatum* and 28 for *C. a. zirahuen*. Obtained sequences were deposited in GenBank (Table 1). The best fit evolutionary models for the *cytb* and *S7* datasets were TrN+G and GTR+I+G, respectively.

3.2 | Phylogenetic relationships and species trees

The phylogenetic trees based on the *cytb* gene and *S7* recovered *C. attenuatum* as a monophyletic assemblage with both BI and ML. For the tree based on the *cytb*, BI and ML produced two highly supported well-differentiated clades, one corresponding to specimens collected in Patzcuaro Lake and the other comprising the specimens from Zirahuen Lake (Figure S1). In contrast, the tree based on the *S7* intron, although supporting the monophyly of *C. attenuatum*, showed unresolved polytomy in within-species relationships with both BI and ML (Figure S2). The species tree based on the two loci showed two well-supported lineages (Figure 1) as the a priori assignments of specimens. The time of separation of the lineages was estimated at 600,000 years ago (HPD 95% 319,000–939,000; Figure 1).

3.3 | Haplotype network

The *cytb* haplotype network showed well-differentiated haplogroups corresponding to *C. a. attenuatum* and *C. a. zirahuen*, separated by 10 mutation steps. The Patzcuaro population showed a network with a central haplotype (frequency = 7) and several peripheral unique haplotypes. The Zirahuen population showed one haplotype with high frequency (*n* = 22) and three unique peripheral haplotypes (Figure 2A). The *S7* haplotype network displayed separation of the *C. a. attenuatum* haplotypes from *C. a. zirahuen*, with only one of the central haplotype shared between subspecies. Fifteen unique haplotypes were found for the Patzcuaro population and seven for the Zirahuen population.

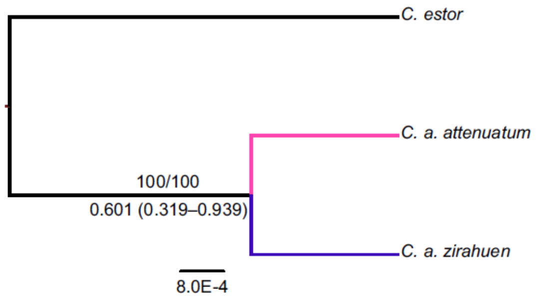


FIGURE 1 Species tree and divergence times in *Chirostoma attenuatum*; 9 individuals of *Chirostoma a. attenuatum* and 11 individuals of *Chirostoma a. zirahuen* were included. The analysis was performed with both molecular markers. Numbers on branches indicate median divergence time, and numbers in parentheses show confidence limits (HPD >95%) in millions of years ago. The number above the branch represents the posterior probability in the species tree and divergence time tree

3.4 | Genetic diversity

For the mitochondrial *cytb* gene, the complete *C. attenuatum* dataset revealed high haplotype diversity (0.785) and low nucleotide diversity (0.00619), with 35 segregating sites. *Chirostoma a. attenuatum* showed high haplotype diversity (0.8933) and low nucleotide diversity (0.00210) with 21 segregating sites, whereas *C. a. zirahuen* exhibited low haplotype diversity (0.18), low nucleotide diversity (0.00025), and three segregating sites (Table 2).

For *S7*, a similar diversity pattern was obtained. The complete dataset of *C. attenuatum* showed high haplotype diversity (0.9935) and low nucleotide diversity (0.00882) with 29 segregating sites. *Chirostoma a. attenuatum* presented high haplotype diversity (0.987) and low nucleotide diversity (0.00698) with 18 segregating sites, whereas *C. a. zirahuen* showed high haplotype diversity (0.989) and low nucleotide diversity (0.00852) with 21 segregating sites (Table 2).

3.5 | Genetic differentiation and structure

For *cytb*, the mean genetic distance between *C. a. attenuatum* and *C. a. zirahuen* was calculated as 1.1%. For *S7*, it was 0.4%. The AMOVA showed significant values for both markers. While for the *cytb* marker (*cytb* = 0.88, α = .000), most variation was observed between populations, for the *S7* intron, the primary source of variation was within-population, with lower, but significant, between-population variation (S7 intron = 0.01, α = .023; Table S1).

3.6 | Historical demography and gene flow

In the neutrality tests using the *cytb* marker, Tajima's D and Fu's Fs showed significant negative values, −2.22923 and −10.06747, respectively, for *C. a. attenuatum*. *Chirostoma a. zirahuen* showed significant negative Tajima's D value (-1.82093) and a negative, non-

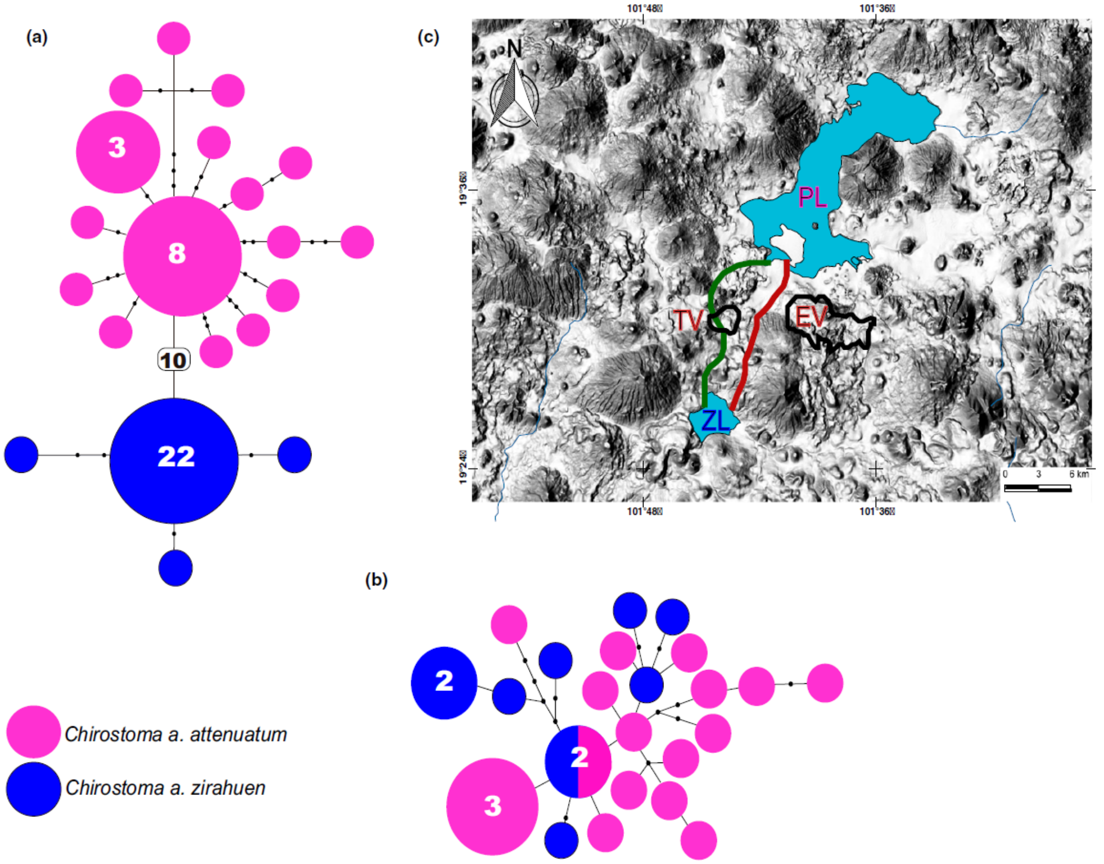


FIGURE 2 Haplotype networks and Patzcuaro and Zirahuen regions: (a) *cytochrome b gene* network and (b) *first intron of S7 ribosomal protein* gene haplotype network. White number indicates the haplotype frequency (number of individuals with the same haplotype), and circle without number is unique haplotype. Black number into the box and small black circle indicate the mutational steps. (c) Patzcuaro and Zirahuen regions. PL, Patzcuaro Lake; ZL, Zirahuen Lake; TV, Las Tazas volcano; EV, Estribo volcano. Red line = hypothetical connection (De Buen, 1943) between Patzcuaro and Zirahuen lakes 700,000 years ago. Green line = paleoconnection between Patzcuaro and Zirahuen *ca.* 30,000–8,000 years ago (Bradbury, 2000; Israde-Alcántara et al., 2005)

TABLE 2 Genetic diversity indices and historical demographic parameters in *Chirostoma attenuatum*, based on mitochondrial and nuclear locus

	n	nH	π	h	S	DT	FS
Mitochondrial (*cytb*)							
C. a. attenuatum	25	16	0.00210	0.8933	21	−2.22923*	−10.06747*
C. a. zirahuen	25	4	0.00025	0.18	3	−1.82093*	−0.06249
Nuclear (*S7*)							
C. a. attenuatum	11	20	0.00698	0.987	18	−0.19231	−16.47126*
C. a. zirahuen	14	24	0.00852	0.989	21	0.22923	−17.59861*

nH, number of haplotypes; π, nucleotide diversity; h, haplotype diversity; S, polymorphic sites; DT, Tajima's D; FS, Fu's Fs

*Significance value $p < .05$

significant, Fu's Fs value (-0.06249). For *S7* in *C. a. attenuatum*, Tajima's D was negative but not significant (-0.19231), and a significant negative Fu's Fs value (-17.59861) was obtained (Table 2).

The Bayesian skyline plot indicated expansion of *C. a. attenuatum ca.* 25,000 years ago (Figure 3A) and of *C. a. zirahuen ca.* 15,000 years ago (Figure 3B). Migration rates for *cytb* were lower ($m_{\text{Zirahuen-Patzcuaro}}$ = 0.01 and $m_{\text{Patzcuaro-Zirahuen}}$ = 0.012) than those for *S7* ($m_{\text{Zirahuen-Patzcuaro}}$ = 0.13 and $m_{\text{Patzcuaro-Zirahuen}}$ = 0.06); while *cytb* indicates symmetry in magnitude and direction between the two populations, the rates recorded for *S7* show an asymmetrical rate from Zirahuen to Patzcuaro.

4 | DISCUSSION

4.1 | *Chirostoma a. attenuatum* and *C. a. zirahuen*, incipient or complete speciation?

Results show significant structure in the paired Φ_{st} and AMOVA (Table S1), as well as genetic distance between *C. a. attenuatum* and *C. a. zirahuen* (*cytb* = 1.1% and *S7* = 0.4%). The genetic distance is relatively high for population level compared with the cyprinid *Algansea lacustris* endemic to Patzcuaro Lake, which presents a divergence of 1.5% from *A. tincella*, its sister species inhabiting other areas (Pérez-Rodríguez, Domínguez-Domínguez, Pérez Ponce de León, & Doadrio, 2009), and the 0.03% found between the Goodeinae *Allotoca meeki* endemic to Zirahuen and its sister species *A. diazi* endemic to Patzcuaro Lake (Corona-Santiago et al., 2015), suggesting isolation of the ancestors of the subspecies in their respective lakes. In contrast, whereas the *cytb* showed reciprocal monophyly at the subspecies level, *S7* demonstrated unresolved relationships and a mixture of haplotypes in the genetic tree and haplotype network (Figure 2A and B; Figures S1 and S2). Disparities between mitochondrial and nuclear trees can be associated with horizontal transfer, lineage sorting, introgression, gene duplication/extinction (Goncalves, Martínez-Solano, Ferrand, & García-París, 2007; Holland, Benthin, Lockhart, Moulton, & Huber, 2008), and the differing mutation rates in nuclear and mitochondrial loci (Egge, Nicholson, & Stark, 2015). In this case, the lack of resolution and mixed haplotypes in the *S7* genetic tree reflected the combined effects of low variation and incomplete lineage sorting that is consistent with the low evolutionary rate and high coalescence time for nuclear loci (Egge et al., 2015). These variation patterns are usually demonstrated by species complexes that have undergone relatively recent divergence (Beltrán-López et al., 2017; Buj, Šanda, Marčić, Ćaleta, & Mrakovčić, 2014; Buj et al., 2015), which is consistent with the most recent common ancestor of *C. a. attenuatum* and *C. a. zirahuen* populations dated *ca.* 600,000 years ago (Figure 1), considered a recent isolation event compared to divergence of the Patzcuaro endemic *A. lacustris* dated 1.9 Ma ago.

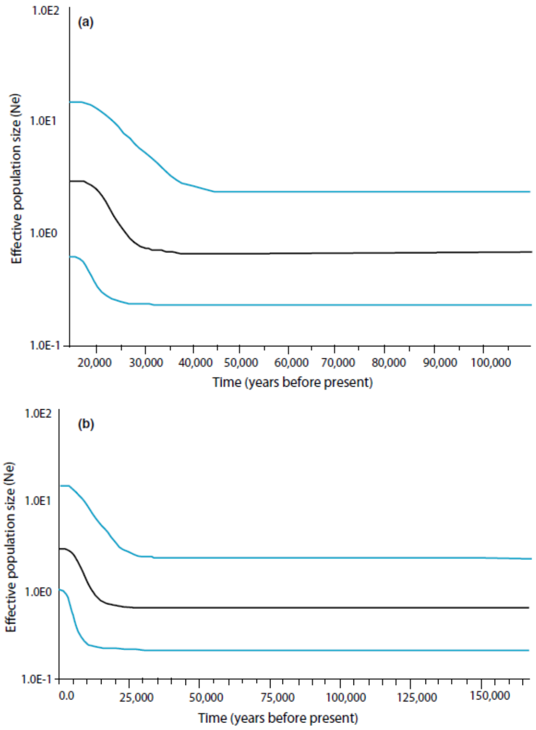


FIGURE 3 Demographic history reconstruction using Bayesian skyline plot based on the *cytochrome b* gene. Black line is the median effective population size. Blue lines indicate confidence intervals. (a) *Chirostoma a. attenuatum* and (b) *Chirostoma a. zirahuen*

Since the *BEAST coalescent-based approach provides the ability to model incomplete lineage sorting and ancestral polymorphism, the species tree produced strongly supported two reciprocally monophyletic lineages (Figure 1), implicating defined barriers to gene flow (Heled & Drummond, 2010). The differentiation pattern of the subspecies *C. a. attenuatum* from *C. a. zirahuen* lineages is consistent with their considerable reported morphological differentiation, including the number of lateral predorsal scales, caudal peduncle length, snout-to-first dorsal fin distance, and mouth shape (Barbour 1973; Barriga-Sosa, 2001; Meek, 1902; Miller et al., 2005). Based on the forgoing evidence, along with the geographic isolation of the lakes, we suggest that these subspecies be considered independent evolutionary units, and argue that a species level of *Chirostoma attenuatum* from Patzcuaro Lake and *Chirostoma zirahuen* from Zirahuen Lake must be recognized.

4.2 | Persistence of a barrier versus ecological isolation

The date of the most recent common ancestor of *C. attenuatum* and *C. zirahuen* was estimated from 319,000 to 939,000 years ago. The most plausible biogeographic hypothesis of the early cladogenetic event separating the species is the existence of a paleoriver connecting the lakes that disappeared ~700,000 years ago (Figure 2C). A similar biogeographic hypothesis has been proposed based on taxa of freshwater fishes codistributed in Patzcuaro and Zirahuen lakes, arguing that a paleotributary connected the Lerma River with Cuitzeo, Patzcuaro, and Zirahuen lakes 700,000–1,000,000 years ago (Álvarez Del Villar, 1972; De Buen, 1943). This is supported by stratigraphic information (Israde-Alcántara, Wade, Garduño-Monroy, & Barron, 2008), limnologic data (Bradbury, 2000; Israde-Alcántara, Garduño-Monroy, & Ortega, 2002), and geological evidence (Israde-Alcántara & Garduño-Monroy, 1999). The Patzcuaro and Zirahuen lakes are located in an area of high volcanic activity known as the "Tarasco Corridor," where more than 1,000 active volcanic cones have been recorded since the Pliocene (Corona-Santiago et al., 2015). The volcanic dynamism was produced by tectonic activity that increased in the area ~700,000 years ago (Johnson & Harrison, 1990; Luhr & Simkin, 1993), shaping the physiography of the Zirahuen and Patzcuaro basins, and corresponding to the time of the early cladogenetic event.

A hypothesis of a more recent connection between Zirahuen and Patzcuaro lakes, from 30,000 to 8,000 years ago (Garduño-Monroy et al., 2009; Israde-Alcántara et al., 2005, 2010), proposes a paleostream flowing from Zirahuen to Patzcuaro until tecto-volcanic events, such as activity in the Tazas volcano, disrupted the connection (Figure 2C). These dates are supported by the genetic flow demonstrated in the sister goodeid species *A. diazi* (Patzcuaro) and *A. meeki* (Zirahuen; Corona-Santiago et al., 2015). In this scenario, the symmetrical migration rates in mtDNA = 0.01 migrants per generation and the asymmetrical migration rates in $\text{nDNA}_{\text{Zirahuen-Patzcuaro}}$ = 0.013 versus $\text{nDNA}_{\text{Patzcuaro-Zirahuen}}$ = 0.06% migrants per generation between lineages, indicating that the recent connection of the lakes allowed migration but was sufficiently low to maintain genetic differentiation, as is supported by the significant values in Φ_{st} and AMOVA, as well as the haplotype network and species trees. These results support the suggestion that the speciation process is better explained by ecological speciation than by the existence of a barrier, as has been proposed in *A. meeki* with respect to *A. diazi*, in which migration was found, but was not significant and was low enough to maintain the genetic differentiation of the species (Corona-Santiago et al., 2015).

Evolutionary, biogeographic, and phylogenetic studies of *Chirostoma* demonstrate that *C. attenuatum* is member of a clade of species restricted to lake habitats (Bloom et al., 2009, 2013). The environmental conditions of lotic and lentic systems exert unique ecological constraints on aquatic organisms (Letsch, Gottsberger, & Ware, 2016). Studies of speciation focusing on transition between streams and lakes are scarce. They include characterization of the divergence of lake and stream ecotypes in the three-spined stickleback *Gasterosteus aculeatus*, in which differing predation regimes, dietary resources, light conditions, and parasite communities led to substantial morphological differences (Seehausen & Wagner, 2014). For *Chirostoma* from Patzcuaro, the diversity of food resources, including zooplankton, fish, insects, and microcrustaceans, can be associated with interspecific morphological differences (Soria-Barreto & Paulo-Maya, 2005), suggesting food resources as a potential ecological constraint. The disparate environmental conditions of Zirahuen and Patzcuaro lakes, the former deep, monomictic, and oligotrophic (Bernal-Brook & MacCrimmon, 2000) and the latter shallow, polymictic, and eutrophic (Bernal-Brook, Gomez-Tagle, & Alcocer, 2002), may have acted to spur rapid differentiation in *C. attenuatum* and *C. zirahuen* traits during the first allopatric stage *ca.* 600,000 years ago, as supported by our genetic and previous morphologic evidence (Barbour 1973; Meek, 1902; Miller et al., 2005). The natural history of a species adapted to lake ecosystems suggests that a recent connection via a paleostream (Figure 2C) may have represented an environmental constraint resulting in a low dispersal rate between lakes. In this scenario, the unfavorable lotic habitat represented a barrier sufficient to maintain a low genetic flow and prevent the establishment of migrants, maintaining the genetic differentiation of *C. attenuatum* and *C. zirahuen* during a secondary drainage connection. This corresponds to a classic scenario of ecological speciation (Schluter, 2001; Seehausen & Wagner, 2014), in which reproductive isolation of two populations has its source in allopatry, during which a population accumulates adaptations to unique aspects of its environment, preventing genetic homogenization in a secondary contact (Schluter, 2001; Domínguez-Domínguez, 2008).

4.3 | Demography and its conservation implications

Since the first allopatric stage 600,000 years ago, the studied lineages were exposed to different environmental conditions, as well as to different evolutionary forces and demographic processes. With respect to historical demography, the high genetic diversity and polymorphism of *C. attenuatum* suggests the persistence of a high effective population size (Buj et al., 2015), whereas the Fu's Fs, Tajima's D statistics, and the Bayesian skyline plot imply a population expansion dated *ca.* 25,000 years ago (Figure 3A), consistent with the early glacial stage (Caballero, Lozano-García, Vázquez-Selem, & Ortega, 2010). Paleoclimatic information from Patzcuaro indicates that during this period *ca.* 25,000 to 13,000 years ago, the basin was dominated by high humidity and precipitation. The lake was turbid with high productivity and deep with an extended shallow littoral zone, as evidenced by the abundance of fern *Isoetes* sp. (Bradbury, 2000; Caballero et al., 2010), facilitating the reproduction of *Chirostoma* and growth of larvae (Rojas-Carrillo, 2006). The deep open areas would support high populations of mature fish (García-De León, Ramírez-Herrejón, García-Ortega, & Hendrickson, 2014; Soria-Barreto & Paulo-Maya, 2005), promoting the population expansion shown by the Bayesian skyline plot for that period.

The demographic history of *C. zirahuen* is more complex, as indicated by the lack of consensus in the two molecular markers, with *cytb* showing low genetic diversity ($H = 0.18$; $\pi = 0.00025$) and a bottleneck effect, while the nuclear gene showed high diversity ($H = 0.989$; $\pi = 0.00852$) and population expansion. This inconsistency could be explained by the formation of Zirahuen Lake via damming of a stream that flowed to Patzcuaro Lake, producing a biogeographic scenario of peripheral isolation. Accordingly, the founder population was low, allowing stochastic processes, such as genetic drift and selection, to occur more slowly in nDNA than in mtDNA (Funk & Omland, 2003; Zink & Barrowclough, 2008), influencing the *C. zirahuen* demographic history (Lacy, 1987), as was proposed for the *A. diazi* complex (Corona-Santiago et al., 2015). The Bayesian skyline plot showed a population expansion *ca.* 15,000 years ago (Figure 3B), during the late glacial stage (Caballero et al., 2010; Clark et al., 2009). Paleoclimatic information demonstrates that 15,000 years ago, Zirahuen Lake underwent considerable increase in surface area, the littoral zone, and productivity (Caballero et al., 2010; Metcalfe, O'Hara, Caballero, & Davies, 2000; Ortega et al., 2010; Torres-Rodríguez, Lozano-García, Figueroa-Rangel, Ortega-Guerrero, & Vázquez-Castro, 2012; Vázquez, Ortega, Davies, & Aston, 2010). As with the *C. attenuatum* population, this paleoclimate scenario may have facilitated the population expansion of the *C. zirahuen* in Zirahuen Lake 15,000 years ago.

From a conservation perspective, the mitochondrial structure, the considerable morphological differentiation, and the historical isolation of the lake populations are sufficient evidence to consider the lineages as separate species, one endemic to Patzcuaro Lake (*C. attenuatum*) and the other endemic to Zirahuen Lake (*C. zirahuen*), representing a unique and irreplaceable genetic pool (Crandall, Bininda-Edmonds, Mace, & Wayne, 2000). This is especially important when considering (i) the restricted geographic range of each species, since for freshwater organisms in general (Ribera, 2008), and fishes in particular (Rosenfield, 2002), the likelihood of the extinction increases as geographic range is reduced; (ii) that *Chirostoma* have represented the most important fishery in Patzcuaro and Zirahuen lakes since pre-Hispanic times (Barbour, 1973; Berlanga-Robles, Luna, Nepita, & Vera, 1997; Berlanga-Robles, Madrid-Vera, & Ruiz-Luna, 2002; Hernández-Batista, Ramírez-Torrez, Azaola-Espinoza, Mayorga-Reyes, & Monroy-Dosta, 2015; Miller, 2005) and it is considered overfished (Chacón-Torres, 1993; Hernández-Batista et al., 2015; Rojas-Carrillo, 2006); and 3) that habitat degradation has been widely documented since the pre-Hispanic time (Guzmán, Polaco, & Pollard, 2001; Nichols & Pool, 2012; Williams & Weigand, 1996) and continues to the present (Lyons, González-Hernández, Soto-Galera, & Guzmán-Arroyo, 1998; Ramírez-Herrejón et al., 2014; Zambrano et al., 2001).

ACKNOWLEDGEMENTS

IBR would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for granting a scholarship. We thank the Ministerio de Economía y Competitividad from Spain (CGL-2013 41375-P) and Coordinación de la Investigación Científica-UMSNH, México, for funding the project. We thank the División de estudios de Posgrado de la Universidad Michoacana de San Nicolás de Hidalgo for providing economic resources for English editing of the manuscript. We thank D.K. Santiago Corona, F. Anguiano Rodríguez, O. Valencia, B. Andrade-García, and A. González Alejo for their laboratory and field support. I.B.R. thanks Dr. I. Barriga-Sosa, Dr. I. Doadrio, and two anonymous reviewers for helpful comments.

ORCID

Isaí Betancourt-Resendes http://orcid.org/0000-0002-3337-6878

REFERENCES

Álvarez Del Villar, V. J. (1972). Ictiología Michoacana V. Origen y distribución del a ictiofauna dulceacuícola michoacana. *Anales de la Escuela Nacional de Ciencias Biológicas*, *19*, 155–161.

Barbour, C. D. (1973). The systematics and evolution of the genus *Chirostoma*, Swainson (Pisces: Atherinidae). *Tulane Studies in Zoology and Botany*, *18*, 97–141.

Barbour, C. D., & Chernoff, B. (1984). Comparative morphology and morphometric of the pescados blancos (genus *Chirostoma*) from Lake Chapala Mexico. In A. A. Echelle & I. Kornfield (Ed.), *Evolution of fish species flock* (pp. 111–127). Orono, ME: Univ Maine Orono.

Barrett, J. C., Fry, C., Maller, J., & Daly, J. M. (2005). Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics*, *21*(2), 263–265. https://doi.org/10.1093/bioinformatics/bth457

Barriga-Sosa, I. (2001). *Variabilidad Morfométrica, Merística y Molecular de especies del género Chirostoma (Pisces: Atherinopsidae)*. Unpublished PhD Thesis, Universidad Autónoma Metropolitana-Iztapalapa.

Beltrán-López, R. G., Domínguez-Domínguez, O., Guerrero, J. A., Corona-Santiago, D. K., Mejia-Mojica, H., & Doadrio, I. (2017). Phylogeny and taxonomy of the genus *Ilyodon* Eigenmann, 1907 (Teleostei: Goodeidae), based on mitochondrial and nuclear DNA sequences. *Journal of Zoological Systematics and Evolutionary Research*, *54*, 85–105. https://doi.org/10.1111/jzs.12175

Berlanga-Robles, C. A., Luna, A. R., Nepita, M. R., & Vera, J. M. (1997). Estabilidad y diversidad de la composición de peces del Lago de Pátzcuaro, Michoacán, México. *Revista de Biologia Tropical*, *45*, 1553–1558.

Berlanga-Robles, C. A., Madrid-Vera, J., & Ruiz-Luna, A. (2002). Fish abundance and trophic structure from the commercial catch in Lake Patzcuaro, Mexico. *Hidrobiología*, *467*, 117–122. https://doi.org/10.1023/A:1014965504486

Bernal-Brook, F. W., Gomez-Tagle, A., & Alcocer, J. (2002). Lake Patzcuaro (Mexico): A controversy about the ecosystem water regime approached by field references, climatic variables, and GIS. *Hidrobiología*, *467*, 187–197. https://doi.org/10.1023/A:1014919032228

Bernal-Brook, F. W., & MacCrimmon, H. R. (2000). Lake Zirahuen (Mexico): An assessment of the morphometry change based on evidence of water level fluctuations and sediment inputs. In M. Munawar, S. Lawrence, I. Munawar & D. Malley (Eds.), *Ecovision World Monograph Series* (pp. 61–76). The Netherlands: Backhuys Publishers. In Press.

Berreli, P. (2009). How to use migrate or why are Markov chain Monte Carlo programs difficult to use? In G. Bertorelle, M. W. Bruford, H. C. Hauffe, A. P. Rizzoli & C. Vernesi (Eds.), *Populations genetics for animal conservation* (pp. 42–79). Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/CBO9780511626920

Bloom, D. D., Piller, K. R., Lyons, J., Mercado-Silva, N., & Medina-Nava, M. (2009). Systematics and biogeography of the silverside tribe Menidiini (Teleostomi: Atherinopsidae) based on the mitochondrial ND2 gene. *Copeia*, *2009*(2), 408–417. https://doi.org/10.1643/CI-07-151

Bloom, D. D., Weir, J. T., Piller, K. R., & Lovejoy, N. R. (2013). Do freshwater fishes diversify faster than marine fishes? A test using state-dependent diversification analyses and molecular phylogenetics of New World silversides (Atherinopsidae). *Evolution; International Journal of Organic Evolution*, *67*(7), 2040–2057. https://doi.org/10.1111/evo.12074

Bouckeart, R., Heled, J., Kuhner, D., Vaughan, T., Wu, C., Xie, D., . . . Drummond, A. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PloS Computational Biology*, *10*, e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Bradbury, J. P. (2000). Limnological history of lago de Pátzcuaro, Michoacan, Mexico for the past 48,000 years: Impacts of climate and man. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *163*, 69–95.

Buj, I., Ćaleta, M., Marčić, Z., Šanda, R., Vukić, J., & Mrakovčic, M. (2015). Different histories, different destinies-impact of evolutionary history and population genetics structure on extinction risk of the Adriatic spined loaches (genus *Cobitis*; Cypriniformes, Actinopterygii). *PLoS One*, *10*, e0131580. https://doi.org/10.1371/journal.pone.0131580

Buj, I., Šanda, R., Marčić, Z., Ćaleta, M., & Mrakovčić, M. (2014). Combining morphology and genetics in resolving taxonomy-A systematic revision of spined loaches (genus *Cobitis*; *Cypriniformes*, *Actinopterygii*) in the Adriatic Watershed. *PLoS One*, *9*(6), e99833. https://doi.org/10.1371/journal.pone.0099833

Caballero, M., Lozano-García, S., Vázquez-Selem, L., & Ortega, B. (2010). Evidencias del cambio climático y ambiental en registros glaciales y en cuencas lacustres del centro de México durante el último máximo glacial. *Boletín de la Sociedad Geológica Mexicana*, *62*, 359–377. https://doi.org/10.18268/BSGM2010v62n3a4

Campanella, D., Hughes, C., Unmack, P., Bloom, D., Piller, K., & Ortí, G. (2015). Multi-locus fossil-calibrated phylogeny of Atheriniformes (Teleostei, Ovalentaria). *Molecular Phylogenetics and Evolution*, *86*, 8–23. https://doi.org/10.1016/j.ympev.2015.03.001

Chacón-Torres, A. (1993). *Pátzcuaro, un Lago Amenazado. Bosquejo limnológico*. Unpublished Lic Thesis Universidad Michoacana de San Nicolás de Hidalgo Morelia México.

Chow, S., & Hazama, K. (1998). Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecology*, *7*, 1247–1263.

Clark, P. U., Dyke, A. S., Shakun, J. D., Carlson, A. E., Clark, J., Wohlfarth, B., . . . McCabe, A. M. (2009). The last glacial maximum. *Science*, *325*, 710–714. https://doi.org/10.1126/science.1172873

Corona-Santiago, D. K., Doadrio, I., & Domínguez-Domínguez, O. (2015). Evolutionary history of the live-bearing endemic *Allotoca diazi* species complex (Actinopterygii, Goodeinae): Evidence of founder effect events in the Mexican pre-Hispanic period. *PLoS One*, *10*(5), e0124138. https://doi.org/10.1371/journal.pone.0124138

Crandall, K. A., Bininda-Edmonds, O., Mace, G. M., & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, *15*, 290–295. https://doi.org/10.1016/S0169-5347(00)01876-0

De Buen, F. (1943). Los lagos michoacanos. I. Caracteres generales el Lago de Zirahuén. *Revista de la Sociedad Mexicana de Historia Natural*, *4*, 211–232.

Domínguez-Domínguez, O. (2008). *Filogeografía de Zoogoneticus quitzeoensis, Xenotoca variata y Alloophorus robustus (Cyprinodontiformes: Goodeidae) en el Centro de México: Implicaciones taxonómicas y de conservación*. Unpublished PhD thesis, Is Universidad Autónoma de México.

Domínguez-Domínguez, O., Doadrio, I., & Pérez-Ponce de León, G. (2006). Historical biogeography of some river basins in central Mexico evidence by their goodeine freshwater fish: A preliminary hypothesis using secondary Brooks parsimony analysis. *Journal of Biogeography*, *8*, 1437–1447. https://doi.org/10.1111/j.1365-2699.2006.01526.x

Drummond, A. J., Rambaut, A., Shapiro, B., & Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, *22*(5), 1185–1192. https://doi.org/10.1093/molbev/msi103

Echelle, A. A., & Echelle, A. F. (1984). Evolutionary genetics of a "species flock" Atherinid fishes on mesa central of Mexico. In A. A. Echelle & I. Kornfield (Eds.), *Evolution of fish species flock* (pp. 93–110). Orono, ME: Univ Maine Orono.

Egge, J. J. D., Nicholson, P. W., & Stark, A. W. (2015). Morphological and molecular variation in the least madtom *Noturus hildebrandi* (Siluriformes: Ictaluridae), a Mississippi Embayment endemic: Evidence for a cryptic lineage in the Hatchie River. *Journal of Fish Biology*, *186*, 493–526. https://doi.org/10.1111/jfb.12574

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analysis under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distance among DNA haplotypes application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479–491.

Ferrari, L., Orozco-Esquivel, T., Maena, V., & Maena, M. (2011). The dynamic history of the Trans-Mexican Volcanic Belt the Mexico subduction zone. *Tectonophysics*, *522–523*, 122–149. https://doi.org/10.1016/j.tecto.2011.09.018

Fu, Y. X. (1997). Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, *147*, 915–925.

Fu, Y. X., & Li, W. H. (1993). Statistical test of neutrality of mutations. *Genetics*, *133*, 693–709.

Funk, D. L., & Omland, K. E. (2003). Species level paraphyly and polyphyly: Frequency, causes and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics*, *34*, 397–423. https://doi.org/10.1146/annurev.ecolsys.34.011802.132421

García-De León, F. J., Ramírez-Herrejón, J. P., García-Ortega, R., & Hendrickson, D. A. (2014). Foraging patterns of four sympatric species of silversides (Atheriniformes: Atherinopsidae) in lago de Pátzcuaro, Central Mexico. *UNED Research Journal/Cuadernos de Investigación UNED*, *6*, 127–139.

García-Martínez, R. M., Mejia, O., García -De León, F. J., & Barriga-Sosa, I. D. L. A. (2015). Extreme genetics divergence in the endemic fish *Chirostoma humboldtianum* (Valenciennes, 1835): Implications for its conservation. *Hidrobiológica*, *25*(186), 95–106.

Garduño-Monroy, V. H., Chávez-Hernández, J., Aguirre-González, J., Vázquez-Rosas, R., Mijares, H., Israde-Alcántara, I., ... Pérez-López, R. (2009). Zonificación de los periodos naturales de oscilación superficial en la ciudad de Pátzcuaro, Mich. México, con base en microtremores y estudios paleosismología. *Revista Mexicana de Ciencias Geológicas*, *26*, 623–637.

Gómez-Tuena, A., Orozco-Esquivel, M. T., & Ferrari, L. (2005). Petrogénesis ígnea de la Faja volcánica Transmexicana. *Boletín de la Sociedad Geológica Mexicana*, *58*, 227–283.

Goncalves, H., Martínez-Solano, I., Ferrand, N., & García-París, M. (2007). Conflicting phylogenetic signal of nuclear vs mitochondrial DNA markers in midwife toads (Anura, Discoglossidae, Alytes): Deep coalescence or ancestral hybridization? *Molecular Phylogenetics and Evolution*, *44*, 494–500. https://doi.org/10.1016/j.ympev.2007.03.001

Guzmán, A. F., Polaco, Ó. J., & Pollard, H. P. (2001). Ofrendas de peces asociadas a entierros del Clásico-Epiclásico en Urichu, Michoacán, México. *Archaeofauna*, *10*, 149–162.

Heled, J., & Drummond, A. J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, *27*, 570–580. https://doi.org/10.1093/molbev/msp274

Hernández-Batista, A. F., Ramírez-Torrez, J. A., Azaola-Espinoza, A., Mayorga-Reyes, L., & Monroy-Dosta, M. C. (2015). The genus *Chirostoma* (Actinopterygii: Atheriniformes) in Mexico: Challenge for conservation and aquaculture technology. *International Journal of Aquatic Science*, *6*, 67–83.

Holland, B. R., Benthin, S., Lockhart, P. J., Moulton, V., & Huber, K. T. (2008). Using super networks to distinguish hybridization from lineage sorting. *BMC Evolutionary Biology*, *8*, 202. https://doi.org/10.1186/1471-2148-8-202

Israde-Alcántara, I., & Garduño-Monroy, V. H. (1999). Lacustrine record in a volcanic intra-arc setting: The evolution of the late Neogene Cuitzeo basin system (central-western Mexico, Michoacan). *Palaeogeography, Palaeoclimatology, Palaeoecology*, *151*, 209–227.

Israde-Alcántara, I., Garduño-Monroy, V. H., Fisher, C. T., Pollard, H. P., & Rodríguez-Pascua, M. A. (2005). Lake level change, climate, and the impact of natural events: The role of seismic and volcanic events in the formation of the Lake Patzcuaro Basin, Michoacan, Mexico. *Quaternary International*, *135*(1), 35–46. https://doi.org/10.1016/j.quaint.2004.10.022

Israde-Alcántara, I., Garduño-Monroy, V. H., & Ortega, M. R. (2002). Paleoambiente lacustre del cuaternario tardío en el centro del lago de Cuitzeo. *Hidrobiología*, *12*, 61–78.

Israde-Alcántara, I., Velázquez-Durán, R., Lozano, G. S. M., Bischoff, J., Domínguez, G., & Garduño-Monroy, V. (2010). Evolución paleolimnológica del lago de Cuitzeo, Michoacán durante el Pleistoceno-Holoceno. *Boletín de la Sociedad Geológica Mexicana*, *62*, 345–357.

Israde-Alcántara, I., Wade, M., Garduño-Monroy, V. H., & Barron, J. (2008). Estratigrafía y encuadramiento geodiná- mico de las cuencas lacustres del centro de México. Unión Mexicana de Estudios del Cuaternario. Fondo de Cultura Económica, México, DF.

Johnson, C. A., & Harrison, A. (1990). Neotectonics in Central Mexico. *Physic of the Earth and Planetary Interiors*, *64*, 187–210. https://doi.org/10.1016/0031-9201(90)90037-X

Lacy, R. C. (1987). Loss of genetic diversity from managed population: Interacting effects of drift, mutation, inmigration, selection and population subdivision. *Conservation Biology*, *1*, 143–158. https://doi.org/10.1111/j.1523-1739.1987.tb00023.x

Letsch, H., Gottsberger, B., & Ware, J. L. (2016). Not going with the flow: A comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. *Molecular Ecology*, *25*, 1340–1353. https://doi.org/10.1111/mec.13562/full

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Luhr, J. F., & Simkin, T. (1993). *Paricutín, the volcano born in a mexican cornfield* (p. 427). Phoenix, AZ: Geoscience Press.

Lyons, J., González-Hernández, G., Soto-Galera, E., & Guzmán-Arroyo, M. (1998). Decline of freshwater fishes and fisheries in selected drainages of West-Central Mexico. *Fisheries*, *23*, 10–18. https://doi.org/10.1577/1548-8446(1998)023<0010:DOFFAF>2.0.CO;2

Meek, S. E. (1902). *A contribution to the Ichthyology of México*. Zoological series (Vol. *3*, pp. 63–128). Chicago, IL: Field Columbian Museum.

Metcalfe, S. E., O'Hara, S. L., Caballero, M., & Davies, S. J. (2000). Records of late Pleistocene-Holocene climatic change in Mexico-a review. *Quaternary Science Reviews*, *19*, 699–721. https://doi.org/10.1016/S0277-3791(99)00022-0

Miller, R. R., Minckley, W. L., Norris, S. M. (2005). *Freshwater fishes of Mexico*. The University of Chicago Press, Chicago.

Miller, M. A., Schwartz, T., Pickett, B. E., He, S., Klem, E. B., Scheuermann, R. H., ... O'Leary, M. A. (2015). A RESTful API for access to phylogenetic tools via the CIPRES Science Gateway. *Evolutionary Bioinformatics*, *11*, 43–48. https://doi.org/10.4137/EBO.S21501

Nichols, D. L., & Pool, C. A. (2012). *The Oxford handbook of Mesoamerican archaeology-google books*. Oxford, UK: Oxford University Press. https://doi.org/10.1093/oxfordhb/9780195390933.001.0001

Niemiller, M. L., Near, T. J., & Fitzpatrick, B. M. (2012). Delimiting species using multilocus data: Diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (Teleostei: Amblyopsidae). *Evolution*, *66*(3), 846–866. https://doi.org/10.1111/j.1558-5646.2011.01480.x

Ortega, B., Vázquez, G., Caballero, M., Israde, I., Lozano-García, S., Schaaf, P., & Torres, E. (2010). Late Pleistocene: Holocene record of environmental changes in Lake Zirahuen, central Mexico. *Journal of Paleolimnology*, *62*, 325–343. https://doi.org/10.1007/s10933-010-9449-x)

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L., Grabosky, G., & Stice, L. (1991). *Simple fool's guide to PCR, version 2*. Honolulu, HI: Department of Zoology, University of Hawaii.

Perdices, A., Bermingham, E., Montilla, A., & Doadrio, I. (2002). Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. *Molecular Phylogenetics and Evolution*, *25*, 172–189. https://doi.org/10.1016/S1055-7903(02)00224-5

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Doadrio, I., Cuevas-Gacía, E., & Pérez-Ponce de León, G. (2015). Comparative historical biogeography of three groups of Nearctic freshwater fishes across central Mexico. *Journal of Fish Biology*, *86*, 993–1015. https://doi.org/10.1111/jfb.12611

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Pérez Ponce de León, G., & Doadrio, I. (2009). Phylogenetic relationships and biogeography of the genus Algansea Girard (Cypriniformes: Cyprinidae) of central Mexico inferred from molecular data. *BMC Evolutionary Biology*, *9*, 223. https://doi.org/10.1186/1471-2148-9-223

Posada, D. (2008). JModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, *25*, 1253–1256. https://doi.org/10.1093/molbev/msn083

Rambaut, A., & Drummond, A. (2007). Tracer v1.5. Retrieved from http://tree.bio.ed.ac.uk/software/tracer/

Ramírez-Herrejón, J. P., Zambrano, L., Mercado-Silva, N., Torres-Téllez, A., Pineda-García, F., Caraveo-Patiño, J., & Balart, E. F. (2014). Long term changes in the fish fauna of lago de Pátzcuaro in central Mexico. *Latin American Journal of Aquatic Research*, *42*, 137–149. https://doi.org/10.3856/vol42-issue1-fulltext-11

Ribera, I. (2008). Habitat constraints and the generation of diversity in freshwater macroinvertebrates. In J. Lancaster & R. A. Briers (Eds.), *Aquatic insects: Challenges to populations* (pp. 289–311). Wallingford, UK: CAB International. https://doi.org/10.1079/9781845933968.0000

Rojas-Carrillo, P. M. (2006). Aspectos reproductivos del "charal Prieto" *Chirostoma attenuatum* (Meek, 2002) del lago de Pátzcuaro, Michoacán. Nuevas líneas de investigación en atherinopsidos de México. *Hidrobiológica*, *16*, 1–9.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Hohna, S., … Huelsenbeck, P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Rosenfield, J. A. (2002). Pattern and process in the geographical ranges of freshwater fishes. *Global Ecology and Biogeography*, *11*, 323–332. https://doi.org/10.1046/j.1466-822X.2002.00287.x

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: A laboratory manual (2nd ed.). New York, NY. Cold Spring Harbor Laboratory.

Schluter, D. (2001). Ecology and the origin of species. *Trends in Ecology & Evolution*, *16*, 372–380. https://doi.org/10.1016/S0169-5347(01)02198-X

Seehausen, O., & Wagner, C. E. (2014). Speciation in freshwater fishes. *Annual Review of Ecology, Evolution, and Systematics*, *45*, 621–651. https://doi.org/10.1146/annurev-ecolsys-120213-091818

Silvestro, D., & Michalak, I. (2012). raxmlGUI: A graphical front-end for RAxML. *Organisms Diversity & Evolution*, *12*, 335–337. https://doi.org/10.1007/s13127-011-0056-0

Soria-Barreto, M., & Paulo-Maya, J. (2005). Morfometría comparada del aparato mandibular en especies de *Chirostoma* (Atheriniformes: Atherinopsidae) del lago de Pátzcuaro, Michoacán, México. *Hidrobiológica*, *15*, 161–168.

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, *57*, 758–771. https://doi.org/10.1080/10635150802429642

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, *68*, 978–989. Retrieved from http://ac.els-cdn.com/S0002929707614244/1-s2.0-S0002929707614244-main.pdf?_tid=b41bd0f6-9747-11e7-b8d1-00000aab0f02&acdnat=1505172167_59082c79b0c0358100067f8d4b794c1e

Tajima, T. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585–595.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Thompson, D. J., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The Clustal_X windows interface: Flexible strategies for multiple sequences alignment aided by quality analysis tools. *Nucleic Acid Research*, *25*, 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Torres-Rodríguez, E., Lozano-García, S., Figueroa-Rangel, B. L., Ortega-Guerrero, B., & Vázquez-Castro, G. (2012). Cambio ambiental y respuesta de la vegetación de los últimos 17,000 años en el centro de México: el registro del lago de Zirahuén. *Revista Mexicana de Ciencias Geológicas*, *29*, 764–778.

Tows, D., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*, 3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x

Vázquez, G., Ortega, B., Davies, S. J., & Aston, B. J. (2010). Registro sedimentario de los últimos ca. 17,000 años del lago de Zirahuén, Michoacán, México. *Boletín de la Sociedad Geológica Mexicana*, *62*, 325–343.

Williams, E., & Weigand, P. C. (1996). *Las cuencas del occidente de México*. Zamora, Mexico: Colegio de Michoacán.

Zambrano, L., Córdova-Tapia, F., Ramírez-Herrejón, J. P., Mar-Silva, V., Bustamantel-Camargo, T., & Bustamante, E. (2001). Las especies exóticas en el lago de Pátzcuaro, Michoacán, México. In R. Huerto-Delgadillo, S. Vargas-Velázquez & C. F. Ortiz-Paniagua (Eds.), *Estudio Ecosistémico del lago de Pátzcuaro. Aportes en gestión ambiental para el fomento del desarrollo sustentable* (pp. 133–156). Morelos, Mexico: Instituto mexicano de tecnología del agua.

Zink, R. M., & Barrowclough, G. (2008). Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, *17*, 2107–2121. https://doi.org/10.1111/j.1365-294X.2008.03737.x

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Betancourt-Resendes I, Pérez-Rodríguez R, Domínguez-Domínguez O. Speciation of silverside *Chirostoma attenuatum* (Pisces: Atheriniformes) in Central Mexico. *J Zool Syst Evol Res*. 2018;00:1–12. https://doi.org/10.1111/jzs.12216

Supporting information

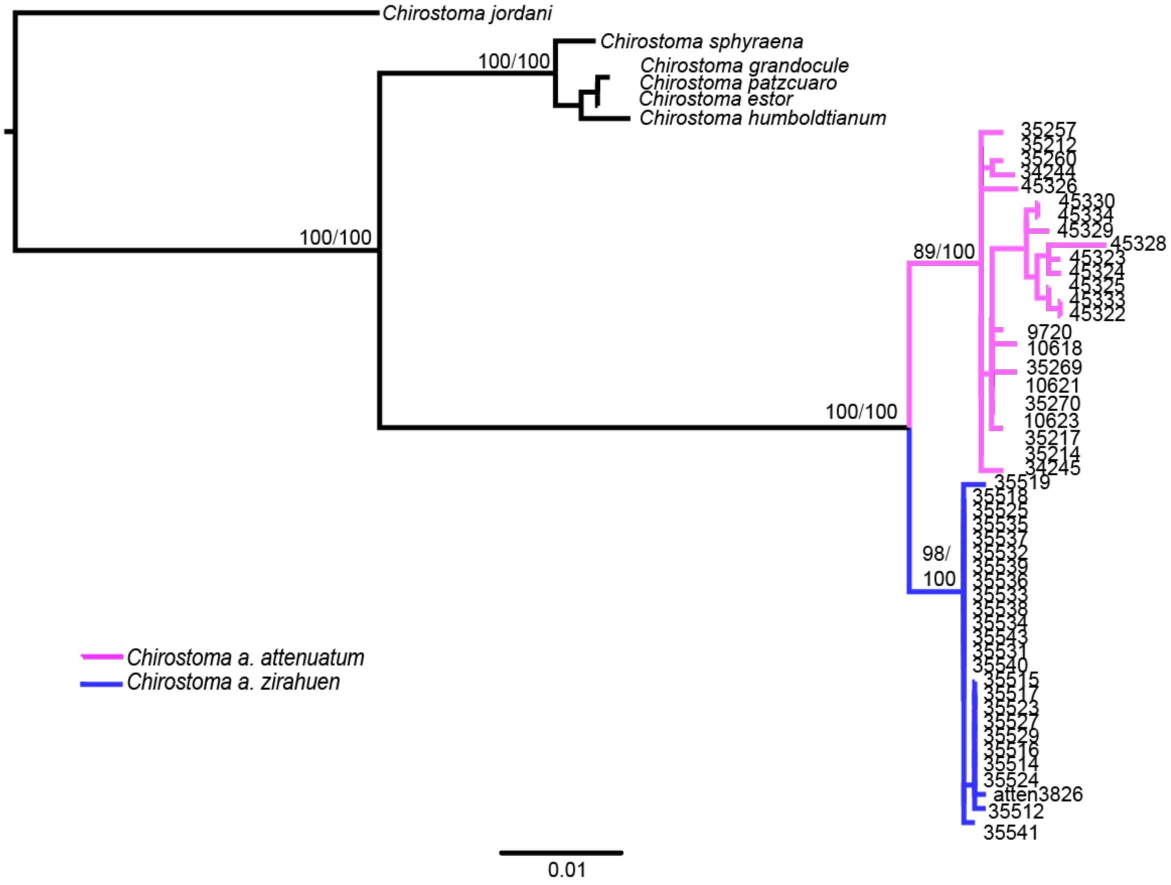


Figure S1. Phylogenetic relationship by maximum likelihood and Bayesian inference of *Chirostoma attenuatum* based on the *cytochrome b* gene. Numbers on branches represent bootstrap values and posterior probability (ML/BI).

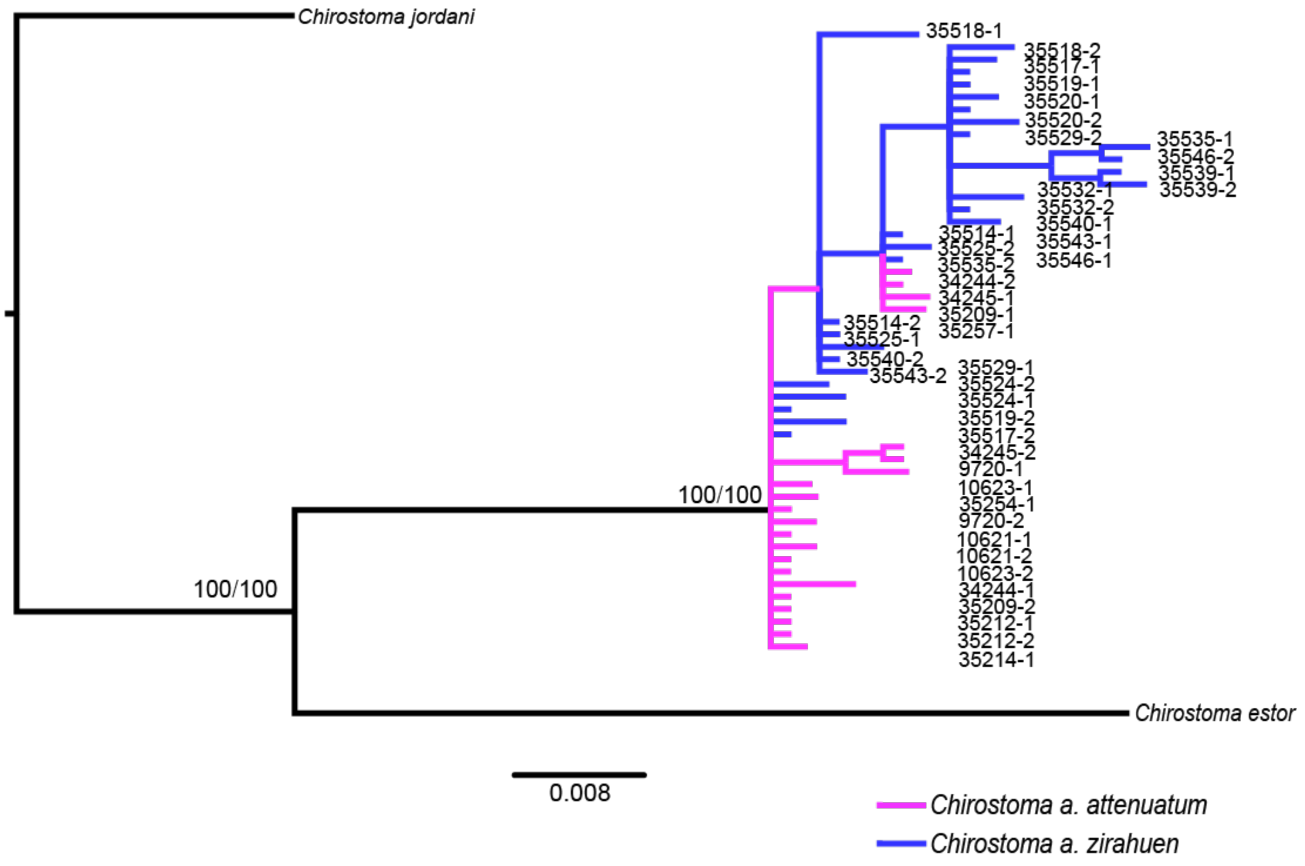


Figure S2. Phylogenetic relationship by maximum likelihood and Bayesian inference of *Chirostoma attenuatum* based on *first intron of S7 ribosomal protein* gene. Numbers on branches represent bootstrap values and posterior probability (ML/BI)

Table S1. Analysis of molecular variance (AMOVA) for *Chirostoma attenuatum*

Source of variation	**d.f**	**Sum of squares**	**Variance components**	**% variation**
Cytochrome b				
Among populations	1	126.280	5.02417 Va	*88.14
Within population	48	32.440	0.67583 Vb	11.86
Total	49	158.720	5.70000	
Firs intron of S7 ribosomal protein				
Among populations	1	0.619	0.00507 Va	*1.02
Within population	48	23.721	0.49418 Vb	98.98
Total	49	24.340	0.49926	

* Value with statistical significance α < 0.05

Capítulo II

Artículo escrito en el formato de la revista Molecular Phylogenetics and Evolution

https://www.journals.elsevier.com/molecular-phylogenetics-and-evolution

3. Filogenia y Filogeografía del grupo de especies *Chirostoma humboldtianum* (Actinopterygii: Atherinopsidae) en el centro de México: Entendiendo su historia evolutiva

Phylogenetic and phylogeographic patterns of endangered *Chirostoma humboldtianum* species group (Actinopterygii: Atherinopsidae): Understanding their evolutionary history

[1]Isaí Betancourt-Resendes, **[2]**Rodolfo Pérez-Rodríguez, **[3]**Irene de los Ángeles Barriga-Sosa, **[4]**Kyle R. Piller and **[2]**Omar Domínguez-Domínguez.

[1]Programa Institucional de Doctorado en Ciencias Biológicas, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mujica S/N, Morelia Michoacán, 58030, México

[2]Laboratorio de Biología Acuática, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mujica S/N, Morelia Michoacán, 58030, México

[3]Laboratorio de Genética y Biología Molecular de la Planta Experimental de Producción Acuícola Departamento de Hidrobiología Universidad Autónoma Metropolitana Unidad Iztapalapa, Av. San Rafael Atlixco 186 Col. Vicentina, Iztapalapa, D. F. 09340. México.

[4]Department of Biological Sciences, Southeastern Louisiana University, Hammond, LA, USA.

Abstract

The atherinopsids is the second largest group of freshwater fish that occurred in Central Mexico. The genus *Chirostoma* records the highest species richness and presents high biological, cultural and economic importance. The *Chirostoma humboldtianum* species group is phylogenetically the most derivate group into *Chirostoma* genus that inhabit lacustrine ecosystems, and nine morphological species have been recognized and these species have been consider as “species flock”. However, the high morphological polymorphism found in *Chirostoma humboldtianum* species group may difficult the correct species identification and limiting the development of research studies mainly in species inhabiting lacustrine ecosystems. In this study, we used the combined phylogenetic and phylogeography approach based in two mitochondrial markers and one nuclear for understand: the phylogenetic relationships between the *Chirostoma humboldtianum* species group and, that process govern the genealogic lineage distribution. The results showed several taxonomic inaccuracy and monophyly of species was not recovered. There were genetic admixture between recognized species. Therefore, there are a genetic segregation according to geography recognizing five genetic lineages closely relationships. Two evolutionary stage was found. First, a cladogenetic scenario between five lineages, and a recent diversification that occurred in Pleistocene less to 1 M.a. The second evolutionary stage showed a recent intra lacustrine genetic differentiation that could be associated to sympatric or ecological speciation. Our genetic data did not support the current classification in *Chirostoma humboldtianum* species group and new biogeography and evolutionary insight have arisen. The discrepancies found between genetic and morphological data can be attributed to the high phenotypic plasticity within the group, related to the pressures of the habitat and to rapid adatative divergences.

Keywords: Species delimitation; phenotypic plasticity; recent divergences; Taxonomic inaccuracy; polymorphism.

3.1 Introduction

The freshwater hydrographic systems in Central Mexico have been strongly influenced by intense tectonic and volcanic activity since the Miocene (Gomez-Tuena et al., 2007; Ferrari et al., 2012). This intense geologic activity has generated a complex hydrological system that, together with the climate fluctuation during the Pleistocene, have promoted dispersal and vicariant events that shaped the richness and diversity of freshwater fishes in Central Mexico (Miller et al., 2005). For the freshwater fishes, allopatric speciation is considered as the major factor of diversification (Dominguez-Dominguez et al., 2004, 2008, 2010; García-Martinez et al., 2014; Perez-Rodriguez et al., 2015; Beltrán-Lopez et al., *in press*), while the sympatric ecological segregation has been suggested for those species inhabiting lacustrine ecosystems, mainly in the family Atherinopsidae (Barbour, 1973; Betancourt-Resendes et al. 2018).

The atherinopsids is the second largest group of freshwater fishes in Central Mexico and are represented by the genera, *Poblana* Bonaparte and *Chirostoma* Swaison (Barbour, 1973; Miller et al., 2005). *Chirostoma* reaches its highest species richness and is a biologically, culturally and economically important group of fishes in Central Mexico (Barbour, 1973; Miller et al., 2005; Moncayo-Estrada et al., 2012). *Chirostoma* has been part of fisheries resources since pre-hispanic times, being the main subsistence activity of different ethnic groups in the region (Soria-Barreto et al., 1988; Paulo-Maya et al., 2000).

The *Chirostoma humboldtianum* species group consists of nine morphological species, which have been recognized considered a “species flock”, originally proposed by Barbour, (1973). This has been supported with morphology and osteology (Barbour and Chernoff, 1984), enzymatic characters (Echelle and Echelle, 1984), as well as combined analyses based on morphological, meristic and genetic data (Barriga-Sosa et al., 2005, 2002). However, the high morphological polymorphism found within the *Chirostoma humboldtianum* species group make accurate identification of species difficult, thereby limiting the development of research studies mainly in species inhabiting lacustrine ecosystems (Alaye, 1996; Barriga-Sosa et al., 2005, García de Leon et al., 2014; Mercado-Silva et al., 2015, Vital-Rodríguez et al., 2016).

This species in the group are distributed along the Lerma system in Central Mexico. *Chirostoma humboldtianum* Valenciennes has the widest distribution. *Chirostoma chapalae* Jordan & Snyder, *Chirostoma consocium* Jordan & Hubbs, *Chirostoma lucius* Boulenger, *Chirostoma promelas* Jordan & Snyder, and *Chirostoma sphyraena* Boulenger are endemic and sympatric within Lake Chapala, the largest natural lake in Mexico. *Chirostoma estor* Jordan, *Chirostoma grandocule* Steindachner, and *Chirostoma patzcuaro* Meek are sympatric and endemic to Lake Patzcuaro.

To date, molecular markers have been unable to resolve the phylogenetic relationship of the species within the *Chirostoma humboldtianum* species group (Bloom et al., 2009; 2012; 2013). These molecular studies have suggested that this species group has evolved very recently in Central Mexico (Bloom et al., 2013; Garcia-Martinez et al., 2015), compared with other freshwater fish, such as goodeids and cyprinids (Dominguez et al., 2004, 2006, 2010; Perez-Rodríguez et al., 2015). This recent diversification has been argued to occur through sympatric speciation due to trophic specialization or to unknown intrinsic mechanism of the species (Barbour, 1973; Echelle and Echelle, 1984; Bloom et al., 2013). Nevertheless, several studies of trophic segregation in sympatric species of *Chirostoma* from Patzcuaro and Chapala Lake fail to show a pattern of trophic differentiation among sympatric species (Moncayo-Estrada et al., 2012; Garcia de Leon et al., 2014; Mercado-Silva et al., 2015). The overlap in trophic ecology among sympatric species from Chapala and Patzcuaro lakes raises questions about the evolutionary processes in this morphologically diverse but genetically similar group.

As a result, a combined phylogenetic and Phylogeographic approach could be useful to understand and resolve the taxonomic and phylogenetic conflict within the *C. humboldtianum* species group and provide information on the processes that govern the distributions of the genealogic lineages give valuable information related to the evolutionary history of the *C. humboldtianum* species group. Therefore, in the present study, mitochondrial and nuclear markers were used to: 1) elucidate the phylogenetic relationships among the recognized species, 2) understand their evolutionary history, and link this to the geologic and climat history of central México and 3) describe how the vicariant events and habitat fragmentation in central Mexico have been important factors in their preset genetic configuration of the *C. humboldtianum* species group.

3.2 Materials and Methods

3.2.1 Specimen collections

Specimens of all the species in the *C. humboldtianum* species group were collected from sixteen localities of central Mexico (Fig. 1 and Table 1) and were obtained from local fisheries. Geographical units were established as discrete regions following Domínguez-Domínguez et al., (2006, 2010) and based on watershed hydrography. Fin clips were obtained for each specimen and preserved in 96% ethanol. Voucher specimens were identified using the diagnostic morphological and meristic characters (Barbour 1973; Barriga-Sosa et al., 2002, 2005; Miller et al., 2005) and preserved in the Coleccion de Peces de la Universidad Michoacana (CPUM) (Table 1). Additionally, sequences were obtained from GenBank (Table 2).

3.2.2 Sequence data

Total genomic DNA was extracted from fin clips using Phenol-Chloroform protocol (Sambrook et al., 1989). Two mitochondrial genes, *cytochrome b* (*Cytb*), and a 350 bp fragment of the hypervariable control region (*D-loop*), and one nuclear locus, the first *intron of S7 ribosomal protein* gene (*S7*) were amplified using the following primers: Cytb Glud-G (Palumbi 1996) and H16460 (Perdices et al., 2002), for the *D-loop* was used RCA and RCE (Lee et al., 1995) and for S7 S7RPEX1F and S7RPEX1R (Chow and Hazama, 1998). Amplification parameters are described in Appendix 1. Amplified products were sequenced in both directions by High-throughput Genomics Center, of Washington University, United States. Nucleotide sequences were edited and manually aligned with MEGA, using the complete mitochondrial genome of *Chirostoma humboldtianum* (GenBank reference NC_024883; Barriga-Sosa et al., 2014.) in order to determinate the position of both mitochondrial region for gene *Cytb* and the non-coding fragment-*D-loop*. The S7 intron was aligned by CLUSTAL_W 1.83 (Thompson et al., 1997). The alignment of *Cytb* was translated to verify the absence of stop codons along the sequence. Nucleotide saturation was tested for *Cytb* using the software DAMBE v5 (Xia, 2013) and free-recombination of non-coding S7 intron was performed by software DNAsp. Also, the sister species of the group, *C. attenuatum* Meek, (Bloom et al., 2013, 2009; Campanella et al., 2015; Betancourt-Resendes et al., 2018) in the phylogenetic analysis. *Chirostoma jordani* was including as outgroup. Alignments for each locus were used to estimate and select the substitution model that best fit the datasets using jModeltest v. 1.7 (Posada, 2008) using the Akaike information criterion (AIC).

3.2.3 Gene tree and haplotype network

Gene trees were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI) with separate analyses consisting of each gene and concatenated mtDNA (*Cytb* and *D-loop*) and an analysis based on *S7*. The ML was run in RaxML (Stamakis et al., 2008, Silvestro and Michalak 2012). Bayesian analyses were run in MrBayes 3.2.2 (Ronquist et al. 2012). Two independent runs were implemented with four MCMC and 10,000,000 generations, sampling every 100 trees. Chain convergence was verified by suitable effective sample size (ESS) for all parameters in Tracer 1.5 (Rambaut and Drummond 2007), discarding 10% of generations as burn-in.

Due the lack of resolution in genes tree in previous phylogenetic analyses (Bloom et al., 2013, 2012, 2009), haplotype networks were generated in order to examine the intra-specific relationships of the *C. humboldtianum* species group. Haplotypes analyses were conducted in Network 4.6.1.3 using a median-joining algorithm (Bandelt et al., 1999).

3.2.4 Species tree calibrated analysis and Bayes Factor species delimitation test

A multi-locus species tree was generated under a coalescent model in *BEAST v.1.8 (Heled and Drummond, 2010). Four scenarios corresponding to distinct classificatory assignments were tested including: 1) morphological species criteria proposed in the *Chirostoma humboldtianum* species group (Barbour, 1973; Barriga-Sosa et al., 2005; Bloom et al., 2013), and the groupings based on 2) *D-loop* 3) *Cytb* and 4) *S7* haplotype networks performed herein. A Yule model specie tree prior and a constant species tree population size was chosen, using an uncorrelated relaxed molecular clock. The mutation rate for *Cytb* gene was of 1% according to rate estimate for Atheriniformes (Campanella et al., 2015). While for *D-loop* and S7 the mutation rate was estimated according to *Cytb* mutation rate. Two independent runs with 100,000,000 generations sampling each 1000 generations were run. The convergence was evaluated with –InL values and effective sample size >200 in Tracer v.5 (Rambaut and Drummond, 2008). The maximum credibility tree was generated in TreeAnnotator v.1.8. The support value for the branch length was evaluated by posterior probability value. Analyses were run in Cripres Science Gateway v 3.3.

In order to calculate and compare the relatives support for the three well supported genetic group found in species tree analyses via Bayes factor (BF), the marginal-likelihood values (MLE) were estimated. This method is a helpful tool for species delimitation by comparing the support for each model relative to the model with the highest ranking (Caviedes-Solis and Nieto-Montes de Oca, 2018; Grummer et al., 2014; Leaché et al., 2014). MLE were estimated using the path sampling (PS) (Lartillot and Philippe, 2006), and stepping-stone (SS) (Xie et al., 2011) methods. Each assignment was run for a chain length of 100,000,000 generations for 200 path steps. Though (BF = 2 (model1-model2) using the MLE for two models, the BF were calculated, and then were compared and assessed using the framework of Kass and Raftery (1995). A negative BF value indicates support in favor of model 1, while a positive BF values indicates support in favor of model 2. The BF scale is as follows:

$0 < \ln$ (BF) < 1 is not worth more than a bare mention, $1 < \ln$ (BF) < 3 is positive evidence, $3 < \ln$(BF) < 5 is strong support, and $\ln$(BF) > 5 is decisive.

3.2.5 Genetic distance and structure

The uncorrelated genetic distance (D_p) were calculated between mains groups found in species tree results in MEGA v5. Bootstrapping was performed with 1,000 repetitions.

Analysis of Molecular Variance (AMOVA) was performed using five *a priori* population assignments based on: 1) morphological species; 2) hydrological discrete regions from Central Mexico according to Dominguez-Dominguez et al. (2006, 2010); 3) *D-loop* haplotype network; 4) *Cytb* haplotype network and 5) *S7* haplotype network. Distribution was generate from 10 000 random permutation to estimate the significances of the variance components. These analyses was performed with ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). Additionally, pairwise intra-lacustrine population differentiation (pairwise Φst) was evaluated (Excoffier et al., 1972) between different segregated groups found in Chapala and Patzcuaro Lake according with *D-loop* marker. The assays were carried out in ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). The significance of Φst value was evaluated by performing a randomization test of 10,000 replications with a significance level of $\alpha = 0.05$.

3.3 Results

3.3.1 Sequence data

Two-hundred four sequences of *Cytb*, 176 for *D-loop* and 113 for *S7* were generated from 16 localities throughout Central Mexico basins (Fig. 1), including all morphological species in the *C. humboldtianum* species group (Table 1). In addition, 10 sequences of *Cytb* of all members of *C. humboldtianum* species group (*sensu* Bloom et al., 2013), and 48 sequences of *C. humboldtianum* for *D-loop* (García-Martínez et al., 2015) were retrieved from GenBank (Table 2) and included in the analyses. The alignment length for *Cytb* were 1087 bp, that records 135 variables sites, 70 were parsimoniously informative*,* D-*loop* total length 284 bp, including 115 variables sites, of which 95 were parsimoniously informative. Finally, the *S7* alignment length was of 671 bp including 21 variable sites of which 12 were parsimoniously informative. The best-fit substitution models were T1M1+I+G, GTR+I+G and TPM3 for *Cytb*, *D-loop* and *S7*.

3.3.2 Gene trees

The current morphological species classification of *Chirostoma humboldtianum* species group was not recovered by any of the three loci used. Nevertheless, there were some geographical structuring for the mitochondrial genes (mtDNA). Mitochondrial DNA recovered a basal polytomy that included the four sympatric species from Lake Chapala including *C. promelas, C. consosium, C. chapala* and *C. lucius*, as well as samples of *C. lucius* from Los Negritos Lake, *C. humboldtianum* from Tepuxtepec dam and San Pedro Lagunillas Lake (Fig. 2, Supplementary Fig. 1, 2, 3, 4). In addition, three geographically segregated, major lineages were recovered. Lineage one clustered individuals of *C. sphyraena* from Lake Chapala with high posterior probability. Lineage two, consisted of *C. humboldtianum* from Zacapu, Santiaguito Maxada, San Juanico and Tiacaque localities. Finally, lineage three contained *Chirostoma estor* from Lakes Patzcuaro and Zirahuen, as well as individuals identified as *C. patzcuaro* and *C. grandocule* from Lake Patzcuaro; *C. estor* from Cointzio dam; *C. humboldtianum* from Villa Victoria dam and an unidentified *Chirostoma* sp. from Atenango dam (Fig. 2). The S7 topology failed to resolve relationships among the *C. humboldtianum* species group. An unresolved polytomy was recovered, with the exception of a monophyletic group of *C. spyraena* from Lake Chapala (Supplementary Fig. 5).

3.3.3 Haplotype nerwork

D-loop resulted in five major haplogroups corresponding to geography of Central Mexico rather than morphological species hypotheses (Fig. 3). Haplogroup A consisted of all individuals of *C. sphyranea* and two individuals from San Juanico Dam (access numbers: KF652020, KF652023) identify by García-Martínez et al.,

(2015) as *C. humboldtianum*. This was separated by 14 mutation steps from the nearest haplogroup. The haplogroup B is consists of specimens identified as *C. promelas, C. lucius, C. consocium* and *C. chapalae* from Lake Chapala Lake, including specimens from the reservoirs Tepuxtepec, Juanacatlan, San Juanico, and San Pedro Lagunillas Lake identified as *C. humboldtianum* by García-Martínez et al., (2015). It should be noted that San Pedro Lagunillas population is segregated within this haplogroup by three mutational steps, although with two haplotypes shared with the major haplogroup B (Fig. 3). Haplogroup C is separated by 11 mutation steps from the nearest haplogroup and includes specimens of *C. humboldtianum* from Zacapu Lake, Tiacaque, and Santiaguito Maxda dam, as well as specimens from reservoirs, San Juanico, Cointzio and San Pedro Lagunillas Lake. Haplogroup D, separated by 12 mutational steps from the haplogroup C, clusters all specimens sampled from Lake Patzcuaro identified as *C. estor, C. grandocule* and *C. patzcuaro.* In addition to specimens identified as *C. estor* from Cointzio dam, *C. humboldtianum* from the reservoirs of Villa Victoria and San Juanico, and unique divergent haplotype with specimens of *C. estor copandaro* from Zirahuen Lake that includes one haplotype of *C. estor* from Lake Patzcuaro (Fig. 3). Finally, haplogroup E was separated by 10 mutational steps and includes samples of *C. estor copandaro* from Zirahuen Lake and one specimen of *C. estor* from Lake Patzcuaro (Fig. 3).

The *Cytb* network shows four genetic groups consistent with the haplogroups found in *D-loop*, but with lower variation. The main difference of this network relative to *D-loop* network is that specimens of *C. estor* from Lakes Zirahuen and Patzcuaro are included in the same haplogroup (Fig. 4). Like *D-loop*, samples are grouped according to geographic location rather than morphological species. The central haplotype (19) of haplogroup B is shared among *C. chapala, C. consosium, C. promelas, C. lucius* and *C. humboldtianum.* Haplotype 34 is shared among *C. lucius*, *C. promelas* and *C. humboldtianum*. Haplotype 35 is shared between *C. consosium* and *C. humboldtianum*, and haplotype 38 is shared between *C. lucius* and *C. humboldtianum*. The haplogroup D also showed shared haplotype between species, as haplotype (1) that is shared between *C. patzcuaro, C. grandocule, C. estor and C. humboldtianum* (Fig. 4).

The nuclear *S7* network recovered samples in two haplogroups, separated by two mutational steps. One group corresponds to samples of *C. sphyraena* from Lake Chapala, and the second group includes the remaining morphological species from Central Mexico. Within latter haplogroup, two central haplotype with peripheral haplotypes were found, but both lack geographic or species-level congruence (Fig. 5).

3.3.4 Species tree and Bayes Factor species delimitation test

Morphological species hypotheses currently recognized as *C. sphyraena, C. promelas, C. lucius, C. chapala, C. consosium, C. humboldtianum, C. patzcuaro, C. grandocule,* and *C. estor*, was poorly supported (Fig. 6A). While the arrangements based on the haplotype networks obtained by *S7*, *Cytb* and *D-loop*, that assess, two, four and five differentiated lineages respectively, show branches highly supported in all cases (Fig. 6B-D). The first corresponding to *S7* arrangement (Fig. 6B) includes *C. sphyraena* and other lineages conformed by remaining morphological species of *C. humboldtianum* species group. The second scenario corresponding to *Cytb* arrangement (Fig. 6C), supported four differentiated lineages related as follows: a Zacapu lineage plus Lerma-Chapala lineage, were related with Patzcuaro lineage, and three together were related with *C. sphyraena*. Zacapu lineages include *C. humboldtianum* from Zacapu and two localities from upper Lerma basin (Tiacaque and Santiago de Maxda dam); Lerma-Chapala group includes specimens of *C. humboldtianum* from two localities in upper Lerma basin (Tepuxtepec and Juanacatlan dam) and samples identified as *C. consocium, C. chapala, C. promelas* and *C. lucius* from Chapala. The Patzcuaro lineage conformed to *C. patzcuaro*, *C. grandocule* and *C. estor* sampled in the Patzcuaro and Zirahuen basins, as well as *C. estor* from Cointzio dam in Cuitzeo drainage, an unidentified *Chirostoma* from Atenango dam of the Tecolutla basin, and *C. humboldtianum* from San Juanico and Villa Victoria dams in Balsas drainage.

The fourth scenario corresponding to the *D-loop* arrangement (Fig. 6D), supported five lineages, including the same four lineages found in *cytb*, but with the Patzcuaro lineage divided into two sister lineages. One included *C. estor* from Zirahuen, and the other consisted of all three species included from Patzcuaro.

The marginal likelihood of the highly-supported three arrangements, S7, *Cytb* and *D-loop* show the lowest SS and PS values for the S7 arrangement (SS = -1207.41 and PS = -1215.24), while the *Cytb* and *D-loop* arrangements recorded higher and similar values each other (SS = -875.17; PS = -875.18 and SS = -875.47; PS = -875.46, respectively). The 2lnBf of *Cytb* arrangement relative to *D-loop* arrangement was 0.60 for SS, and 0.57 for PS. Following Kass and Raftery (1995), these results are considered “not worth more than a bare mention”, indicating a lack of support to choice a best arrangement (Fig. 6 C, D. Table 3).

3.3.5 Divergence times, genetic distance, and structure

The divergence time results obtained using four and five genetic groups according with the high support found in the species tree analyses were the same. The most common recent ancestor (MRCA) of *C. humboldtianum* species group was estimated at *ca* 0.58 Ma. (HPD 95 % 0.25-1 Ma.). The separation of lineages from Patzcuaro and Zirahuen with respect to lineages from Lerma-Chapala and Zacapu was estimated at *ca* 0.27 Ma (HDP 95 % 0.13-0.45 Ma). The separation between Lerma-Chapala and Zacapu lineages was estimated at *ca* 0.13 Ma (HDP 95 % 0.06-0.21 Ma). Finally, the most recent split were between Patzcuaro and Zirahuen species, dated at *ca* 0.076 Ma. (HDP 95 % 0.133-0.033 Ma.) (Fig. 7).

The un-correlated mean genetic distance ($\bar{D}_p$) between *C. sphyraena* with respect to the *C. humboldtianum* species group by *Cytb* was 1.2 %. The $\bar{D}_p$ calculated among the Patzcuaro lineage and the Lerma-Chapala and Zacapu lineages was of 0.4 % and 0.3 %, respectively. The closest group pairs recorded $\bar{D}_p$ were 0.05 % and 0.02 % between Lerma-Chapala and Zacapu lineages, and Patzcuaro and Zirahuen lineage, respectively. Whereas with the *D-loop* the higher $\bar{D}_p$ was between *C. spyraena* with respect to *Chirostoma humboldtianum* species group recording 6.2 % and the lowest D_p was between Patzcuaro with respect to Zirahuen lineages recording 4.2 %.

The AMOVA results based on *Cytb* showed a significant value for the four lineage hypothesis tested in the species tree analyses (nine, five, four and two lineages). The major Φct value was when samples were grouped according to four and five lineages with same Φct value of 0.72, two lineages arrangement showed a Φct value of 0.50, and the nine lineages showed the Φct value of 0.32. Arrangement testing the discrete hydrographic basin recorded the lower and no significant value of Φct (0.30) (Table 3). With *D- loop*, the major percent of variation and significate values were recorded in the five lineages arrangement (Φct = 0.52), followed by four lineages, hydrographic basin and nine lineages arrangement (Φct = 0.48, Φct =0.37 %, Φct =0.29, respectively). Two lineages arrangement recorded the lowest and not significant values (Table 3). Finally, with the *S7* four and five lineages arrangements recorded the same major percent of variation and significant (Φct = 0.50), with the remaining three arrangements showing not significant Φct value (Table 4). In Chapala Lake a high Φst value between some sub-groups was found, the Φst value of sub-groups B1 respect with others (B2, B3 and B4) was high and significant, also the sub-group B4 had high and significate Φst value respect with others sub-groups (B1, B2 and B3), while the Φst value of sub-group B2 respect to B3 was no significant (Table 5). Within Patzcuaro Lake a high and significant Φst values (0.303) between both segregate sub-groups (D1 and D2) ware recorded (Table 5).

3.4 Discussion

The *C. humboldtianum* species group is composed of nine currently recognized species (*sensu* Barbour, 1973). However, several recent molecular studies with mitochondrial and nuclear markers showed a lack of resolution in phylogenetic relationships with the species not recovered as a monophyletic entities (Bloom et al., 2013, 2012, 2009). The identity of the *C. humboldtianum* species group is highly controversial due the overlap in morphological features, making phylogentic studies challenging. This situation has been exacerbated since several species, as

C. humboldtianum, *C. lucius* and *C. estor* have been translocated out of their natural distributional ranges (Barriga-Sosa et al., 2005). Several translocations were made into areas where other species of *Chirostoma* already occurred. The findings of the present work, using several methods, as well as mitochondrial and nuclear markers, do not support the current taxonomy. Instead, a genetic mixture of some recognized species was recovered. The genetic signature was found to have a high geographic correlation, rather than supporting species level hypotheses, demonstrating that the current taxonomy of the group need to be revised.

3.4.1 *Species boundaries and taxonomic implications*

The data generated in this study support the monophyly of the *C. humboldtianum* species group, however the species proposed by morphological features (Barbour, 1973), electrophoretic assays (Echelle and Echelle, 1984) and morphometric data (Barriga-Sosa et al., 2005, 2002) were not supported. Based on the results from several analyses, including gene and species trees, Bayes factor, haplotype network, genetic distances and structure, four scenarios of species delimitation into the *Chirostoma humboldtianum* species group will be discussed. First, the morphological arrangement that includes nine recognized species, was least not supported with the genetic data. It had low levels of genetic divergence for *Cytb* gene and no variation with the *S7* intron. The value found in AMOVA assay was lower with mitochondrial markers and there was statistical significance with *S7* intron (Table 4), the monophyly of species were not recovered with any genes tree (Fig. 2, Supplementary Fig. 1), a low support values was obtained in Star Beast (Fig. 6A), and genetic mixture was found among morphological species in genes tree and haplotype networks.

Arrangement 2 is the most conservative hypothesis, clustering two differentiate lineages based on *S7* consisting of *C. sphyraena* and a second group containing all of the species in the *Chirostoma humboldtianum* species group. Two differentiated lineages were recovered as monophyletic groups without genetic mixture based on the *S7* gene tree and haplotype network (Fig. S1, Fig. 5). The species tree assay was well-supported, however this scenario had a lower marginal likelihood estimation value (SS = -1207.41 and PS = -1215.24), and the value of Φct was not statistical significant with the non-coding *D-loop* and *S7* intron when clustering with two groups in AMOVA assays (Fig. 3). Nevertheless, the genetic distance between two groups were 1.3 % with *cytb* gene.

The third arrangement showed four differentiated lineages according to *Cytb*, which were highly congruent geographically and well supported by the Star Beast analyses (Fig. 6C) with high and significant values for all genes (Φct = 0.72, 0.48, 0.50 with *cytb*, *D-loop* and *S7* respectively) in the genetic structure analyses (Fig. 4) and the highest marginal likelihood estimation value (SS = -875.17; PS = -875.47). *Chirostoma sphyraena* is the most divergent, and in general, the genetic groups found within the *Chirostoma humboltianum* species group had a high geographic congruence, segregating the species from Lerma-Chapala basin, Zacapu Lake, and Patzcuaro-Zirahuen lakes respectively. The genetic divergences were low with *Cytb* gene (<0.4 %).

The fourth scenario showed five genetic differentiated lineages with high supported (>99 %) values with Star Beast (Fig. 6D), with high and significant value for all genes (Φct = 0.72, 0.52, 0.50 with *cytb*, *D-loop* and *S7* respectively) in AMOVA analyses (Fig. 4). There are high genetic and significant differentiation values between them Φst = >0.4 (Table 5) and marginal likelihood estimation value was closely to three arrangement (SS = -875.18; PS = -875.46). In this scenario based on *D-loop* hypothesis showed the separation of the *C. estor copandaro* from Zirahuen Lake respect to *C. estor estor* from Patzcuaro Lake (Fig. 6D).

The speciation scenarios 2, 3 and 4 based on genetic data presented differences with respect to the morphological hypothesis, and did not recover the nine currently recognized species (Star Beast analysis Fig. 6). Complete speciation seems to have occurred in *C. sphyraena*, with complete lineage sorting in mtDNA and *S7* nDNA and additional evidence in morphology (Barbour, 1973; Barbour and Chernoff, 1984). *Chirostoma sphyraena* was recovered as a monophyletic assemblage in gene and species trees (Fig. 2, 6 and Supplementary Fig. 1, 2, 3, 4, 5). The mean genetic distance calculated with *Cytb* is 1.2 % similar to that calculated between sister species *C. attenuatum* and *C. zirahuen* (Betancourt-Resendes et al., 2018). *Chirostoma sphyraena* was recovered as sister to the *C. humboldtianum* species group in species tree assays (Fig. 6), and did have any shared haplotypes in the network analysis (Fig. 3, 4 and 5). Based on this genetic evidence, the morphological identity of *C. sphyraena* is corroborated with this genetic data. The rest of species, however, our genetic data showed a species complex, supporting until five species according to *D-loop* molecular marker (Fig. 3), Star Beast (Fig. 6D), and AMOVA analysis (Table 3). Besides, recent cladogenetic events in an isolated basin since the Pleistocene, and high genetic structure supported the hypothesis to consider as separate species. While the low genetic distances between them (<0.4 %) could be a signal of an incipient speciation process. However the discrepancy observed between mtDNA and nDNA, could be explicated for recent divergences (< 0.265 M.a) and the coalescent time in nDNA is not complete. The mtDNA was structured according to geographical area, while S7 intron did not show genetic structure between the divergent groups due to a lower mutation rate (Almeida and De Salle, 2016). So the recent divergence within the *C. humboldtianum* species group did not show the separation between them in nDNA. The discrepancies between them seem to be due by recent divergences and not hybridization, given that this study did not find genetic mixture in mtDNA among them. The Bayes Factor species delimitation test (Table 3) does not allows for the support on of scenario three and four (four and five species Fig. 6 C and D) (Caviedes-Solis and Nieto-Montes de Oca, 2018; Grummer et al., 2014; Leaché et al., 2014). The differences between two speciation models is the separation of *Chirostoma estor* from Zirahuen with respect to Patzcuaro, however there are further evidence about isolation between both basin, as geological (Israde-Alcántara and Garduño-Monroy, 1999), biogeographic (De Buen, 1943; Alvarez, 1972; Corona-Santiago et al., 2015; Betancourt-Resendes et al. 2018) and biological (Corona-Santiago et al., 2015; Betancourt-Resendes et al. 2018) evidence that support that *C. estor estor* and *C. estor copandaro* could be cataloged as different taxonomic entities.

Other important taxonomic implications is that this genetic data supports the occurrence of two species in Patzcuaro Lake, *C. attenuatum* and one species of the *C. humboltianum* species group, not three as species hypothesis support. The monophyly of *C. humboldtianum* was not supported, showing specimens identified as *humboltianum* in different genetic lineages, however the Zacapu lineage that includes specimens identified as *C. humboldtianum,* also including specimens from Santiaguito Máxda and Tiacaque dams, is well supported in mtDNA gene tree (Fig. 2) and Star Beast analysis (Fig. 6 C, D), showing that *C. humboldtianum* seems to be more geographically restricted. Whereas for Chapala, the genetic data support the occurrence of two species, *C. sphyraena* and one species within the *C. humboltianum* species group, and rather than five as previously known.

The principal morphological features used to differentiate among this species of *Chirostoma* are related to measures in the head and swimming structure (Barbour, 1973; Barriga-Sosa et al., 2004;), and the variation detected in morphological measures that have been associated with food resources and swimming requirements in the water column (Barriga-Sosa et al., 2002). The high variability in inter- and intraspecific morphological measures have been associated with a variety of selective pressures, such as competition for food resources, and space, as well as fishing pressure (Barriga-Sosa et al., 2002). No evidence of trophic segregation in sympatric species of *Chirostoma* from Lakes Patzcuaro and Chapala have been found (Moncayo-Estrada et al., 2012; Garcia de Leon et al., 2014; Mercado-Silva et al., 2015). The meristic and morphometric characters are influence by environmental conditions and geographical distribution in *Menidia* and *Chirostoma* species (Miller et al., 2005), as is the case of the inland silverside *Menidia audens* and *Menidia beryllina* in which the meristic and morphometric characters are sensitive to habitat (Chernoff, 1982). The discordance between morphological and genetic data could be explained by several aspects, such as hybridization (Pérez-Miranda et al., 2017), recent diversification (Tarvin et al., 2017), the lack of resolution of the molecular markers (Bloom et al., 2009), secondary contact (Emerson and Faria, 2014; Faria et al., 2016), taxonomic inaccuracy or morphological homoplasy (Duellman, 2001; Ornelas-García et al., 2008) and phenotypic plasticity (Gulisija et al., 2016; Rosenblum et al., 2014; Tarvin et al., 2017; Zamudio et al., 2016).

These explanations proposed for the high morphological polymorphism in *Chirostoma* have been hybridization (Alaye, 1996; Martin del Campo, 1940).

Although, this process may predispose populations to adaptive radiation (Seehausen and Wagner, 2014) and that occurred after colonization events. However, the low colonization capacity, due the high association of those species to lacustrine ecosystems, could be a barrier for dispersal after the cladogenetic events (Betancourt-Resendes et al., 2018). The lack of resolution in the molecular markers used is less likely, since previously have been demonstrated usefulness to elucidate recent events of speciation in *C. attenuatum* and *C. zirahuen* (Betancourt-Resendes et al. 2018). So, the discrepancies between molecular markers and morphological characters in the *C. humboldtianum* species group could be related to the high phenotypic plasticity within the group, mainly related to habitat pressures and rapid adaptive divergences, as have occurred in inland silverside *Menidia beryllina* population in adjacent environmental regimes (Fluker et al., 2011).

3.4.2 *Evolutionary history of Chirostoma humboldtianum species group*

The evolution of freshwater fishes is further associated with geological activity, climate fluctuation, effective population size, and diverse ecological pressures (Buj et al., 2015; Fluker et al., 2011; Schluter and Conte, 2009; Seehausen and Wagner, 2014). In central Mexico, the evolution of freshwater systems is mainly promoted by the high dynamism of genesis, destruction and change in hydrographic basins configuration, influenced by geological events and climatic change. The tectonism and volcanism occurred in central Mexico allowed deep change in hydrographic basins configuration, with differential response of the ichthyofaunal to these changes (Beltrán-López et al., 2017; Betancourt-Resendes et al. 2018; Corona-Santiago et al., 2015; Doadrio and Domínguez, 2004; Dominguez-Dominguez et al., 2006, 2008; García-Martínez et al., 2015; Pérez-Rodríguez et al., 2009; Piller et al., 2015). The *Chirostoma humboldtianum* species group is adapted exclusively to lacustrine ecosystems, therefore the low dispersal capacity through lotic systems, may influence the reproductive isolation and genetic differentiation within the group, even with evidence of interbasin connection via rivers and streams.

The model that has been proposed to explain the evolution of the *C. humboldtianum* species group is the flock speciation (Barbour, 1973; Barbour and

Chernoff, 1984; Echelle and Echelle, 1984). However, the genetic data provided evidence to conclude that the early evolution of the *Chirostoma humboldtianum* species group was highly associated with vicariant events that occurred in Central Mexico, and not to sympatric speciation. The colonization of the TMRCA of *Chirostoma humboldtianum* species group to Central México occurred with the invasion of *C. humbultianum*-like ancestor (*sensu* Barbour, 1973; Echelle and Echelle, 1984), and the age of this colonization was, dated since Miocene (Barbour, 1973). Nevertheless, the most recent molecular studies propose the colonization age of TRMCA dated less than 1 M.a. (Bloom et al., 2013; Campanella et al., 2015). Our divergence time for the split of *C. sphyraena* with respect to other lineages within the *Chirostoma humboldtianum* species group was dates around 0.6 m.a. (Fig. 7), and is consistent with the hypothesis of a recent diversification. The high volcanic activity and climatic fluctuation allow several connection and disconnection between hydrological Basin of central México during the late Pleistocene (Israde-Alcantara et al. 2005; Garduño-Monroy et al., 2009). These events of connection and disconnection between hydrological basins have been supported as the causes of fish population separation, as the case of goodeids *Zoogoneticus quitzeoensis* (Domínguez-Domínguez et al., 2008) and *Xenotoca variata* (Domínguez-Domínguez, 2008). Therefore, based in the geographic congruence of lineage found in the present study, these geologic and climatic events seems to be the explanation of the isolation and molecular differentiation between the major lineages of *C. humboldtianum* species group. The colonization route of ancestor is unclear, but is more likely it was thought the Lerma Basin as was proposed by Barbour, (1973). The colonization of Patzcuaro basin could be for ancient connection thought of Cuitzeo basin that kept an ancient connection (Álvarez, 1972). There are geological (Moncayo et al., 2001; Israde-Alcantara et al., 2005) and biological (Dominguez-Dominguez et al., 2006, 2008, 2010; Pérez-Rodríguez et al., 2009) evidence the support the ancient connection between Patzcuaro and Cuitzeo basin and at the same time they had a connection with the Lerma basin, bedsides the occurrence the *C. grandocule* in Cuitzeo Lake, extinct during the temporary dryness of Cuitzeo Lake (Barriga-Sosa et al., 2002) and the fossil record found one large *Chirostoma* sp.

dated c.a. 30,000 years ago could be explained the colonization of Patzcuaro Lakes through the connection occurred between Cuitzeo and Patzcuaro basin. The separation of Zirahuen respect to Patzcuaro groups was occurred *ca.* 0.076 Ma. (HDP 95 % 0.133-0.033 Ma.) (Fig. 7). Several connection and isolated events between both basins have been described (De Buen, 1947; Israde-Alcántara et al., 2005; Garduño-Monroy et al., 2010). But the divergence time between shared ichthyofaunal was not synchronic (Domínguez-Domínguez, 2008; Corona-Santiago et al., 2015; Betancourt-Resendes et al., 2018).

The separation between Zacapu and Lerma-Chapala groups was dated ca. 0.13 Ma ago (HDP 95 % 0.06-0.21 Ma). The Zacapu Cienega was connected with Lerma system in two independent ways; 1) the ancestral connection through the Angulo river tributary of Middle Lerma (Moncayo-Estrada et al., 2001) and 2) ancestral connection through the Cuitzeo basin for the Villa Morelos and the Chucandiro-Huaniqueo corridors. Seemingly, both connections were produced by the activity of the pliocenic Northeast-Southwest fault system of the area (Israde-Alcántara, 1999). However, the part of Lerma system present important alluvial and volcano sedimentary deposits due to the tectonic instability which is typical in the volcanic arc and active faulting.

3.4.3 *Population structure*

The freshwater fish display high levels of genetic differentiation and population subdivision compared with marine fish, mainly due to the island like model of the basins and hydrological complexity of freshwater systems, as well as small population sizes (Ward et al., 1994, Seehausen, 2004). In the *C. humboldtianum* species group, in addition to the high and significant genetic structure between the major groups found with *D-loop* marker (0.476 to 0.694) (Table 5), explained by the geological and climatic activity of Central Mexico, there are genetic segregation within the haplogrup B (Chapala-Lerma) and D (Patzcuaro-Zirahuen) (Fig. 3). In Chapala, some haplotypes were separated by at least by four mutational steps (Subgroups B1 to B4 in Fig. 3). The subgroup B1 cluster individuals identify as *Chirostoma lucius*, which presented high and significate Φst value (Table 5) respect to subgroups B2, B3 and B4. The subgroup B4 showed high and significate structure respect to B1, B2, and B3 subgroups (Table 5). There was high Φst value between subgroups B2 and B3 but there was not significate (Table 5). While in Patzcuaro, our genetic data showed the segregation of the haplotype in two subgroups (D1 and D2 in Fig. 3), with significate Φst value (Table 5). The genetic segregation process that promote the separation in four subgroups B1 to B4 in Chapala (Fig. 3) and two subgroups D1 to D2 in Patzcuaro Lake (Fig. 3) could be explained by two ways: 1) the formation of intra-lacustrine barriers or 2) ecological specialization or even both. Lake Chapala is the biggest lake in Mexico, and several environmental and geological events have been impacted the lacustrine basin, even some authors have been proposed that the changes in the paleoclimate regime in the area promoted changes in the water level of the Chapala paleo-Lake, causing the isolation and posterior contact of different regions within the large Chapala paleo-Lake, which could have promoted the genetic differentiation of fish species groups, as have been proposed for Goodeids (Domínguez-Domínguez et al., 2010) or for *C. grandocule* population of Patzcuaro Lake, (Barriga-Sosa et al., 2004).

In the other case, the mechanism involved in sympatric speciation or ecological segregation are the ecological specialization in response to ecological opportunity (Mayr, 1942) and reproductive isolation, as have been reported by Nicaruaga cichlids (Barluenga et al., 2006), African lakes cichlids (Wagner et al., 2013; Seehausen, 2013) and *Chirostoma attenautum* (Betancourt-Resendes et al., 2018). Studies around the trophic ecology in sympatric species of silversides in Chapala and Patzcuaro lakes have not shown a trophic overlap (García-de León et al., 2014; Mercado-Silva et al., 2015). Nevertheless, the morphological differentiation in sympatric species of Chapala and Patzcuaro are related mainly to jaw bones, suggesting a further relation with trophic divergence (Lake et al., 1988; Soria Barreto et al., 2005). The mitochondrial *D-loop* marker feature several limitation to test the sympatric speciation approach, so this issue must to be perform with powerful molecular markers as SNPs.

3.4.4 *Hybridization and out-natural dispersion*

The hybridization between different species translocated mainly from big Lakes (Chapala and Patzcuaro) have been largely proposed as the major problem in species differentiation and suggests that the level of hybridization between species, due the large amount of translocation between lakes, is high enough to homogenize the genetic and morphological pool of the *C. humboltianum* species group (Martín del Campo, 1940; Alaye, 1996; Martínez-Palacios et al., 2007; Navarrete-Salgado and Contreras-Rivero, 2011 Hernandez-Batista et al., 2015), as have been reported for the introduction of *C. lucius* into the Patzcuaro Lake and subsequent hybridization with *C. estor* (Alaye, 1996). Although, the molecular markers used herein, provide no conclusive results in regards to hybridization, the large mitochondrial data set provide no support for the genetic mixture between Chapala and Patzcuaro species, since a high geographic segregation, genetic structure and genetic distance was found in samples collected in both lakes. Due the high number of individuals translocated in each lake, the fact that males and females were introduce and that the introduction initiate since pre-Hispanic times (Hernández-Batista et al., 2015), one would expect a mixture of maternal haplotypes in the analyzed individuals if the translocation events were successful, suggesting that the genetic signal of the new individuals not become part of effective population.

Chirostoma humboldtianum, *C. estor* and *C. lucius* have been translocated out of their natural distribution al ranges (Chacón-Torres and Rosas-Monje, 1995; Barriga-Sosa, 2001). The genetic data in this study could provide insight about the translocation of species and we proposed some no natural population as the population of Villa Victoria dam recognized as *C. humboldtianum* (García-Martinez et al., 2015) however, mtDNA evidence show that this population belong to Patzcuaro lineage more than to other population of *C. humboldtianum* from Lerma basin, also this is supported by the lack of evidence of a connection between Balsas basin with Patzcuaro Basin less than 1 Ma. So the presence of *Chirostoma* in Villa Victoria dam seems to be conformed by specimens introduced from Patzcuaro, The same scenario seems to be occurred in Atenango dam of Tecololutla basin, in which haplotypes were cluster with Patzcuaro lineage (Fig. 4). The San Juanico dam presented genetic mixture from almost all the differentiated genetic lineages found, but with some segregation, so we considered the San Juanico case an unresolved issue.

ACKNOWLEDGEMENTS

IBR would like to thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for granting a scolarship. We thank the Ministerio de Economía y Competitividad de España (CGL-2013 41375-P) and Coordinación de la Investigación Científica-UMSNH, México, for funding the project. We thank B. García-Andrade, A. González-Alejo, and F. Mar-Silva for their laboratory and field support.

Appendix 1. PCR Protocols

Amplification process was conducted in a reaction of 25 µL, containing 50-100ng DNA, 2.5 mM of 1X buffer, 1.5 mM $MgCl_2$, 2.5 µM dNTP mix (µM 10), 10 pmol of each primer, 1 unit of Taq DNA polymerase (Invitrogen), and distilled water to bring the reaction volume to 25 µL. The amplification condition was following: The *cytb* was an initial denaturalization at 94°C for 2 min, 35 cycles of 94°C for 45 s, 48°C for 1 min, and 72°C for 1 min, and final extension at 72°C for 5 min. The *D-loop* amplification procedure was an initial denaturalization at 94°C for 3 min, 35 cycles of 94°C for 30 s, 52.4°C for 45 s, and 72°C for 1 min, and final extension at 72°C for 5 min. The S7 was an initial denaturalization at 94°C for 1 min, 35 cycles of 94°C for 30 s, 56°C for 45 s, and 72°C for 45 s and final extension at 72°C for 5 min.

References

Alaye-Rahy, N., 1996. Estudios del polimorfismo de la hemoglobina para identificar especies del género *Chirostoma* del lago de Pátzcuaro, Michoacán, México. Ciencia Pesquera 13: 1-9.

Alaye-Rahy, N., 1993. El pescado blanco (género *Chirostoma*) del lago de Pátzcuaro, Michoacán. Composición de especies. Ciencia Pesquera 9: 113-128.

Álvarez Del Villar, V. J., 1972. Ictiología Michoacana V. Origen y distribución del a ictiofauna dulceacuícola michoacana. Anales de la Escuela Nacional de Ciencias Biológicas, 19, 155–161.

Almeida, F.C., DeSalle, R., 2016. Genetic differentiation and adaptive evolution at reproductive loci in incipient *Drosophila* species. J. Evol. Biol. 30, 524–537. doi:10.1111/jeb.13021

Bandelt, H.J., Forster, P., Röhl, a, 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Barbour, D., 1973. The Systematics and Evolution of the Genus *Chirostoma* Swainson (Pisces, Atherinidae).

Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439, 719–723. doi:10.1038/nature04325

Barriga-Sosa I., 2001. Variabilidad morfométrica, merística y molecular de especies del género *Chirostoma* (Pisces: Atherinopsidae). PhD Tesis., Universidad Autónoma Metropolitana-Iztapalapa, México, D. F. 199 p.

Barriga-Sosa, I., Pérez-Ramírez, M.Y., Soto-Aguirre, F., Castillo-Rivera, M., Arredondo-Figueroa, J.L., 2005. Inter-specific variation of the mitochondrial r16S gene among silversides, “Peces Blancos”, (Atherinopsidae: Menidiinae) and its utilization for species identification. Aquaculture 250, 637–651. doi:10.1016/j.aquaculture.2005.05.003

Barriga-Sosa, I.D.L.A., Eguiarte, L.E., Arredondo-Figueroa, J.L., 2004. Low but Significant Subdivision among Populations of *Chirostoma grandocule* from Lake Patzcuaro, Mexico. Biotropica 36, 85–98. doi:10.1111/j.1744-7429.2004.tb00299.x

Barriga-Sosa, I.D.L.A., Ibáñez-Aguirre, A.L., Arredondo-Figueroa, J.L., 2002. Morphological and genetic variation in seven species of the endangered *Chirostoma* “*humboldtianum* species group” (Atheriniformes: Atherinopsidae). Rev. Biol. Trop. 50, 199–216.

Barriga-Sosa I., García-Martínez, R.M., Díaz-Larrea, J., Lozano Garza, O.A., García-De-León, F.J., 2014. Isolation and Characterization of 26 Microsatellite Loci for the Shortfin Silverside Fish *Chirostoma humboldtianum* Valenciennes1835 (Atherinopsidae: Menidiinae) Derived from Next Generation Sequencing and Their Cross-Amplification in Central Mexican Ather. Open J. Genet. 04, 355–361. doi:10.4236/ojgen.2014.45032

Beltrán-López, R.G., Domínguez-Domínguez, O., Guerrero, J.A., Corona-Santiago, D.K., Mejía-Mojica, H., Doadrio, I., 2017. Phylogeny and taxonomy of the genus *Ilyodon* Eigenmann, 1907 (Teleostei: Goodeidae), based on mitochondrial and nuclear DNA sequences. J. Zool. Syst. Evol. Res. 1907. doi:10.1111/jzs.12175

Betancourt-Resendes I., Pérez-Rodríguez R., Domínguez-Domínguez O., 2018 Speciation of silverside *Chirostoma attenuatum* (Pisces: Atheriniformes) in Central Mexico. J Zool Syst Evol Res. 2018;00:1 12.https://doi.org/10.1111/jzs.12216

Blair, C., Murphy, R.W., 2011. Recent trends in molecular phylogenetic analysis: where to next? J. Hered. 102, 130–8. doi:10.1093/jhered/esq092

Bloom, D.D., Piller, K.R., Lyons, J., Mercado-Silva, N., Medina-Nava, M., 2009. Systematics and Biogeography of the Silverside Tribe Menidiini (Teleostomi: Atherinopsidae) Based on the Mitochondrial ND2 Gene. Copeia 2009, 408–417. doi:10.1643/CI-07-151

Bloom, D.D., Unmack, P.J., Gosztonyi, A.E., Piller, K.R., Lovejoy, N.R., 2012. It's a family matter: molecular phylogenetics of Atheriniformes and the polyphyly of the surf silversides (family: Notocheiridae). Mol. Phylogenet. Evol. 62, 1025–30. doi:10.1016/j.ympev.2011.12.006

Bloom, D.D., Weir, J.T., Piller, K.R., Lovejoy, N.R., 2013. Do freshwater fishes diversify faster than marine fishes? A test using state-dependent diversification analyses and molecular phylogenetics of new world silversides (atherinopsidae). Evolution 67, 2040–57. doi:10.1111/evo.12074

Buj, I., Ćaleta, M., Marčić, Z., Šanda, R., Vukić, J., Mrakovčić, M., 2015. Different histories, different destinies-impact of evolutionary history and population genetic structure on extinction risk of the adriatic spined loaches (genus *Cobitis*; Cypriniformes, actinopterygii). PLoS One 10, 1–17. doi:10.1371/journal.pone.0131580

Campanella, D., Hughes, L.C., Unmack, P.J., Bloom, D.D., Piller, K.R., Ortí, G., 2015. Multi-locus fossil-calibrated phylogeny of Atheriniformes (Teleostei, Ovalentaria). Mol. Phylogenet. Evol. 86, 8–23. doi:10.1016/j.ympev.2015.03.001

Carstens, B.C., Pelletier, T. a., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. Mol. Ecol. 22, 4369–4383. doi:10.1111/mec.12413

Caviedes-Solis, I.W., Nieto-Montes de Oca, A., 2018. A multilocus phylogeny of the genus *Sarcohyla* (Anura: Hylidae), and an investigation of species boundaries using statistical species delimitation. Mol. Phylogenet. Evol. 118, 184–193. doi:10.1016/j.ympev.2017.09.010

Chacón Torres, A., Rosas Monge, C., 1995. A restoration plan for pez blanco in Lake Patzcuaro, Mexico. Symposium of the American Fisheries Society 15, 122 – 126.

Chernoff, B., 1982 Character variation among populations and the analysis of biogeography. Am Zool 22:425–439

Chow, S., Hazama, K., 1998. Universal PCR primers for *S7* ribosomal protein gene introns in fish. Molecular Ecology, 7, 1247–1263.

Corona-Santiago, D.K., Doadrio, I., Domínguez-Domínguez, O., 2015. Evolutionary History of the Live-Bearing Endemic *Allotoca diazi* Species Complex (Actinopterygii, Goodeinae): Evidence of Founder Effect Events in the Mexican Pre-Hispanic Period. PLoS One 10, e0124138. doi:10.1371/journal.pone.0124138

De Buen, F. (1943). Los lagos michoacanos. I. Caracteres generales el Lago de Zirahuén. Revista de la Sociedad Mexicana de Historia Natural, 4, 211–232.

Doadrio, I., Domínguez, O., 2004. Phylogenetic relationships within the fish family Goodeidae based on cytochrome b sequence data. Mol. Phylogenet. Evol. 31, 416–30. doi:10.1016/j.ympev.2003.08.022

Domínguez-Domínguez, O., Alda, F., de León, G.P.-P., García-Garitagoitia, J.L., Doadrio, I., 2008. Evolutionary history of the endangered fish *Zoogoneticus quitzeoensis* (Bean, 1898) (Cyprinodontiformes: Goodeidae) using a sequential approach to phylogeography based on mitochondrial and nuclear DNA data. BMC Evol. Biol. 8, 161. doi:10.1186/1471-2148-8-161

Dominguez-Dominguez, O., Doadrio, I. y, Perez-Ponce de Leon, G., 2006. Historical biogeography of some river basins in central Mexico evidenced by their goodeine freshwater fishes: a preliminary hypothesis using secondary Brooks parsimony analysis. J. Biogeogr. 33, 1437–1447. doi:10.1111/j.1365-2699.2006.01526.x

Domínguez-Domínguez, O., 2008. Filogeografía de *Zoogoneticus quitzeoensis*, *Xenotoca variata* y *Alloophorus robustus* (Cyprinodontiformes: Goodeidae) en el Centro de México: Implicaciones taxonómicas y de conservación. Thesis, Universidad Autónoma de México.

Domínguez-Domínguez, O., Pedraza-Lara, C., Gurrola-Sánchez, N., Perea, S., Pérez-Rodríguez, R., Israde-Alcántara, I., Garduño-Monroy, V. H., Doadrio, I., Pérez-Ponce de León, G., Brooks, D. R., 2010. Biogeography of the Goodeinae (Cyprinodontiforms). In Viviparous Fishes II (Uribe, M. C.& Grier, H. J., eds), pp. 19–61. Homestead, FL: New Life Publications

Echelle, A. A., Echelle, A. F., 1984. Evolutionary genetics of a "species flock" Atherinid fishes on mesa central of Mexico. In A. A. Echelle & I. Kornfield (Eds.), Evolution of fish species flock (pp. 93–110). Orono, ME: Univ Maine Orono.

Excoffier, L., Lischer, H. E., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analysis under Linux and Windows. Molecular Ecology Resources, 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., Quattro, J. M., 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes application to human mitochondrial DNA restriction data. Genetics, 131,479–491.

Faria, C.M. a, Machado, A., Amorim, I.R., Gage, M.J.G., Borges, P. a V, Emerson, B.C., 2016. Evidence for multiple founding lineages and genetic admixture in the evolution of species within an oceanic island weevil (Coleoptera, Curculionidae) super-radiation. J. Biogeogr. 43, 178–191. doi:10.1111/jbi.12606

Ferrari, L., Orozco-Esquivel, T., Manea, V., Manea, M., 2012. The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico subduction zone. Tectonophysics 522-523, 122–149. doi:10.1016/j.tecto.2011.09.018

Fluker, B.L., Pezold, F., Minton, R.L., 2011. Molecular and morphological divergence in the inland silverside (*Menidia beryllina*) along a freshwater-estuarine interface. Environ. Biol. Fishes 91, 311–325. doi:10.1007/s10641-011-9786-2

García-Andrade, A., 2014. Filogeografía Comparada de Cuatro Especies de Peces Vivíparos en los Lagos del Norte de Michoacán.

García-de León, F.J., Ramírez-Herrejón, J.P., García-Ortega, R., Hendrickson, D. a., 2014. Foraging patterns of four sympatric species of silversides (Atheriniformes : Atherinopsidae) in Lago de Pátzcuaro , Central Mexico. Res. J. Costa Rican Distance Educ. Univ. 6, 127–139.

García-Martínez Rosa María, Omar Mejía, F.J.G.-D.L. and I.D.L.A.B.-S., 2015. " Extreme genetics divergence in the endemic fish *Chirostoma humboldtianum* (Valenciennes , 1835): implications for its conservation." Hidrobiológica 25, 95–106.

Gómez-tuena, A., Orozco-esquivel, M.T., Ferrari, L., 2005. Petrogénesis ígnea de la Faja Volcánica Transmexicana. Bol. la Soc. Geol. Mex. 3, 227–283.

Grummer, J.A., Bryson Jr, R.W., Reeder, T.W., 2014. Species Delimitation Using Bayes Factors: Simulations and Application to the *Sceloporus scalaris* Species Group (Squamata: Phrynosomatidae). Syst. Biol 63, 119–133. doi:10.1093/sysbio/syt069

Gulisija, D., Kim, Y., Plotkin, J.B., 2016. Phenotypic plasticity promotes balanced polymorphism in periodic environments by a genomic storage effect 202, 1437–1448. doi:10.1101/038497

Heled, J., Drummond, A.J., 2010. Bayesian Inference of Species Trees from Multilocus Data. Mol. Biol. Evol 27, 570–580. doi:10.1093/molbev/msp274

Hernández-Batista, A.F., Ramírez-Torrez, J.A., Azaola-Espinosa, A., Mayorga-Reyes, L., Monroy-Dosta, M.D.C., 2015. The genus *Chirostoma* (Actinopterygii:

Atheriniformes) in Mexico: Challenge for conservation and aquaculture technology. Int. J. Aquat. Sci. 6, 67–83.

Israde-Alcántara, I., Garduño-Monroy, V.H., 1999. Lacustrine record in volcanic intra-arc setting.The evolution of the Late Neogene Cuitzeo basin system(central western Michoacan,Mexico).Paleogeography,Paleoclimatology,Paleoecology. Palaeogeogr. Paleoclimatology, Palaeoecol. Vol.15, 209–227. doi:doi.org/10.1016/S0031-0182(99)00024-3

Israde-Alcántara, I., Garduño-Monroy, V.H., Fisher, C.T., Pollard, H.P., Rodríguez-Pascua, M. a., 2005. Lake level change, climate, and the impact of natural events: The role of seismic and volcanic events in the formation of the Lake Patzcuaro Basin, Michoacan, Mexico. Quat. Int. 135, 35–46. doi:10.1016/j.quaint.2004.10.022

Kass, R.E., Raftery, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Lake, C., Rodriguez-ruiz, A., Granado-lorencio, C., 1988. Características del aparato bucal asociadas al régimen alimenticio en cinco especies coexistentes del género *Chirostoma* (Lago de Chapala, México). Rev. Chil. Hist. Nat. 61, 35–51.

Lartillot, N., Philippe, H., 2006. Computing Bayes factors using thermodynamic integration. Syst. Biol. 55, 195–207.

Leaché, A.D., Fujita, M.K., Minin, V.N., Bouckaert, R.R., 2014. Species delimitation using genome-wide SNP Data. Syst. Biol. 63, 534–542. doi:10.1093/sysbio/syu018

Lee, W.-J., Conroy, J., Howell, W.H., Kocher, T.D., 1995. Structure and evolution of Teleost mitochondrial control regions. J Mol Evol 41, 54–66.

Martin Del Campo, R. 1940. Los vertebrados de Pátzcuaro. Anales Inst. Biol. 11: 481-492.

Martínez-Palacios C.A., Ríos-Durán M.G., Ambriz-Cervantes L., Jauncey K.J., Ross L.G., 2007 Dietary protein requirement of juvenile Mexican Silverside *Menidia estor* Jordan 1879), a stomachless zooplanktophagous fish. Aquaculture Nutrition, 13: 304–310.

Martínez, R.M., 2015. Análisis filogeográfico del pez blanco *Chirostoma humboldtianum* (Valenciennes, 1835) (Pisces: Atheriniformes).

Mayr, E., 1984 in Evolution of Fish Species Flocks, eds. Echelle, A. A. & Kornfield, I. (Univ. of Maine Press, Orono, ME), pp. 3–11

Mercado-Silva, N., Lyons, J., Moncayo-Estrada, R., Gesundheit, P., Krabbenhoft, T.J., Powell, D.L., Piller, K.R., 2015. Stable isotope evidence for trophic overlap of sympatric Mexican Lake Chapala silversides (Teleostei: Atherinopsidae: *Chirostoma* spp.). Neotrop. Ichthyol. 00–00. doi:10.1590/1982-0224-20140079

Meyer, B.S., Matschiner, M., Salzburger, W., 2015. Molecular Phylogenetics and Evolution A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. Mol. Phylogenet. Evol. 83, 56–71. doi:10.1016/j.ympev.2014.10.009

Miller, R. R., Minckley, W. L., Norris, S. M., 2005. Freshwater fishes of Mexico. The University of Chicago Press, Chicago.

Moncayo-Estrada, R., Israde-Alcántara, I., Garduño-Monroy V. H., 2001. La cherehuita *Hubbsina Turneri* De Buen (1941) (Pisces: Goodeidae). Origen, distribución y uso de la regionalización de la cuenca del Lerma. Hidrobiológica 11(1): 1-18.

Moncayo-Estrada, R., Escalera-Gallardo, C., Lind, O.T., 2011. Spatial patterns of zooplanktivore *Chirostoma* species (Atherinopsidae) during water-level fluctuation in the shallow tropical Lake Chapala, Mexico: Seasonal and interannual analysis. Neotrop. Ichthyol. 9, 815–824. doi:10.1590/S1679-62252011000400012

Navarrete-Salgado N.A. and Contreras-Rivero G. 2011.Crecimiento y mortalidad del charal (*Menidia jordani*) en el estanque Guillermo Lagunes (gl), Estado de México. Revista Chapingo. Serie Ciencias Forestales y del Ambiente, 17: 407-410.

Niemiller, M.L., Near, T.J., Fitzpatrick, B.M., 2012. Delimiting species using multilocus data: Diagnosing cryptic diversity in the southern cavefish, typhlichthys subterraneus (teleostei: Amblyopsidae). Evolution (N. Y). 66, 846–866. doi:10.1111/j.1558-5646.2011.01480.x

Ornelas-García, C.P., Domínguez-Domínguez, O., Doadrio, I., 2008. Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC Evol. Biol. 8, 340. doi:10.1186/1471-2148-8-340

Ortega, B., V??zquez, G., Caballero, M., Israde, I., Lozano-Garc??a, S., Schaaf, P., Torres, E., 2010. Late Pleistocene: Holocene record of environmental changes in Lake Zirahuen, Central Mexico. J. Paleolimnol. 44, 745–760. doi:10.1007/s10933-010-9449-x

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., Stice, L., Grabosky, G., Stice, L., 1991. Simple fool's guide to PCR, version 2. Honolulu, HI: Department of Zoology, University of Hawaii.

Paulo-Maya J., Figueroa L.G., Soria-Barreto M., 2000 Peces dulceacuícolas mexicanos XIX *Chirostoma humboldtianum* Atheriniformes: Atherinopsidae) ENCB- IPN, Zoología Informa, 43: 59-74.

Perdices, A., Bermingham, E., Montilla, A., Doadrio, I., 2002. Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. Molecular Phylogenetics and Evolution, 25, 172–189. https://doi.org/10.1016/S1055-7903 (02) 00224-5

Pedraza-Lara, C., Doadrio, I., Breinholt, J.W., Crandall, K. a., 2012. Phylogeny and Evolutionary Patterns in the Dwarf Crayfish Subfamily (Decapoda: Cambarellinae). PLoS One 7. doi:10.1371/journal.pone.0048233

Pérez-Miranda, F., Mejía, O., Soto-Galera, E., Espinosa-Pérez, H., Piálek, L., Říčan, O., 2017. Phylogeny and species diversity of the genus *Herichthys* (Teleostei: Cichlidae). J. Zool. Syst. Evol. Res. doi:10.1111/jzs.12197

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Doadrio, I., Cuevas-García, E., Pérez-Ponce de León, G., 2015. Comparative historical biogeography of three groups of Nearctic freshwater fishes across central Mexico. J. Fish Biol. 86, 993–1015. doi:10.1111/jfb.12611

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Mar-Silva, A.F., Doadrio, I., Pérez-Ponce de León, G., 2016. The historical biogeography of the southern group of the sucker genus *Moxostoma* (Teleostei: Catostomidae) and the colonization of central Mexico. Zool. J. Linn. Soc. 177, 633–647. doi:10.1111/zoj.12383

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Pérez Ponce de León, G., Doadrio, I., 2009. Phylogenetic relationships and biogeography of the genus *Algansea* Girard (Cypriniformes: Cyprinidae) of central Mexico inferred from molecular data. BMC Evol. Biol. 9, 223. doi:10.1186/1471-2148-9-223

Piller, K.R., Kenway, C.S., Kenway-Lynch, C.S., Camak, D.T., Domínguez-Domínguez, O., 2015. Phylogeography and Population Structure of the Imperiled Redtail Splitfin (Goodeidae: Xenotoca eiseni): Implications for Conservation. Copeia 103, 440–454. doi:10.1643/CI-14-067

Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–6. doi:10.1093/molbev/msn083

Rambaut, A., & Drummond, A. (2007). Tracer v1.5. Retrieved from http://tree.bio.ed.ac.uk/software/tracer/

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Hohna, S., ... Huelsenbeck, P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542. https://doi.org/10.1093/sysbio/sys029

Rosenblum, E.B., Parent, C.E., Brandt, E.E., 2014. The Molecular Basis of Phenotypic Convergence. Annu. Rev. Ecol. Evol. Syst 45, 203–26. doi:10.1146/annurev-ecolsys-120213-091851

Schluter, D., Conte, G.L., 2009. Genetics and ecological speciation. PNAS 106, 9955–9962. doi:DOI/10.1073/PNAS.0901264106

Seehausen O., 2004 Hybridization and adaptive radiation. Trends in Ecology & Evolution, 19, 198–207.

Seehausen, O., 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Mol. Ecol. 22, 787–798. http://dx.doi.org/10.1111/mec.12023

Seehausen, O., Wagner, C.E., 2014. Speciation in Freshwater Fishes. Annu. Rev. Ecol. Evol. Syst 45, 621–51. doi:10.1146/annurev-ecolsys-120213-091818

Soria Barreto, Miriam, Paulo Maya, J., 2005. Morfometría comparada del aparato mandibular en especies de *Chirostoma* (Atheriniformes : Atherinopsidae) del Lago de Pátzcuaro , Michoacán , México. Hidrobiologica 15, 161–168.

Silvestro, D., Michalak, I., 2012. raxmlGUI: A graphical front-end for RAxML. Organisms Diversity & Evolution, 12, 335–337. https://doi. org/10.1007/s13127-011-0056-0

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology, 57, 758–771. https://doi.org/10.1080/10635150802429642

Tarvin, R.D., Powell, E.A., Santos, J.C., Ron, S.R., Cannatella, D.C., 2017. The birth of aposematism: High phenotypic divergence and low genetic diversity in a young clade of poison frogs. Mol. Phylogenet. Evol. 109, 283–295. doi:10.1016/j.ympev.2016.12.035

Thompson, J. D., T. J. Gibson, F., Plewniak, F., Jeanmougin y D. G., Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876-4882.

Unmack, P.J., Allen, G.R., Johnson, J.B., 2013. Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. Mol. Phylogenet. Evol. 67, 15–27. doi:10.1016/j.ympev.2012.12.019

Vital-Rodríguez, B.E., Ramírez-Herrejón, J.P., Moncayo-Estrada, R., Caraveo-Patiño, J., Domínguez-Domínguez, O., 2017. Feeding strategy of silverside species in eutrophic Lake Pátzcuaro, Mexico. J. Appl. Ichthyol. 33, 93–101. doi:10.1111/jai.13248

Wagner, C.E., Keller, I., Wittwer, S., Selz, O.M., Mwaiko, S., Greuter, L., Sivasundar, A., Seehausen, O., 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Mol. Ecol. 22, 787–798. doi:10.1111/mec.12023

Xia, X., 2013. DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. Mol. Biol. Evol. 30, 1720–1728. doi:10.1093/molbev/mst064

Xie, W., Lewis, P.O., Fan, Y., Kuo, L., Chen, M., 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst. Biol. 60, 150–160.

Yang, Z., Rannala, B., 2012. Molecular phylogenetics: principles and practice. Nat. Rev. Genet. 13, 303–14. doi:10.1038/nrg3186

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. PNAS 107, 9264–9269. doi:10.1073/pnas.0913022107

Zamudio, K.R., Bell, R.C., Mason, N.A., 2016. Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. PNAS 113, 8041–8048. doi:10.1073/pnas.1602237113

Tables

Tabla 1. Sampling localities and sequences information for the three molecular markers *cytb*, *D-loop* and *S7* intron.

Locality	Biogeographic region	Taxon	*Cytb* Sequences	D-*loop* Sequences	*S7* Sequences
Zirahuen Lake	Zirahuen	*C. estor*	14	12	4
Patzcuaro Lake	Patzcuaro	*C. estor*	17	15	7
	Patzcuaro	*C. grandocule*	10	10	10
	Patzcuaro	*C. patzcuaro*	5	5	7
	Patzcuaro	*Chirostoma* sp.	8	11	0
Chapala Lake	Chapala	*C. sphyraena*	44	33	8
	Chapala	*C. lucius*	15	15	10
	Chapala	*C. promelas*	8	9	5
	Chapala	*C. consocium*	10	10	10
	Chapala	*C. chapala*	5	5	5
	Chapala	*Chirostoma* sp.	18	12	7
Zacapu Lake	Zacapu	*C. humboldtianum*	8	1	6
Santiaguito Maxda dam	Alto Lerma	*C. humboldtianum*	4	4	5
San Juanico dam	San Juanico	*C. humboldtianum*	2	2	2
Garabato dam	Bajo Lerma	*C. consocium*	1	0	0
Negritos Lake	Chapala	*C. lucius*	3	3	2
San Pedro Lagunillas dam	San Pedro Lagunillas	^*C. humboldtianum*	4	3	4
Juanacatlán dam	Alto Lerma	*C. humboldtianum*	5	3	4
Tepuxtepec dam	Alto Lerma	*C. humboldtianum*	9	2	7
Tiacaque dam	Alto Lerma	*C. humboldtianum*	4	1	4
Villa Victoria dam	Balsas	*C. humboldtianum*	3	3	2
Atenango dam	Tecololutla	*Chirostoma* sp.	3	5	3
Cointzio dam	Cuitzeo	*C. estor*	4	5	0
Total			**204**	**176**	**113**

[1] Discrete regions proposed for Domínguez- Domínguez *et al.* 2010. Based in goodeids endemism.

^Samples give for the PExPA (UAM-I)

Table 2. Sequences obtained for *C. humboldtianum* species group retrieve from GenBank database.

Taxon	Accession number	Locality	Sequences
[1]*"Citocrome b"*			
C. *grandocule*	KC736369	Patzcuaro Lake	1
C. estor	KJ921739	Patzcuaro Lake	1
C. patzcuaro	JQ282029	Patzcuaro Lake	1
C. chapala	KC736397	Chapala Lake	1
C. consocium	KC736401	Chapala Lake	1
C. consocium	JQ282025	Chapala Lake	1
C. humboldtianum	KC736402	San Pedro Lagunillas lake	1
C. promelas	KC736368	Chapala Lake	1
C. humboldtianum	JQ282026	Zacapu Lake	1
C. sphyraena	KC736400	Chapala Lake	1
Total			**10**
[2]*"D-loop"*			
C. humboldtianum-SP	KF652026-KF652041	San Pedro Lagunillas Lake	15
C. humboldtianum-SJ	KF652014-KF652025	San Juanico dam	11
C. humboldtianum-Z	KF652006-KF652013	Zacapu Lake	7
C. humboldtianum-Tx	KF651996-KF652005	Tepuxtepec dam	7
C. humboldtianum-VV	KF651988-KF651995	Villa Victoria dam	7
C. humboldtianum-T	KF651987	Tiacaque dam	1
Total			**48**

[1] Reference: Bloom *et al.* 2013

[2] Reference: García-Martínez *et al.* 2014

Table 3. Marginal Likelihood estimates (ML) and empirical results for Bayes Factor value (2lnBf) species delimitation in the *Chirostoma humbodtianum* species group.

Model	Stepping Stone		Path Sampling	
	MLE	**2lnBF**	**MLE**	**2lnBF**
4 species	-875.17	NA	875.18	NA
5 species	-875.46	0.60	-875.47	0.57

Table 4. Analysis of Molecular Variance (AMOVA) in *Chirostoma humboldtianum* species group

Arrangement	Φct	Φst	Φsc	% of variation among groups	% of variation among population within groups	% of variation within populations
Cytb gene						
according to morphological species	0.32*	0.71*	0.57*	31.82	39.52	28.65
according to *cytb* hypothesis	0.72*	0.76*	0.13*	72.80	3.74	23.45
according to *D-loop* hypothesis	0.72*	0.75*	0.13*	72.12	3.65	24.23
according to S7 hypothesis	0.50*	0.81*	0.62*	50.19	31.12	18.6
according to discrete region basin	0.30 ^{ns}	0.66*	0.51*	30.86	35.14	34.00
D-loop no coding gene						
according to morphological species	0.29*	0.58*	0.41*	29.10	29.64	41.25
according to *cytb* hypothesis	0.48*	0.60*	0.22*	48.35	11.30	40.35
according to *D-loop* hypothesis	0.52*	0.59*	0.14*	52.18	6.66	41.15
according to S7 hypothesis	0.26 ^{ns}	0.63*	0.50*	26.73	37.00	36.26
according to discrete region basin	0.37*	0.53*	0.25 ^{ns}	36.92	16.27	46.81
S7 no coding gene						
according to morphological species	0.30 ^{ns}	0.45*	0.21*	30.75	14.84	54.41
according to *cytb* hypothesis	0.50*	0.53*	0.69*	49.68	3.45	46.87
according to *D-loop* hypotesis	0.50*	0.53*	0.045^{ns}	50.43	2.27	47.31
according to S7 hypothesis	0.75^{ns}	0.78*	0.13*	75.49	3.26	21.24
Grouped according to discrete region basin	-0.77 ^{ns}	0.43*	0.68*	-76.76	120.17	56.59

*_P_ value<0.01; **_P_ value<0.05; ns no significance.

Table 5. Genetic differentiation (Φst pairwaise) between the major lineages and intra lacustrine genetic population differentiation using the *D-loop* marker.

Φst pairwaise between the major lineages	Lerma-Chapala lineage	Zacapu lineage	Patzcuaro lineage
Zacapu lineage	0.498**	0	
Patzcuaro lineage	0.587**	0.561**	0
Zirahuen lineage	0.585**	0.555**	0.476**
Intra-lacustine differentiation			
Chapala Lake	Sub-group B1	Sub-group B2	Sub-group B3
Sub-group B2	0.721*	0	
Sub-group B3	0.784**	0.897ns	0
Sub-group B4	0.458**	0.583**	0.447*
Patzcuaro Lake	Sub-group D1		
Sub-group D2	0.303**	0	

*Significance value *P*>0.05

Figures

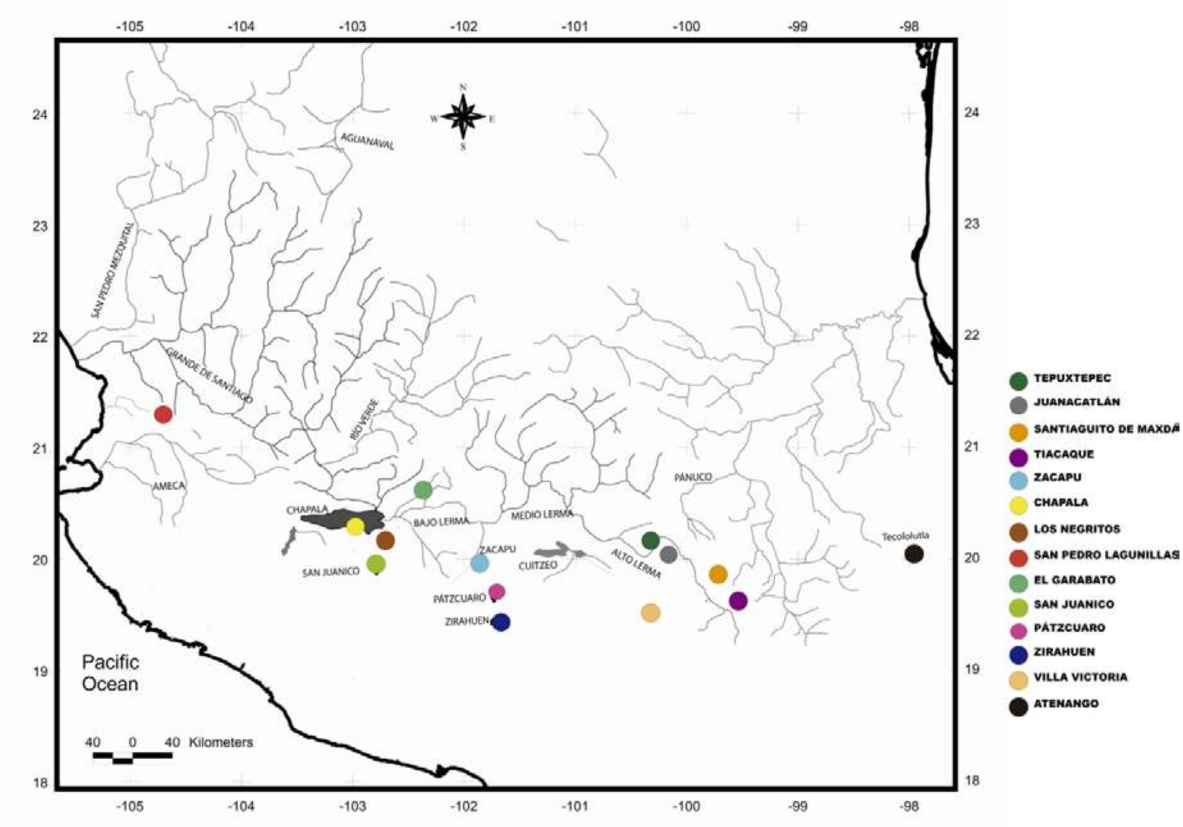


Figure 1. Sample location for *Chirostoma* species in central Mexico. Color circles represents the discrete regions propose by Domínguez-Domínguez et al., (2006, 2010).

Figure 2. Phylogenetic relationship between *C. humboldtianum* species group using a concatenated mitochondrial data set (*cytb* and *D-loop*). The posterior probability is represented by number on node. The branch color represent each samples locality.

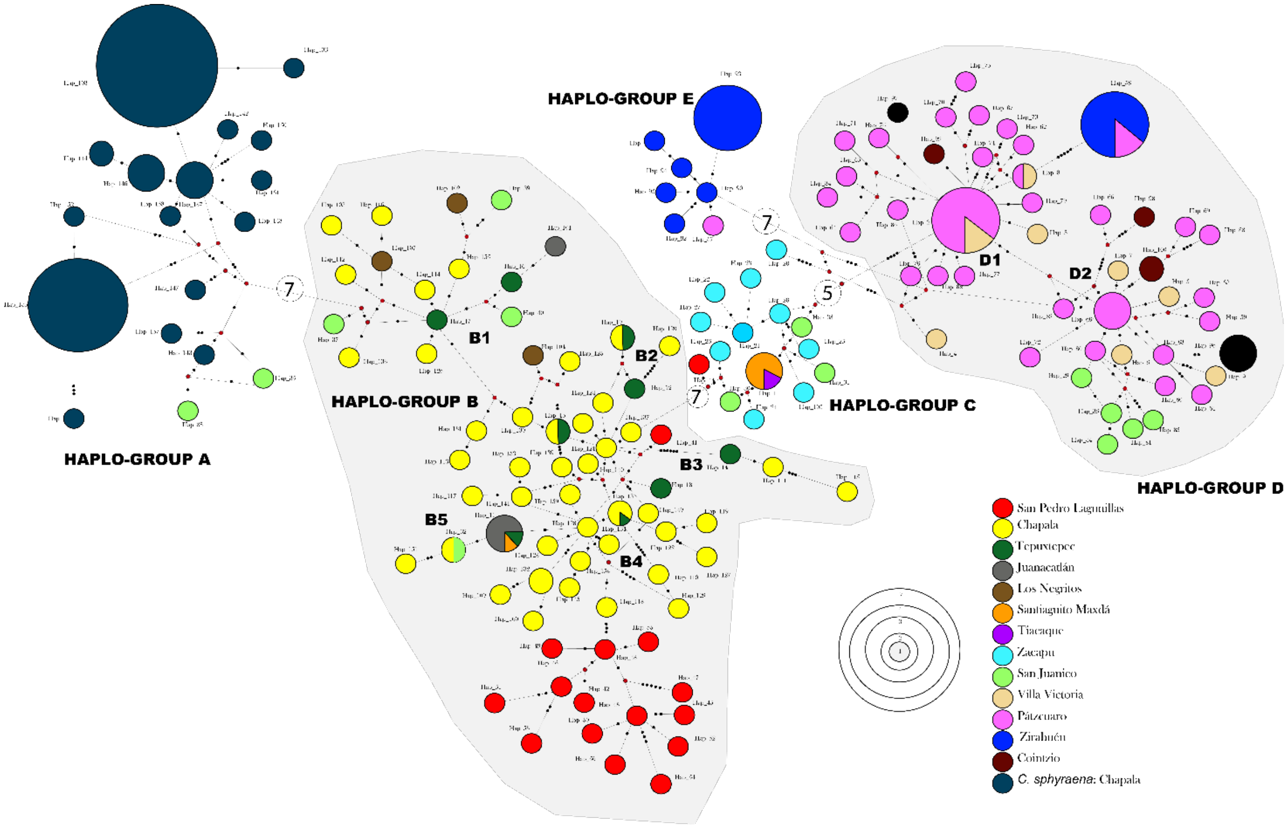


Figure 3. Haplotype network based on *D-loop* no coding gene. The major haplogrups are represented by A-E and intra-lacustrine groups is represented by subfije 1-5. Circle represent the haplotype and the size of circle are proportional to frequency. The color indicates the locality as indicate in the asociated legend. The small black point are the mutational steps. Finally, the small red point represent the median vectors.

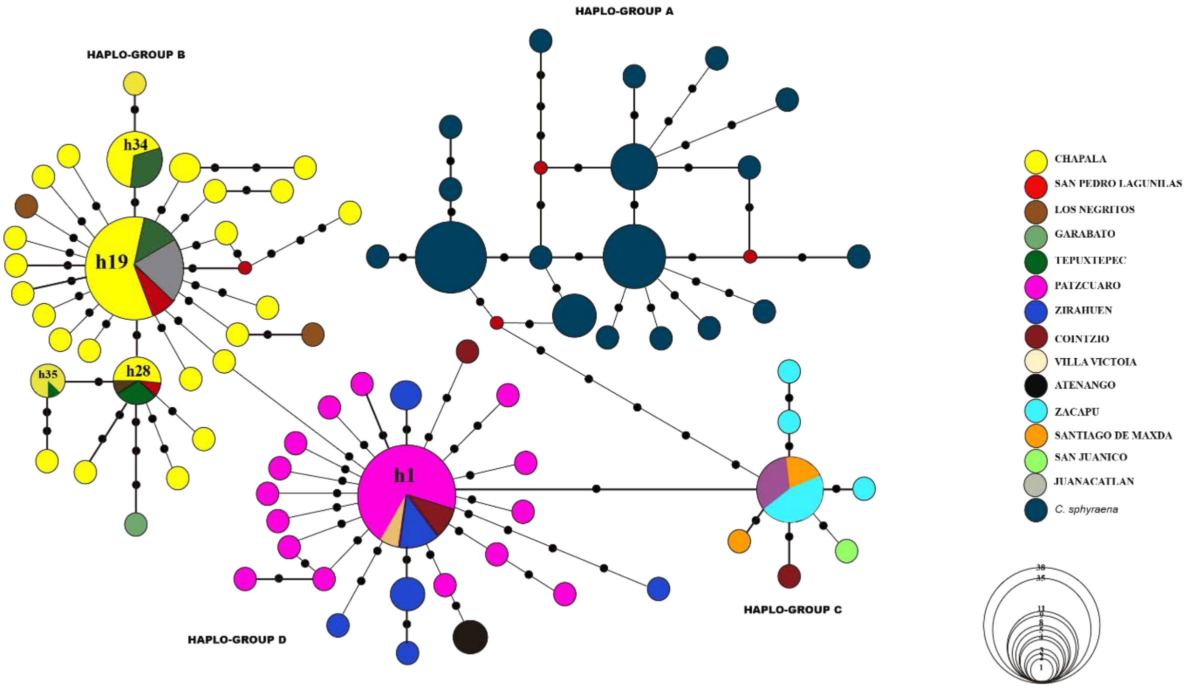


Figure 4. Haplotype network based on *cytb* gene. The major haplo-groups are represented by A-D. Circle represent unique haplotype and the size of the circle are proportional to frequency, Number black into the some haplotype represented number of haplotype. The color indicates the locality as indicate in the associated legend. The small black points are the mutational steps. Finally, the small point Red represent the median vectors.

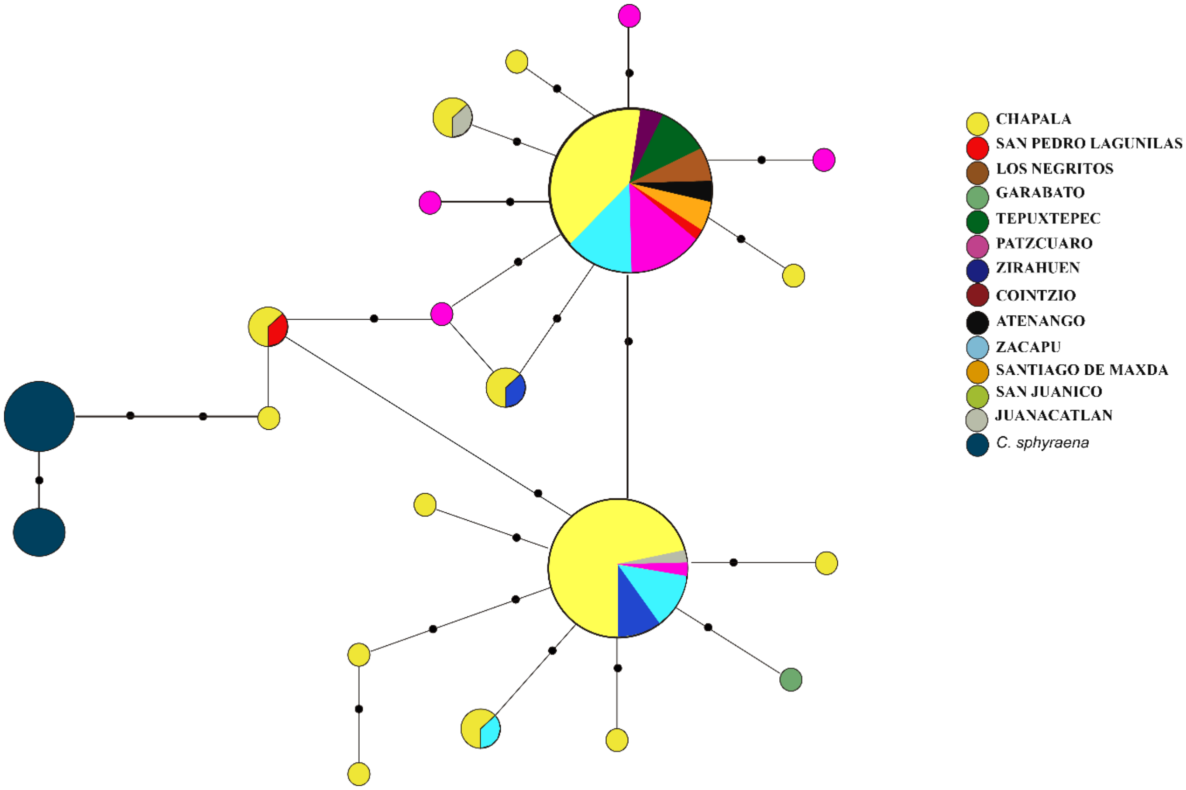


Figure 5. Haplotype network based on *first intron of S7 ribosomal protein* gene. Circle represent the haplotype and the size of circles are proportional to the frequency. The color indicate the locality, as indicates in the associated legend. The small black points are the mutational steps.

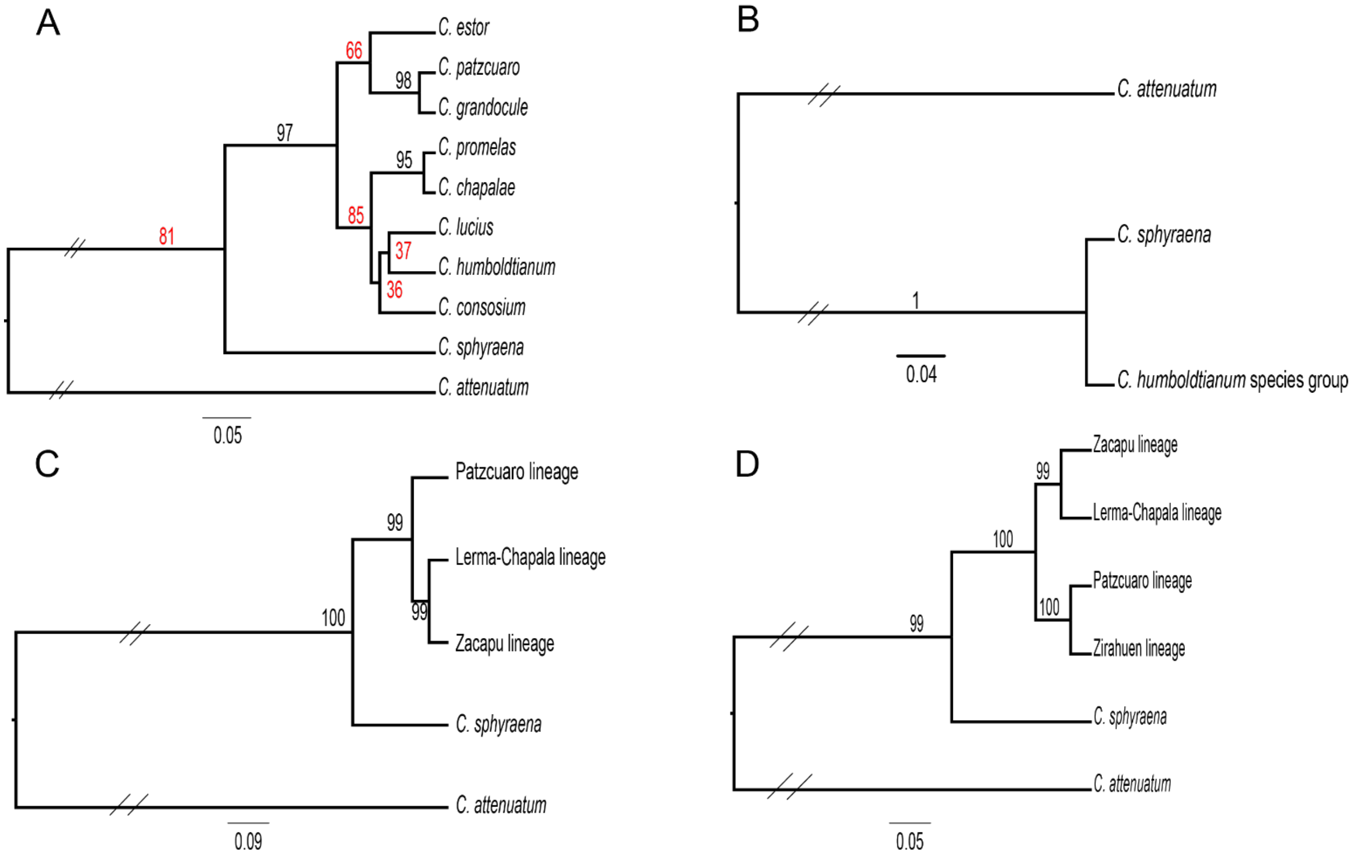


Figure 6. Species tree based in: A) the morphologic species arragement, and the haplogroups found for B) *S7*, C) *cytb* and D) *D-loop* locus in *Chirostoma humboldtianum* species group. The number above branches represent the length posterior probability. The red number were value with low support value (<95%).

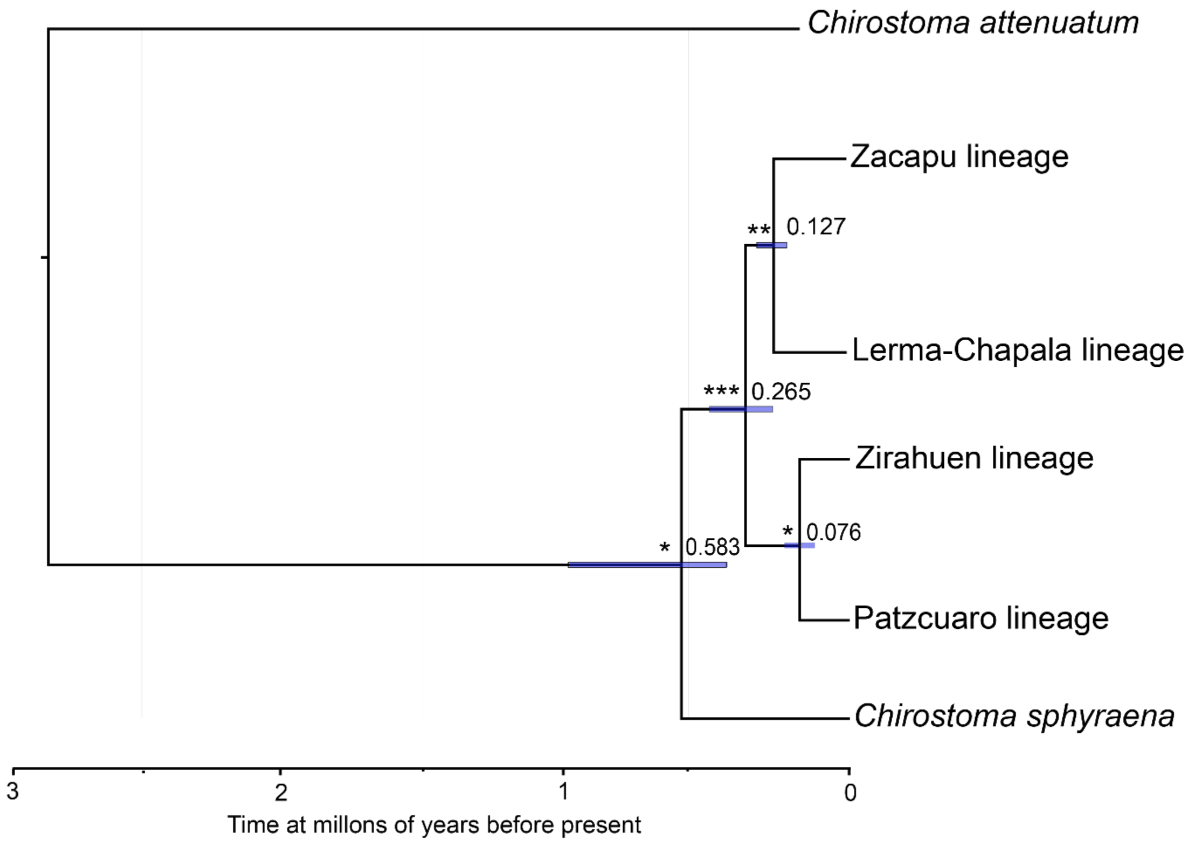
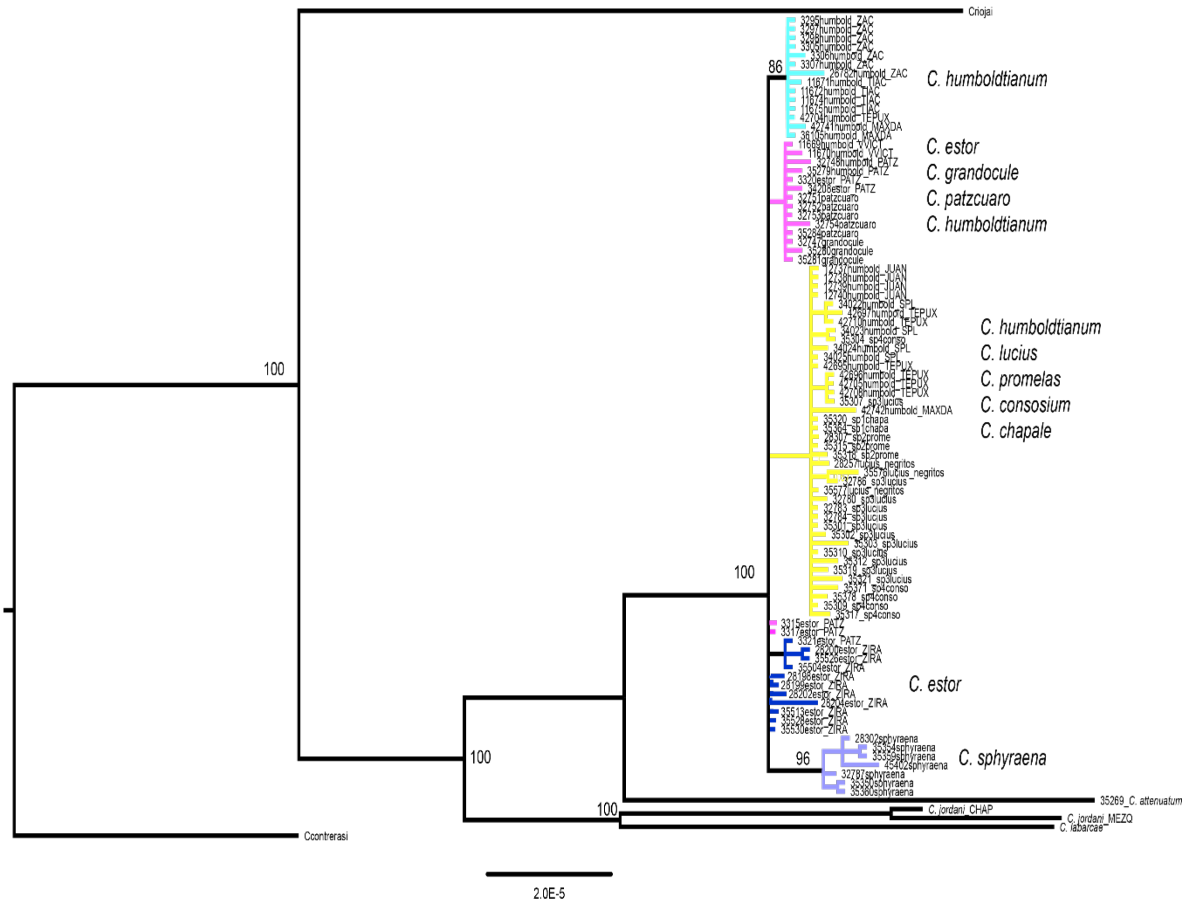
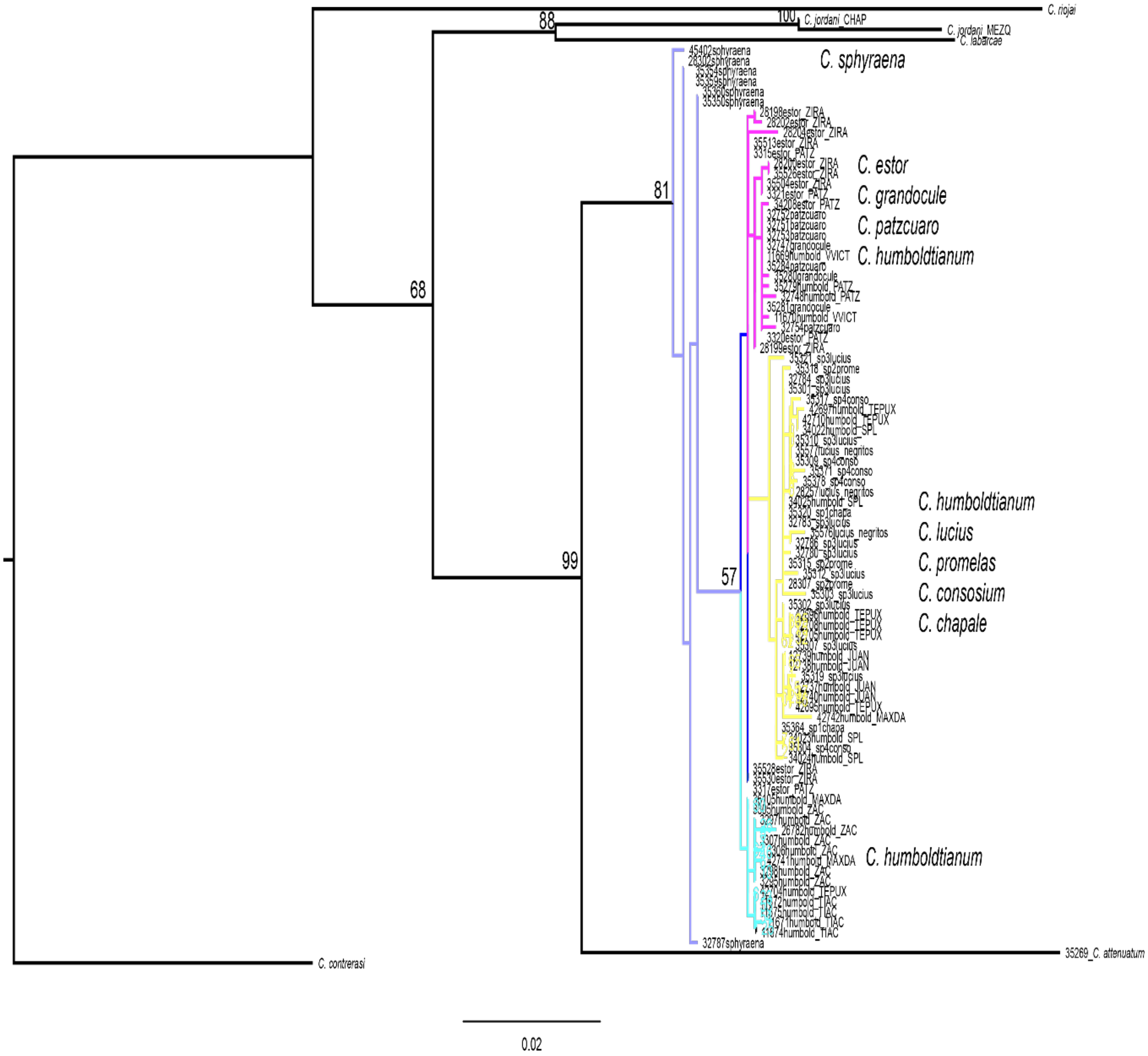


Figure 7. Divergence time estimated in *BEAST for the major lineages of *Chirostoma humboldtianum* species group, based on species three assays with five arrangement. The number on the nodes are the mean age at millions years. The bars represented the high posterior density (HDP 95 %). The Bayesian posterior probabilities are represented by * (* = 100 %; ** = 99 % and *** = 96 %).

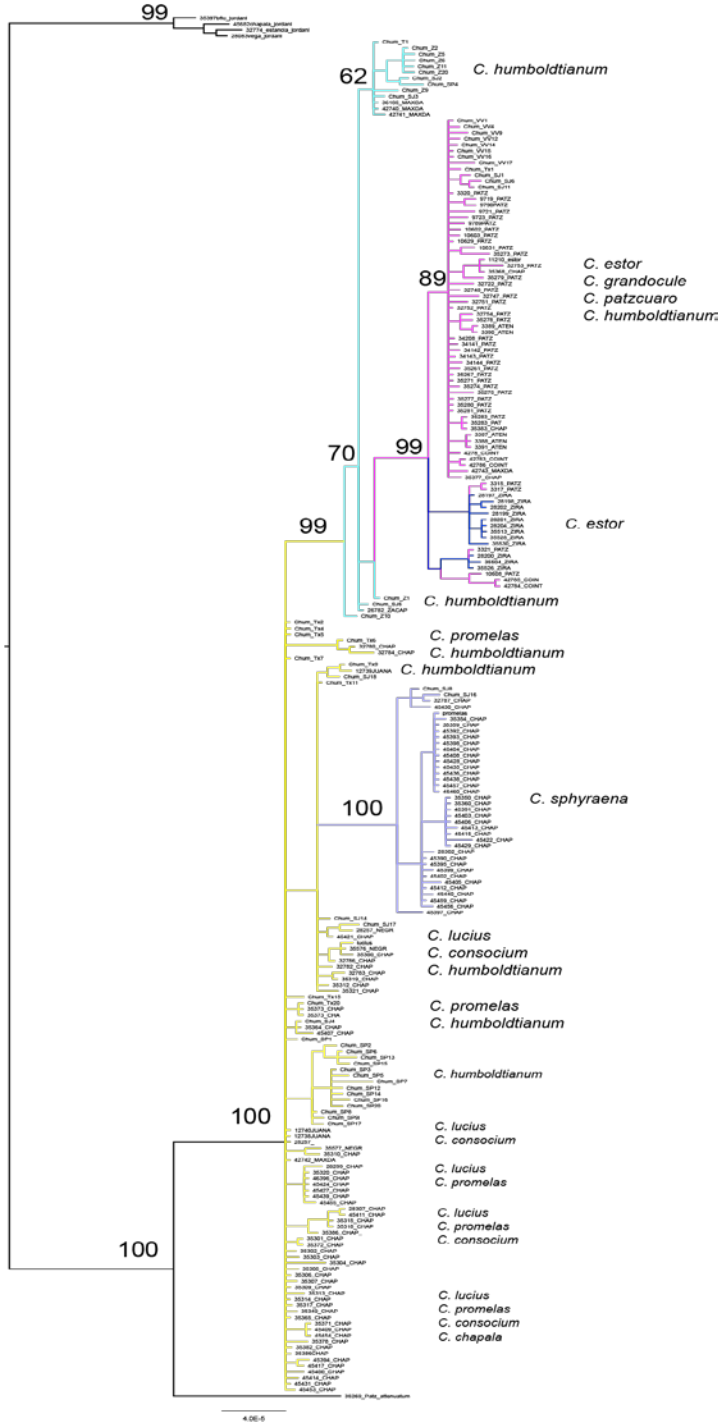
Supplementary information



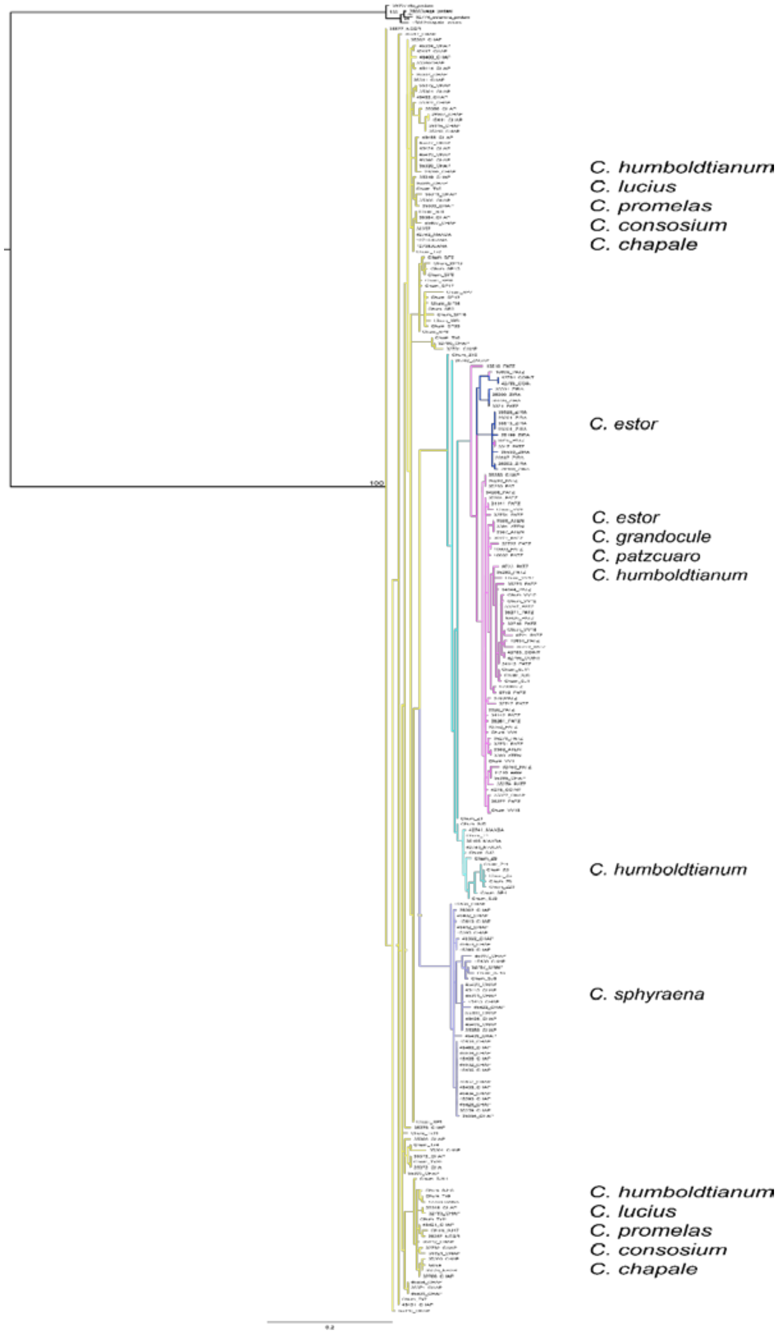
Supplementary Fig. 1. Phylogenetic tree based on the *cytochrome b* gene of *Chirostoma humboldtianum* species group. The assays was performed by Bayesian inference. The number on nodes represent the posterior probability.



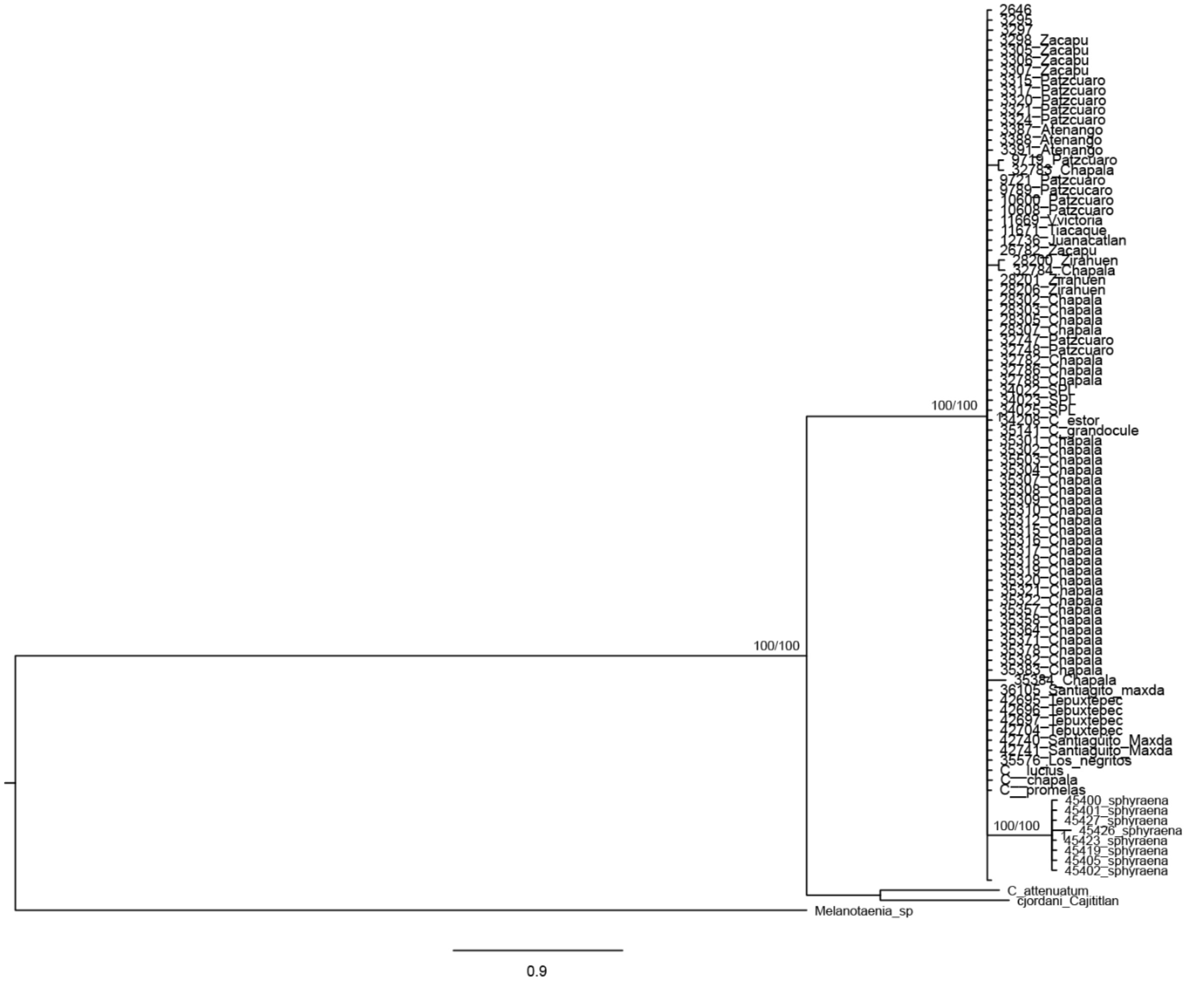
Supplementary Fig. 2. Phylogenetic tree based on the *cytochrome b* gene of *Chirostoma humboldtianum* species group. The assays was performed by Maximum Likelihood. The number on nodes represent the bootstrap value.



Supplementary Fig. 3. Phylogenetic tree based on the *D-loop* no coding gene of *Chirostoma humboldtianum* species group. The assays was performed by Bayesian inference. The number on nodes represent the posterior probability.



Supplementary Fig. 4. Phylogenetic tree based on the *D-loop* no coding gene of *Chirostoma humboldtianum* species group. The assays was performed by Maximum Likelihood. The number on nodes represent the bootstrap value.



Supplementary Fig. 5. Phylogenetic tree based on the *first intron S7 ribosomal protein* gene of *Chirostoma humboldtianum* species group. The assays was performed both Maximum Likelihood and Bayesian Inference. The number on nodes represent the bootstrap value and posterior probability, respectively.

4. Discusión general

La historia evolutiva de las especies del grupo *Chirsotoma* adaptados a los lagos del centro de México es compleja. Sin embargo, en gran medida concuerda con la mayoría de los estudios evolutivos desarrollados en peces del centro de México, donde se menciona a los eventos geológicos y climáticos como el motor de la radiación evolutiva de los diferentes grupos estudiados (Corona-Santiago et al., 2015; Doadrio and Domínguez, 2004; Domínguez-Domínguez et al., 2008; Pérez-Rodríguez et al., 2009). Sin embargo, otros procesos evolutivos están ocurriendo posterior al aislamiento geográfico, lo que ha permitido la diferenciación genética entre y dentro de las poblaciones.

4.1 Implicaciones taxonómicas y delimitación de especies

Los estudios aquí realizados, basados en el marco contextual, filosófico y metodológico de dos aproximaciones como son la filogenética y la filogeográfica, permiten dilucidar aspectos controversiales en la sistemática y taxonomía de las especies actualmente reconocidas, así como la distribución geográfica de los linajes genéticos. El caso del charal prieto *Chirostoma attenuatum*, los resultados de estudios genéticos basados en secuencias de ADNmt y ADNn permitieron discernir y apoyar la hipótesis morfológica de la separación de dos entidades taxonómicas propuestas (Meek, 1904; Barbour, 1973; Barriga-Sosa, 2002). Sin embargo, la evidencia genética otorga los elementos necesarios para considerarse a las dos subespecies previamente descritas como dos entidades taxonómicas, con su propia trayectoria evolutiva. Por el contrario, dentro de las especies del grupo *Chirostoma humboldtianum,* la evidencia genética no se correlaciona con la descripción morfológica. En este sentido, a la luz de la investigación aquí realizada, ahora se tienen dos hipótesis contrastantes, por un lado la morfológica, previa a esta investigación y por otro lado la molecular, resultado de esta investigación. Esta última soporta cinco de las nueve especies reconocidas dentro del grupo. En ambos casos se demuestra que la taxonomía actual de las especies estudiadas tiene que ser revisada.

Los caracteres morfológicos y merísticos utilizados en la descripción de las especies pueden estar sujetos a evolución convergente, o bien estar siendo afectados por diversas presiones selectivas (Yang y Rannala, 2010). Sin embargo, lo mismo podría ocurrir con los marcadores moleculares, por lo tanto, la elección de marcadores moleculares debe regirse bajo ciertos criterios, como el principio de ortología, libres de recombinación y que cumplan el supuesto de una evolución neutral (Li, 1997). La delimitación de la diversidad biológica a nivel de especie es una tarea importante en los estudios de sistemática y evolución, el no tener un entendimiento claro, puede fungir como una barrera para dilucidar las relaciones filogenéticas, y por lo tanto la historia evolutiva es incierta. Además, la incorporación de diferentes metodologías, ayuda a esclarecer los límites a nivel específico de la diversidad biológica, que son indispensables para proponer hipótesis más robustas (Carstens et al., 2013).

Los datos genéticos aquí analizados fueron útiles para proponer evidencia a favor de reconocer la identidad taxonómica de *C. attenuatum* y *C. zirahuen* cada una con una historia demográfica y evolutiva independiente. Además, las metodologías de delimitación de especie son una herramienta practica para poner a prueba diversos escenarios posibles de especiación, como lo fue en el caso del grupo de especies de *Chirostoma humboldtianum*, donde se pusieron a prueba 4 escenarios. Finalmente, con los resultados obtenidos basados en marcadores moleculares la actual taxonomía no se soporta, debido a que existe una mezcla genética entre las especies reconocidas. Sin embargo, existe una segregación genética que se correlaciona con la geografía, por lo tanto existen nuevas implicaciones de especiación, biogeográficas y evolutivas en las especies de *Chirostoma* adaptadas a hábitats lacustres.

4.2 Historia evolutiva e implicaciones biogeográficas.

La historia evolutiva y biogeográfica de los peces dulceacuícolas del centro de México está principalmente asociada con eventos geológicos y el cambio climático ocurridos en el centro de México (Doadrio y Domínguez, 2004; Dominguez-Dominguez et al., 2006,; Pérez-Rodríguez et al., 2009; Corona-

Santiago et al., 2015; García-Martínez, et al., 2015; Beltrán-López et al., 2017;). El conocimiento existente de aislamiento y reconexión de cuencas hidrológicas ocurridos en el centro de México, permiten proponer hipótesis en la evolución de los peces dulceacuícolas en el centro de México. El caso de las especies filogenéticamente más derivadas de *Chirostoma* adaptadas exclusivamente a hábitats lacustres, el conocimiento histórico de estos procesos permitieron dilucidar los mecanismos de especiación ocurridos en *C. attenuatum* y *C. zirahuen*, concluyendo que los mecanismos que llevaron a la diferenciación tanto morfológica como genética, puede ser explicada mediante procesos de especiación ecológica. Este mecanismo de especiación está asociado a la interacción de los organismos con los factores bióticos y abióticos de su entorno (Orr y Smith, 1998; Doebeli y Dieckmann, 2000; Schluter, 2001).

Los Lagos de Pátzcuaro y Zirahuén tuvieron recurrentes periodos de aislamiento y reconexión en diferentes tiempos durante el Pleistoceno (Israde-Alcántara et al., 2005; Ortega et al., 2010; García-Andrade, 2014; Corona-Santiago et al., 2015;), durante los periodos de reconexión entre ambos lagos, el flujo y entrecruzamiento de fauna fue posible, al encontrar haplotipos compartidos en las poblaciones del goodeino *Alloophorus robustus* (Domínguez-Domínguez, 2008), *Goodea atripinnis* (García-Andrade, 2014; Beltrán-López et al., en preparacion) y en *Allotoca diazi* con *A. meeki* (Corona-Santiago, 2013; Corona-Santiago et al., 2015) . Sin embargo, la respuesta del charal prieto ante estos eventos de aislamiento y reconexión entre los lagos fue contrastante con lo observado en los peces vivíparos. El tiempo transcurrido en el evento de aislamiento geográfico entre *C. attenuatum* y *C. zirahuen*, propició la acumulación de adaptaciones únicas en las poblaciones aisladas, que durante un contacto secundario las poblaciones diferenciadas no tuvieron éxito reproductivo o, si lograron tenerlo, los híbridos resultantes tuvieron baja capacidad de adecuación y no llegaron a ser parte del tamaño efectivo de la población. Los supuestos para considerar un proceso de especiación ecológica sigue tres componentes fundamentales: 1) hay una fuente de selección natural divergente o perjudicial, lo que implica una menor adecuación en los híbridos resultantes debido a diversos factores ecológicos, 2) un mecanismo de aislamiento reproductivo y 3) un contacto entre las dos entidades biológicas (Rundle y Nosil, 2005). La evidencia biológica entre la separación de dos especies en las cuencas aisladas de Pátzcuaro y Zirahuén apoyan la hipótesis de las conexiones en el pasado entre ambos lagos. Sin embargo, estas conexiones no fueron sincrónicas, mostrando diferentes tiempos de divergencia de la fauna compartida entre los lagos de Pátzcuaro y Zirahuén, como es el caso de los Atherinopsidos estudiados, los goodeinos *A. diazi-meeki* (Corona-Santiago et al., 2015), *Alloophorus robustus* (Domínguez-Domínguez, 2008), *Goodea atripinnis* (García-Andrade, 2014; Beltrán-Lopez en preparación) y el decápodo de río *Cambarellus patzcuarensis* (Pedraza-Lara et al., 2012).

En las especies del grupo *humboltianum* los mecanismos de especiación están fuertemente asociados a los eventos vicariantes ocurridos en el centro de México, sin embargo, la variación morfológica en estudios previos encuentran que las diferencias entre las especies estriba en la morfología de la mandíbula, sugiriendo especiación ecológica en simpatría (Barbour, 1973b; Barriga-Sosa, 2002; Barriga-Sosa et al., 2002; 2005). Las diferencias en la morfología de la mandíbula están asociadas con la especialización trófica en especies simpátricas (Terai et al., 2002; Alberson, et al., 2003, 2005; Puebla, 2009;). Por lo tanto la variación genética encontrada dentro de las poblaciones en el interior de los lagos (la segregación genética observada en los lagos de Chapala Pátzcuaro con el ADNmt), está asociada procesos demográficos, y a factores ecológicos, como la especialización trófica (García-de León et al., 2014; Mercado-Silva et al., 2015). A pesar de que los resultados de los trabajos de especialización trófica con las especies simpátricas de los lagos de Chapala y Pátzcuaro no son concluyentes. La evidente diferenciación en la morfología de la mandíbula, sugiere que la especialización trófica funge como el motor de la diferenciación entre los morfotipos (Barbour, 1973b; Barriga-Sosa, et al., 2002, 2005), como occurre en otros peces, en los cuales la diferenciación genética de especies simpátricas y la diferenciación de las estructuras asociadas con la alimentación esta correlacionada (Barluenga et al., 2006; Wagner et al., 2013; Meyer et al., 2015; Burress et al., 2018).

Los eventos cladogenéticos ocurridos en el grupo más derivado de *Chirostoma* que incluyen a la especie *C. spyraena* y el grupo de especies *C. humboldtianum* fue impulsado por la intensa actividad volcánica y a los cambios climáticos ocurridos en el pleistoceno (Barbour, 1973a; Miller et al., 2005; Caballero et al., 2010;). El evento cladogenético que separó a *C. sphyraena* del grupo *Chirostoma humboldtianum* está fechado *c.a* 0.583 M.a., edad que es congruente con estudios previos de diversificación de este grupo (Bloom et al., 2013; Campanella et al., 2015). El escenario biogeográfico y evolutivo inicial es similar a lo reportado para otros grupos de peces del sistema del Lerma en espacio más no en tiempo (Domínguez-Domínguez et al., 2006, 2008; Pérez-Rodríguez et al., 2009, 2015; Corona-Santiago et al., 2015). Los diversos periodos de aislamiento y conexión entre los diferentes sistemas hidrológicos (Barbour, 1973a; Israde-Alcantara y Garduño-Monroy, 1999; Moncayo et al., 2001; Miller et al., 2005; Domínguez-Domínguez et al., 2008) fueron determinantes en la separación de la ictiofauna de la región. Además el aislamiento geográfico de las cuencas hidrológicas y la asociación de estos ateinopsidos a hábitats lacustres, en conjunto propiciaron una diferenciación genética en las especies estudiadas. La propuesta de una conexión en el pleistoceno entre el Lago de Pátzcuaro con Cuitzeo a través del corredor Tarasco, y de Cuitzeo con Zacapu a través del corredor Chucandiro-Huaniqueo y estos sistemas a su vez con el Lerma ha sido bien documentada (Israde-Alcantara y Garduño-Monroy, 1999; Moncayo-Estrada et al., 2011; Corona-Santiago et al., 2015). Estos corredores posibilitaron las conexiones entre los cuerpos de agua aislados en periodos glaciales, ante estos fenómenos históricos la ictiofauna aislada respondió de dos maneras: 1) las conexiones entre cuerpos de agua coadyuvaron al intercambio de migrantes entre las poblaciones aisladas con alta capacidad de dispersión por lo tanto el flujo génico fue exitoso entre las poblaciones históricamente aisladas, mostrando haplotipos compartidos entre las cuencas hidrográficas y 2) en aquellas especies con baja o nula capacidad de dispersión el intercambio de migrantes fue en bajas proporciones o nulo, permitiendo un aislamiento reproductivo y diferenciación genética entre las poblaciones restringiéndose el flujo genético (Domínguez et al., 2008; Pérez-

Rodríguez et al., 2009). La baja diferenciación y estructura genética encontrada en el *S7* está directamente asociada con las divergencias recientes del grupo (menores a 0.5 M.a). Además, las pérdidas de los polimorfismos es más lenta en el ADNn (Funk y Omland, 2003; Zink y Barrowclough, 2008) y el tiempo de coalescencia se incrementa con tamaños efectivos poblacionales grandes (Hudson y Turnelli, 2003) como es el caso del grupo bajo estudio.

La estructura genética encontrada entre las diferentes regiones hidrológicas en diversos grupos de peces como los goodeinos (Doadrio & Domínguez, 2004, 2008; Corona-Santiago et al., 2015; Piller et al., 2015; Beltrán-López et al., 2017), ciprinidos (Pérez-Rodríguez et al., 2009, 2015) y en el catostomido *Moxostoma austrinum* (Pérez-Rodríguez, et al., 2016), está asociada a barreras geográficas existentes, la estructura genética se evidencia mayormente en aquellas especies adaptadas exclusivamente a hábitats lacustres como algunos miembros del género *Algansea* (Pérez-Rodríguez et al., 2009). Por lo tanto la intensa actividad volcánica, los periodos de cambio climático ocurridos en el pleistoceno aunado con la asociación de las especies a hábitats lacustres son factores importantes para entender la biogeografía histórica y evolución del grupo más derivado de *Chirostoma*.

4.3 Implicaciones de conservación

Los estudios de genética en peces son importantes para proponer medidas de conservación. México cuenta con alrededor de 500 especies de peces de agua dulce de las cuales el 60 % de ellas son consideradas como especies nativas (Miller et al., 2005). La extinción de ciertas especies puede deberse a dos factores: 1) Impactos mediados por el hombre (contaminación, fragmentación de hábitats, introducción de especies exóticas y sobre explotación) y, 2) eventos estocásticos ocurridos cuando el tamaño efectivo poblacional es pequeño (Shaffer, 1981). La evidencia genética demuestra que *C. attenuatum* y *C. zirahuen*, son un grupo recíprocamente monofiletico, y pueden ser considerados como linajes evolutivos independientes con historias demográficas distintas, estos procesos evolutivos deben ser prioritarios para la conservación (Keith et al., 2010).

En el grupo de especies de *Chirostoma humboldtianum* también se presenta linajes evolutivos con estructura genética, lo que debe ser tomado en cuenta para plantear medidas de conservación. Además, estas especies son de gran importancia ecológica, primero porque son peces endémicos del centro de México, y parte de la cadena trófica manteniendo la estabilidad en los ecosistemas donde se encuentran (Moncayo-Estrada et al., 2011; Vital-Rodríguez, et al., 2017). También tienen una gran importancia cultural, ya que han sido parte de los recursos que las etnias P´urepechas, nahuas y otomíes, siendo las pesquerías autóctonas más antiguas en el centro de México, considerados como el manjar de los dioses, utilizados como ofrendas por las culturas prehispánicas (Alaye, 1993b; Hernández, 2006). A pesar de su importancia, abundancia y diversidad las especies están amenazadas por diversos factores, principalmente por la pérdida y contaminación de hábitat, introducción de especies exóticas y sobre-explotación pesquera (Ram et al., 2008; Hernández-Batista et al., 2015) . Sin embargo, no se considera a estas especies en ninguna categoría de riesgo por las normas mexicanas, con la excepción de *Chirostoma promelas* que se encuentra enlistada en algún estatus de riesgo por la normatividad mexicana (NOM-ECOL-059-94). La pérdida y contaminación de hábitat, la introducción de especies exóticas y la sobrepesca podrían ser un factor determinante en la existencia de la especie (Rosas-Moreno, 1976; Chacón-Torres, 1993; Rojas-Carrillo, 2006) Por lo tanto, es necesario la implementación de un manejo efectivo de conservación que incluya la conservación de los sistemas lacustres.

5. Conclusiones generales

- En la historia evolutiva del grupo filogenéticamente más derivado de *Chirostoma,* que está confinado exclusivamente a hábitats lacustres los eventos geológicos y climáticos han modelado la historia evolutiva de los diferentes grupos. Sin embargo, posterior al aislamiento geográfico, la especiación ecológica ha tenido un papel muy importante en la separación de los linajes genéticos y no se descarta que esté ocurriendo la especiación simpátrica en los lagos de Chapala y Pátzcuaro.

- Los estudios filogenéticos y la filogeográficos permitieron dilucidar aspectos controversiales en la sistemática y taxonomía de las especies analizadas, las cuales deben ser revisadas, aportando nuevas directrices acerca de las inferencias biogeográficas y evolutivas dentro del grupo de estudio.

- La alta variación de los polimorfismos morfológicos descritos para las especies, pero no sustentados por los datos genéticos, evidencian a un grupo con una gran plasticidad fenotípica que están fuertemente asociados con presiones selectivas de los ambientes donde habitan.

- Las especies de *Chirostoma* estudiadas en este trabajo presentan una baja pero significativa estructura genética entre las poblaciones aisladas, con una significativa variación dentro de las poblaciones en el interior de los lagos, por lo que estas especies son un modelo interesante para estudios de la radiación adaptativa, los cuales tendrán que ser abordados con marcadores moleculares que den mayor resolución como los SNPs.

- Los estudios genéticos pueden aportar argumentos tangibles para la conservación de la diversidad biológica. En este grupo de estudio, a pesar de las implicaciones en la sistemática, biogeografía y evolución del grupo, además de la importancia ecológica, cultural y pesquera, no existe un plan de conservación para las especies y debe ser necesario la implementación de un manejo efectivo de conservación que incluya la información genética para llevar un manejo adecuado de las especies y de su hábitat.

6. Referencias

Anderson E. Thompson E., 2002 A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160, 1217–1229.

Alaye-Rahy, N., 1996a. Estudios del polimorfismo de la hemoglobina para identificar especies del género *Chirostoma* del lago de Pátzcuaro, Michoacán, México. Ciencia Pesquera 13: 1-9.

Alaye-Rahy, N., 1993b. El pescado blanco (género *Chirostoma*) del lago de Pátzcuaro, Michoacán. Composición de especies. Ciencia Pesquera 9: 113-128.

Almeida, F.C., DeSalle, R., 2016. Genetic differentiation and adaptive evolution at reproductive loci in incipient *Drosophila* species. J. Evol. Biol. 30, 524–537. doi:10.1111/jeb.13021

Alvarez del Villar, J., 1972. Algunos ejemplos de especiación en peces mexicanos. Ciencia 13:81-898.

Avise, J.C., 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge.

Bandelt, H.J., Forster, P., Röhl, a, 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48. doi:10.1093/oxfordjournals.molbev.a026036

Barbour, C. D., 1973a. A biogeographycal history of *Chirostoma* (Pisces: Atherinidae): A species flock from the Mexican Plateau. Copeia 3: 533-556.

Barbour, D.., 1973b. The Systematics and Evolution of the Genus *Chirostoma* Swainson (Pisces, Atherinidae).

Barbour, C. D., y B. Chernoff., 1984. Comparative morphology and morphometric of the pescados blancos (Genus *Chirostoma*) from the lake Chapala, México.

En: A. A. Echelle e I. Konfield (eds.). Evolution of fish species flocks. University of Maine Press, Toronto. p. 11-126.

Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439, 719–723. doi:10.1038/nature04325

Barriga-Sosa, I., 2001. Variabilidad Morfométrica, Merística y Molecular de especies del género *Chirostoma* (Pisces: Atherinopsidae). Unpublished PhD Thesis, Universidad Aut_onoma Metropolitana-Iztapalapa.

Barriga-Sosa, I., Pérez-Ramírez, M.Y., Soto-Aguirre, F., Castillo-Rivera, M., Arredondo-Figueroa, J.L., 2005. Inter-specific variation of the mitochondrial r16S gene among silversides, “Peces Blancos”, (Atherinopsidae: Menidiinae) and its utilization for species identification. Aquaculture 250, 637–651. doi:10.1016/j.aquaculture.2005.05.003

Barriga-Sosa, I.D.L.A., Eguiarte, L.E., Arredondo-Figueroa, J.L., 2004. Low but Significant Subdivision among Populations of *Chirostoma grandocule* from Lake Patzcuaro, Mexico. Biotropica 36, 85–98. doi:10.1111/j.1744-7429.2004.tb00299.x

Barriga-Sosa, I.D.L.A., Ibáñez-Aguirre, A.L., Arredondo-Figueroa, J.L., 2002. Morphological and genetic variation in seven species of the endangered *Chirostoma* “*humboldtianum* species group” (Atheriniformes: Atherinopsidae). Rev. Biol. Trop. 50, 199–216.

Beltrán-López, R.G., Domínguez-Domínguez, O., Guerrero, J.A., Corona-Santiago, D.K., Mejía-Mojica, H., Doadrio, I., 2017. Phylogeny and taxonomy of the genus *Ilyodon* Eigenmann, 1907 (Teleostei: Goodeidae), based on mitochondrial and nuclear DNA sequences. J. Zool. Syst. Evol. Res. 1907. doi:10.1111/jzs.12175

Bermingham, E., McCafferty, S.S., Martin, A.P., 1997. In: Kocher, T.D., Stepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, San Diego, pp. 113–128

Blair, C., Murphy, R.W., 2011. Recent trends in molecular phylogenetic analysis: where to next? J. Hered. 102, 130–8. doi:10.1093/jhered/esq092

Bloom, D.D., Piller, K.R., Lyons, J., Mercado-Silva, N., Medina-Nava, M., 2009. Systematics and Biogeography of the Silverside Tribe Menidiini (Teleostomi: Atherinopsidae) Based on the Mitochondrial ND2 Gene. Copeia 2009, 408–417. doi:10.1643/CI-07-151

Bloom, D.D., Unmack, P.J., Gosztonyi, A.E., Piller, K.R., Lovejoy, N.R., 2012. It’s a family matter: molecular phylogenetics of Atheriniformes and the polyphyly of the surf silversides (family: Notocheiridae). Mol. Phylogenet. Evol. 62, 1025–30. doi:10.1016/j.ympev.2011.12.006

Bloom, D.D., Weir, J.T., Piller, K.R., Lovejoy, N.R., 2013. Do freshwater fishes diversify faster than marine fishes? A test using state-dependent diversification analyses and molecular phylogenetics of new world silversides (atherinopsidae). Evolution 67, 2040–57. doi:10.1111/evo.12074

Bradbury, J.., 2000. Limnologic history of Lago de Pátzcuaro, Michoacán, México for the past 48, 000 years: impacts of climate and man. Palaeogeogr. Paleoclimatology, Palaeoecol. 163(1-2), 65–69. doi:https://doi.org/10.1016/S0031-0182(00)00146-2

Buckley, D., 2009 Toward an organismal, integrative, and iterative phylogeography. BioEssays 31(7):784–793.

Buj, I., Ćaleta, M., Marčić, Z., Šanda, R., Vukić, J., Mrakovčić, M., 2015. Different histories, different destinies-impact of evolutionary history and population genetic structure on extinction risk of the adriatic spined loaches (genus Cobitis;

Cypriniformes, actinopterygii). PLoS One 10, 1–17. doi:10.1371/journal.pone.0131580

Caballero, M., Lozano-García, S., Vázquez-Selem, L., Ortega, B., 2010. Evidencias de cambio climático y ambiental en registros glaciales y en cuencas lacustres del centro de México durante el último máximo glacial. Bol. la Soc. Geol. Mex. 62, 359–377.

Campanella, D., Hughes, L.C., Unmack, P.J., Bloom, D.D., Piller, K.R., Ortí, G., 2015. Multi-locus fossil-calibrated phylogeny of Atheriniformes (Teleostei, Ovalentaria). Mol. Phylogenet. Evol. 86, 8–23. doi:10.1016/j.ympev.2015.03.001

Carstens, B.C., Pelletier, T. a., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. Mol. Ecol. 22, 4369–4383. doi:10.1111/mec.12413

Caviedes-Solis, I.W., Nieto-Montes de Oca, A., 2018. A multilocus phylogeny of the genus *Sarcohyla* (Anura: Hylidae), and an investigation of species boundaries using statistical species delimitation. Mol. Phylogenet. Evol. 118, 184–193. doi:10.1016/j.ympev.2017.09.010

Chernoff, B., 1996. Phylogenetic relationships. Zool. J. Linn. Soc. 117, 1–69.

Clark, P.U., Dyke, a. S., Shakun, J.D., Carlson, a. E., Clark, J., Wohlfarth, B., Mitrovica, J.X., Hostetler, S.W., McCabe, a. M., 2009. The Last Glacial Maximum. Science (80). 325, 710–714. doi:10.1126/science.1172873

Corona-Santiago, D.K., Doadrio, I., Domínguez-Domínguez, O., 2015. Evolutionary History of the Live-Bearing Endemic Allotoca diazi Species Complex (Actinopterygii, Goodeinae): Evidence of Founder Effect Events in the Mexican Pre-Hispanic Period. PLoS One 10, e0124138. doi:10.1371/journal.pone.0124138

Corona-Santiago, D.K., Santiago, 2013. Filogeografía de tres especies de Goodeidos endémicos del Centro de México : Allotoca diazi , A . meeki y A . catarinae (Cyprinodontiformes : Goodeidae).

De Buen, F., 1943. Los lagos michoacanos. I. Caracteres generales. El lago de Zirahuen. Rev. Soc. Mex. Hist. Nat. 4 (3-4), 211– 232

Doadrio, I., Domínguez, O., 2004. Phylogenetic relationships within the fish family Goodeidae based on cytochrome b sequence data. Mol. Phylogenet. Evol. 31, 416–30. doi:10.1016/j.ympev.2003.08.022

Doebeli, M., Dieckmann, U., 2000. Evolutionary Branching and Sympatric Speciation Caused by Different Types of Ecological Interactions Understanding the origin of new species remains one of the core problems in evolutionary biology. Whether one. Am. Nat 156, 77–101

Domínguez-Domínguez, O., Alda, F., de León, G.P.-P., García-Garitagoitia, J.L., Doadrio, I., 2008. Evolutionary history of the endangered fish *Zoogoneticus quitzeoensis* (Bean, 1898) (Cyprinodontiformes: Goodeidae) using a sequential approach to phylogeography based on mitochondrial and nuclear DNA data. BMC Evol. Biol. 8, 161. doi:10.1186/1471-2148-8-161

Domínguez-Domínguez, O., Doadrio, I. y, Perez-Ponce de Leon, G., 2006. Historical biogeography of some river basins in central Mexico evidenced by their goodeine freshwater fishes: a preliminary hypothesis using secondary Brooks parsimony analysis. J. Biogeogr. 33, 1437–1447. doi:10.1111/j.1365-2699.2006.01526.x

Domínguez-Domínguez, O., Boto, L., Alda, F., Pérez-Ponce De León, G., Doadrio, I., 2007. Human impacts on drainages of the Mesa Central, Mexico, and its genetic effects on an endangered fish, *Zoogoneticus quitzeoensis*. Conserv. Biol. 21, 168–180. doi:10.1111/j.1523-1739.2006.00608.x

Domínguez-Domínguez, O. y P.-P. de L.G., 2009. ¿ La mesa central de México es una provincia biogeográfica? Análisis descriptivo basado en componentes bióticos dulceacuícolas Is the Mesa Central of Mexico a biogeographical province? Descriptive analysis based on freshwater biotic components. Rev. Mex. Biodivers. 80, 835–852.

Echelle, A. A., Echelle, A. F., 1984. Evolutionary genetics of a “species flock” Atherinid fishes on mesa central of Mexico. In A. A. Echelle & I. Kornfield (Eds.), Evolution of fish species flock (pp. 93–110). Orono, ME: Univ Maine Orono.

Edwards S., Bensch, S., 2009. Looking forwards or looking backwards in avian phylo- geography? A comment on Zink and Barrowclough 2008. Mol Ecol 18(14):2930–2933, discussion 2934–2936.

Edwards, S. V., Beerli, P., 2000. and the Variance In coalescence Time in Phylogeographic. Evolution (N.Y). 54, 1839–1854. doi:10.1554/0014-3820(2000)054

Edwards, S. V., Potter, S., Schmitt, C.J., Bragg, J.G., Moritz, C., 2016. Reticulation, divergence, and the phylogeography–phylogenetics continuum. Proc. Natl. Acad. Sci. 113, 8025–8032. doi:10.1073/pnas.1601066113

Faria, C.M. a, Machado, A., Amorim, I.R., Gage, M.J.G., Borges, P. a V, Emerson, B.C., 2016. Evidence for multiple founding lineages and genetic admixture in the evolution of species within an oceanic island weevil (Coleoptera, Curculionidae) super-radiation. J. Biogeogr. 43, 178–191. doi:10.1111/jbi.12606

Ferrari, L., Orozco-Esquivel, T., Maena, V., Maena, M., 2011. The dynamic history of the Trans-Mexican Volcanic Belt the Mexico subduction zone. Tectonophysics, 522–523, 122–149. https://doi.org/10.1016/j.tecto.2011.09.018

Fluker, B.L., Pezold, F., Minton, R.L., 2011. Molecular and morphological divergence in the inland silverside (*Menidia beryllina*) along a freshwater-estuarine interface. Environ. Biol. Fishes 91, 311–325. doi:10.1007/s10641-011-9786-2.

Funk, D.J., Omland, K.E., 2003. Species-Level Paraphyly and Polyphyly: Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 34, 397–423. doi:10.1146/annurev.ecolsys.34.011802.132421.

García-Andrade, A., 2014. Filogeografía Comparada de Cuatro Especies de Peces Vivíparos en los Lagos del Norte de Michoacán.

García-de León, F.J., Ramírez-Herrejón, J.P., García-Ortega, R., Hendrickson, D. a., 2014. Foraging patterns of four sympatric species of silversides (Atheriniformes : Atherinopsidae) in Lago de Pátzcuaro , Central Mexico. Res. J. Costa Rican Distance Educ. Univ. 6, 127–139.

García-Martínez Rosa María, Omar Mejía, F.J.G.-D.L. and I.D.L.A.B.-S., 2015. " Extreme genetics divergence in the endemic fish *Chirostoma humboldtianum* (Valenciennes , 1835): implications for its conservation". Hidrobiológica 25, 95–106.

Grummer, J.A., Bryson Jr, R.W., Reeder, T.W., 2014. Species Delimitation Using Bayes Factors: Simulations and Application to the *Sceloporus scalaris* Species Group (Squamata: Phrynosomatidae). Syst. Biol 63, 119–133. doi:10.1093/sysbio/syt069

Gulisija, D., Kim, Y., Plotkin, J.B., 2016. Phenotypic plasticity promotes balanced polymorphism in periodic environments by a genomic storage effect 202, 1437–1448. doi:10.1101/038497

Heled, J., Drummond, A.J., 2010. Bayesian Inference of Species Trees from Multilocus Data. Mol. Biol. Evol 27, 570–580. doi:10.1093/molbev/msp274.

Hernández M.D., 2006 La pesquería en el lago de Pátzcuaro. In: Arreguín S.F., Beléndez M.L., Gómez-Humaran M.I., Solana S.R. and Rangel D.C. (eds.),

Sustentabilidad y pesca responsable en México. Evaluación y manejo. Instituto Nacional de la Pesca. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. México, D. F. 391-422.

Hernández-Batista, A.F., Ramírez-Torrez, J.A., Azaola-Espinosa, A., Mayorga-Reyes, L., Monroy-Dosta, M.D.C., 2015. The genus *Chirostoma* (Actinopterygii: Atheriniformes) in Mexico: Challenge for conservation and aquaculture technology. Int. J. Aquat. Sci. 6, 67–83.

Hudson, R.R., Turelli, M., 2003. Stochasticity overrules the “three-times rule”: genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. Evolution 57, 182–190. doi:10.1016/j.saa.2011.05.102

Israde-Alcántara, I., Garduño-Monroy, V.H., 1999. Lacustrine record in volcanic intra-arc setting.The evolution of the Late Neogene Cuitzeo basin system (central western Michoacan,Mexico).Paleogeography,Paleoclimatology,Paleoecology. Vol.15 Vol.15, 209–227. doi:doi.org/10.1016/S0031-0182(99)00024-3

Israde-Alcántara, I., Garduño-Monroy, V.H., Fisher, C.T., Pollard, H.P., Rodríguez-Pascua, M. a., 2005. Lake level change, climate, and the impact of natural events: The role of seismic and volcanic events in the formation of the Lake Patzcuaro Basin, Michoacan, Mexico. Quat. Int. 135, 35–46. doi:10.1016/j.quaint.2004.10.022

Keith A., Crandall, O.R.., Bininda-Emonds, O.R.R., Mace, G.M., Wayne, R.K., 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. 15, 290–295. doi:10.1016/S0169-5347(00)01876-0

Kocher, T.D., Thomas, W.K., Meyer, a., Edwards, S. V., Paabo, S., Villablanca, F.X., Wilson, a. C., 1989. Dynamics of mitochondrial DNA evolution in animals:

amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. 86, 6196–6200. doi:10.1073/pnas.86.16.6196

Lake, C., Rodriguez-ruiz, A., Granado-lorencio, C., 1988. Características del aparato bucal asociadas al régimen alimenticio en cinco especies coexistentes del género *Chirostoma* (Lago de Chapala, México). Rev. Chil. Hist. Nat. 61, 35–51.

Leaché, A.D., Fujita, M.K., Minin, V.N., Bouckaert, R.R., 2014. Species delimitation using genome-wide SNP Data. Syst. Biol. 63, 534–542. doi:10.1093/sysbio/syu018

Marshall, C.J., Liebherr, J.K., 2000. Cladistic biogeography of the Mexican transition zone. J. Biogeogr. 27, 203–216. doi:10.1046/j.1365-2699.2000.00388.x

Martínez, R.M.G., 2015. Análisis filogeográfico del pez blanco *Chirostoma humboldtianum* (Valenciennes, 1835) (Pisces: Atheriniformes).

Meek, S. E., 1902. A contribution to the Ichthyology of Mexico. Zoological series Vol. 3, pp. 63–128. Chicago, IL: Field Columbian Museum.

Mercado-Silva, N., Lyons, J., Moncayo-Estrada, R., Gesundheit, P., Krabbenhoft, T.J., Powell, D.L., Piller, K.R., 2015. Stable isotope evidence for trophic overlap of sympatric Mexican Lake Chapala silversides (Teleostei: Atherinopsidae: Chirostoma spp.). Neotrop. Ichthyol. 00–00. doi:10.1590/1982-0224-20140079

Metcalfe, S.E., O’Hara, S.L., Caballero, M., Davies, S.J., 2000. Records of Late Pleistocene-Holocene climatic change in Mexico - A review. Quat. Sci. Rev. 19, 699–721. doi:10.1016/S0277-3791(99)00022-0

Meyer A., Kocher, T.D., Basasibwaki, P., Wilson, A.C., 1990 Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature Lond 347:550–553

Meyer, B.S., Matschiner, M., Salzburger, W., 2015. Molecular Phylogenetics and Evolution A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. Mol. Phylogenet. Evol. 83, 56–71. doi:10.1016/j.ympev.2014.10.009

Moncayo-Estrada, R., Escalera-Gallardo, C., Lind, O.T., 2011. Spatial patterns of zooplanktivore Chirostoma species (Atherinopsidae) during water-level fluctuation in the shallow tropical Lake Chapala, Mexico: Seasonal and interannual analysis. Neotrop. Ichthyol. 9, 815–824. doi:10.1590/S1679-62252011000400012

Niemiller, M.L., Near, T.J., Fitzpatrick, B.M., 2012. Delimiting species using multilocus data: Diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus* (teleostei: Amblyopsidae). Evolution (N. Y). 66, 846–866. doi:10.1111/j.1558-5646.2011.01480.x

Ornelas-García, C.P., Domínguez-Domínguez, O., Doadrio, I., 2008. Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC Evol. Biol. 8, 340. doi:10.1186/1471-2148-8-340

Ortega, B., Vázquez, G., Caballero, M., Israde, I., Lozano-García, S., Schaaf, P., Torres, E., 2010. Late Pleistocene: Holocene record of environmental changes in Lake Zirahuen, Central Mexico. J. Paleolimnol. 44, 745–760. doi:10.1007/s10933-010-9449-x

Palumbi, S.R., 1996 Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics, 2nd edn. Sinauer Associates, Sunderland

Paulo-Maya, J., Figueroa Lucero, G., Soria-Barreto, M., 2000. Peces dulceacuícolas mexicanos XIX. *Chirostoma humboldtianum* (Atheriniformes: Atherinopsidae). Zoología Informa 43: 59-74.

Pedraza-Lara, C., Doadrio, I., Breinholt, J.W., Crandall, K. a., 2012. Phylogeny and Evolutionary Patterns in the Dwarf Crayfish Subfamily (Decapoda: Cambarellinae). PLoS One 7. doi:10.1371/journal.pone.0048233

Pérez-Miranda, F., Mejía, O., Soto-Galera, E., Espinosa-Pérez, H., Piálek, L., Říčan, O., 2017. Phylogeny and species diversity of the genus *Herichthys* (Teleostei: Cichlidae). J. Zool. Syst. Evol. Res. doi:10.1111/jzs.12197

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Doadrio, I., Cuevas-García, E., Pérez-Ponce de León, G., 2015. Comparative historical biogeography of three groups of Nearctic freshwater fishes across central Mexico. J. Fish Biol. 86, 993–1015. doi:10.1111/jfb.12611

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Mar-Silva, A.F., Doadrio, I., Pérez-Ponce de León, G., 2016. The historical biogeography of the southern group of the sucker genus Moxostoma (Teleostei: Catostomidae) and the colonization of central Mexico. Zool. J. Linn. Soc. 177, 633–647. doi:10.1111/zoj.12383

Pérez-Rodríguez, R., Domínguez-Domínguez, O., Pérez Ponce de León, G., Doadrio, I., 2009. Phylogenetic relationships and biogeography of the genus *Algansea* Girard (Cypriniformes: Cyprinidae) of central Mexico inferred from molecular data. BMC Evol. Biol. 9, 223. doi:10.1186/1471-2148-9-223

Piller, K.R., Kenway, C.S., Kenway-Lynch, C.S., Camak, D.T., Domínguez-Domínguez, O., 2015. Phylogeography and Population Structure of the Imperiled Redtail Splitfin (Goodeidae: *Xenotoca eiseni*): Implications for Conservation. Copeia 103, 440–454. doi:10.1643/CI-14-067

Pritchard, J.K., Stephens, M., Donnelly, P., 2000 Inference of popu- lation structure using multilocus genotype data. Genetics, 155, 945–959.

Puebla, O., 2009. Ecological speciation in marine vs freshwater fishes. J. Fish Biol. 75, 960–996. doi:10.1111/j.1095-8649.2009.02358.x

Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. 94, 9197–9201. doi:10.1073/pnas.94.17.9197

Rosenblum, E.B., Parent, C.E., Brandt, E.E., 2014. The Molecular Basis of Phenotypic Convergence. Annu. Rev. Ecol. Evol. Syst 45, 203–26. doi:10.1146/annurev-ecolsys-120213-091851

Sanger, F., Nicklen, S., Coulson, a. R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74, 5463–5467. doi:10.1073/pnas.74.12.5463

Schluter, D., Conte, G.L., 2009. Genetics and ecological speciation. PNAS 106, 9955–9962. doi:DOI/10.1073/PNAS.0901264106

Schluter, D., 2001. Ecology and the origin of species. Trends Ecol. Evol. 16, 372–380. doi:10.1016/S0169-5347(01)02198-X

Schönhuth, S., Doadrio, I., 2003. Phylogenetic relationships of Mexican minnows of the genus *Notropis* (Actinopterygii, Cyprinidae). Biol. J. Linn. Soc. 80, 323–337. doi:10.1046/j.1095-8312.2003.00241.x

Seehausen, O., Wagner, C.E., 2014. Speciation in Freshwater Fishes. Annu. Rev. Ecol. Evol. Syst 45, 621–51. doi:10.1146/annurev-ecolsys-120213-091818

Simon, C., Frati, F., Beckenbach, a., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann. Entomol. Soc. Am. 87, 651–701. doi:10.1093/aesa/87.6.651

Soria Barreto, Miriam, Paulo Maya, J., 2005. Morfometría comparada del aparato mandibular en especies de *Chirostoma* (Atheriniformes : Atherinopsidae) del Lago de Pátzcuaro , Michoacán , México. Hidrobiologica 15, 161–168.

Tarvin, R.D., Powell, E.A., Santos, J.C., Ron, S.R., Cannatella, D.C., 2017. The birth of aposematism: High phenotypic divergence and low genetic diversity in a young clade of poison frogs. Mol. Phylogenet. Evol. 109, 283–295. doi:10.1016/j.ympev.2016.12.035

Terai, Y., Morikawa, N., Okada, N., 2002. The Evolution of the Pro-Domain of Bone Morphogenetic Protein 4 (Bmp4) in an. Mol. Biol. Evol. 19, 1628–1632.

Torres-Rodríguez, E., Lozano-García, S., Figueroa-Rangel, B.L., 2012. Cambio ambiental y respuestas de la vegetación de los últimos 17,000 años en el centro de México : el registro del lago de Zirahuén. Rev. Mex. Ciencias Geológicas 29, 764–778.

Unmack, P.J., Allen, G.R., Johnson, J.B., 2013. Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. Mol. Phylogenet. Evol. 67, 15–27. doi:10.1016/j.ympev.2012.12.019

Vital-Rodríguez, B.E., Ramírez-Herrejón, J.P., Moncayo-Estrada, R., Caraveo-Patiño, J., Domínguez-Domínguez, O., 2017. Feeding strategy of silverside species in eutrophic Lake Pátzcuaro, Mexico. J. Appl. Ichthyol. 33, 93–101. doi:10.1111/jai.13248

Wagner, C.E., Keller, I., Wittwer, S., Selz, O.M., Mwaiko, S., Greuter, L., Sivasundar, A., Seehausen, O., 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Mol. Ecol. 22, 787–798. doi:10.1111/mec.12023

Wilson, G.A., Rannala, B., 2003 Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1190

Yang, Z., Rannala, B., 2012. Molecular phylogenetics: principles and practice. Nat. Rev. Genet. 13, 303–14. doi:10.1038/nrg3186

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. PNAS 107, 9264–9269. doi:10.1073/pnas.0913022107

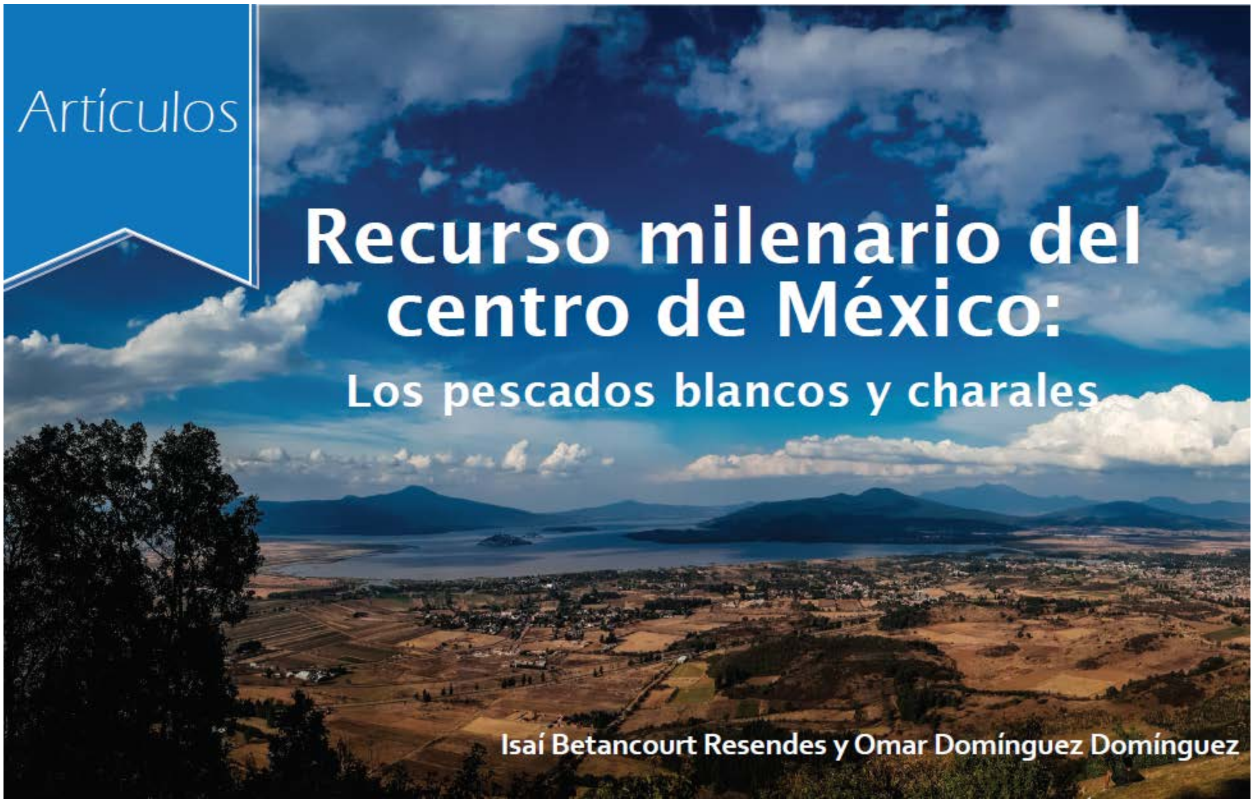
Zamudio, K.R., Bell, R.C., Mason, N.A., 2016. Phenotypes in phylogeography: Species' traits, environmental variation, and vertebrate diversification. PNAS 113, 8041–8048. doi:10.1073/pnas.1602237113

ANEXOS

ARTÍCULO DE DIVULGACIÓN. REVISTA DE DIVULGACIÓN SABER MÁS DE LA UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO N0 36.

Recurso milenario del centro de México: Los pescados blancos y charales

Escrito por Isaí Betancourt Resendes y Omar Domínguez Domínguez



En la tierra han ocurrido grandes cambios a través del tiempo y lo que hoy vemos es solo el reflejo de esos grandes procesos geológicos ocurridos en el pasado. La región central de México no es la excepción, debido a que ha sido testigo de estos procesos, que generaron una gran complejidad topográfica y un singular mosaico de ambientes diferentes, incluyendo los sistemas acuáticos, manantiales, lagos y sistemas fluviales extensos que se convirtieron en el hogar de muchos organismos.

La mayoría de los peces que habita esta región son endémicos, es decir no se encuentran en ningún otro lugar del mundo, esto contrasta con la alta tasa de extinción de peces en la región, siendo una de las más altas del planeta. Dentro de los diferentes grupos de peces del Centro de México destaca la presencia de la familia Atherinopsidae (Aterinópsidos), representada por dos géneros, siendo *Chirostoma* el más característico y abundante, representado por 18 especies conocidas como pescados blancos y charales, los cuales han sido el principal recurso pesquero desde épocas prehispánicas. Un aspecto interesante de estos Aterinópsidos es que tienen un origen marino, atribuido a la invasión de diversos grupos de peces marinos.

¿Cómo llegaron al centro de México?

Por muchos años se consideró que el género *Chirostoma* tenía dos ancestros (uno que llegó del Atlántico y otro del Pacífico), que colonizaron el Centro de México en diferentes tiempos geológicos y evolucionaron de manera independiente. Sin embargo, los nuevos descubrimientos científicos afirman que estos Aterinópsidos comparten un mismo ancestro con otros de origen marino del Atlántico, lo que significa que las especies del Centro de México provienen de un único ancestro.

En la actualidad las especies de *Chirostoma* las podemos encontrar en el sistema hidrológico Lerma-Chapala y regiones contiguas, sin embargo, solamente *Chirostoma jordani* y *Chirostoma humboldtianum*, presentan un amplio rango de distribución, estableciéndose en diferentes tipos de hábitats acuáticos a lo largo de las cuencas del Centro de México, mientras que el resto de las especies tienen una distribución más restringida. El lago de Chapala, el más grande del Centro de México, y el que alberga el mayor número de especies, nueve en total, seis de ellas endémicas a este cuerpo de agua. Mientras que para el lago de Pátzcuaro, quizá el más emblemático de Michoacán, se han registrado cuatro especies, destacando la presencia del pescado blanco de Pátzcuaro (*Chirostoma estor*).

Lago de Chapala. Sistema lentico, hábitat de 9 especies de Chirostoma. Imagen por Isaí Betancourt Resendes

Pero ¿Cómo diferenciamos a los charales del pescado blanco?

La manera más común es por el tamaño. Los charales alcanzan medidas menores a los 150 mm de longitud estándar, aquí se ubican la mayoría de las especies, se alimentan de zooplancton y viven en lagos, ríos, arroyos, riachuelos y manantiales.

Por su parte, los pescados blancos son aquellos que alcanzan medidas de más de 150 mm de longitud estándar, llega alcanzar un peso entre los 200 a 300 g, solo se encuentran en lagos y en etapas juveniles se alimentan de zooplancton mientras que los adultos se alimentan de peces.

Sin embargo, las especies son difíciles de diferenciar incluso para los especialistas, algunos mencionan que esta dificultad se debe a que son especies que evolucionaron de manera muy reciente, otros creen que esto se puede deber a la plasticidad fenotípica que poseen, mientras que otros lo atribuyen a los procesos de hibridación existentes atribuido a la introducción de especies de *Chirostoma* ajenos al sitio.

Por lo que, el tamaño no es útil para diferenciar a los charales de los pescados blancos en etapas juveniles, por lo tanto, al degustar los deliciosos charales a las orillas de los lagos de Chapala y Pátzcuaro, no tendremos la certeza si son charales o juveniles de pescado blanco.

Con los estudios genéticos recientes realizados en el Laboratorio de Biología Acuática de la Facultad de Biología de la Universidad Michoacana de San Nicolás de Hidalgo, se está demostrando que en realidad el número de especies de pescado blanco está sobre estimado, encontrando solo dos grupos genéticamente bien diferenciados: la especie *Chirostoma humboldtianum* no está ampliamente distribuida como se sugería y que la variación fenotípica es tan amplia en este grupo, que los caracteres que usaban los expertos para diferenciar entre especies son poco útiles.

Sin embargo, aún no alcanzamos a comprender los procesos evolutivos que están ocurriendo en los pescados blancos, no sabemos aún cómo un grupo con elevada variación morfológica tenga escasa variación genética, la cual además tiene correspondencia geográfica.

Estas especies son de gran importancia ecológica, primero porque son peces endémicos del Centro de México, además son parte de la cadena trófica manteniendo la estabilidad en los ecosistemas donde se encuentran, siendo los pescados blancos los depredadores tope en los lagos donde habitan.

Los pescados blancos y charales también tienen una gran importancia cultural, ya que han sido parte de los recursos que las etnias purépechas, nahuas y otomíes han usado como sustento, siendo las pesquerías autóctonas más antiguas en el Centro de México, considerados como el manjar de los dioses, sirviendo como ofrendas por las culturas prehispánicas.

A pesar de su importancia, abundancia y diversidad, las especies están amenazadas por diversos factores, principalmente por la pérdida y contaminación de hábitat, introducción de especies exóticas y sobre-explotación pesquera. El Centro de México es el lugar más poblado de todo el territorio nacional (según datos mostrados en el último censo del INEGI), La industria también se concentran en esta región, por lo que el agua superficial del país, y los cuerpos de agua presentan una fuerte presión que los ha afectados severamente, siendo los principales canales de desecho tanto de la industria como de la agricultura, la ganadería y el drenaje doméstico, por lo que, el alto grado de contaminación y destrucción de hábitat ha propiciado la extinción local de algunas poblaciones de *Chirostoma*.

Figura Pescado blanco capturado en el lago de Zirahuén, Michoacán. Los pescados blancos alcanzan tallas mayores a los 150 mm. Solamente se encuentran en lagos. Imagen por Isaí Betancourt Resendes

Figura Charal, capturado en el lago de San Juanico, Michoacán. Los charales no alcanzan tallas mayores a 120 mm. Pueden vivir tanto en lagos como en ríos, arroyos y manantiales. Imagen por Isaí Betancourt Resendes

¿Charales extintos?

Por ejemplo el charal del verde (*Chirostoma arge*) no ha sido reportado en los diez últimos años. Otra especie extinta es el charal Tarasco (*Chirostoma charari*), exclusivo del río grande de Morelia, el cual ha sido convertido en la cloaca de la ciudad, y su grado de contaminación es fácilmente percibido a través del desagradable olor y la insalubre vista. O bien el charal *Chirostoma riojai* restringido a las partes altas del alto Lerma en el estado de México. Estos son algunos de los varios casos que existen en cuanto a desaparición de poblaciones y especies.

Otra causa es la introducción de especies exóticas, considerada como la segunda causa de la pérdida de biodiversidad. En este sentido, por más de 50 años los diferentes órdenes de gobierno han realizado la siembra de peces exóticos en diferentes cuerpos de agua, con fines alimenticios. Las especies invasoras pueden competir por espacio y recursos, o bien depredar a las especies nativas, tal es el caso de la introducción de la lobina negra (*Micropterus salmoides*) en el lago de Zirahuén, desplazando a *Chirostoma estor* como el depredador tope, además ha sido la causa de desaparición de diversas especies nativas, incluso de la extinción de un pez vivíparo *Allotoca meeki* endémico a este lago.

Figura 5. Pescadores en el Lago de Zirahuén, donde se pesca al pescado blanco Chirostoma estor y al charal Chirostoma attenuatum. Imagen por Isaí Betancourt Resendes

Otro problema es la carpa exótica en el lago de Chapala, en la cual se ha identificado que hasta el 90% de los artículos consumidos son huevos de diferentes especies de *Chirostoma*. El pescado blanco *Chirostoma estor* ha sido introducido en presas de la cuenca del Lerma, el cual puede provocar los mismos efectos negativos que cualquier especie traída de tierras lejanas.

La pesca excesiva también es un problema grave para las poblaciones de pescado blanco. En los 80s y 90s las capturas se incrementaron considerablemente, sin embargo en los últimos años la captura de organismos de tallas mayores a 150 mm de longitud ha disminuido para los lagos de Pátzcuaro, Zirahuén y Chapala, esto ha provocado la sobrepesca de los especímenes que incluso no han sido capaces de reproducirse. Además la sobrepesca está afectando los esfuerzos de conservación que han sido escasos así como el conocimiento generado del grupo, incluso algo sorprendente es que ninguno de los miembros del género *Chirostoma*, a excepción de *Chirostoma promelas*, se encuentra enlistados en algún estatus de riesgo por la normatividad mexicana (NOM-ECOL-059-94).

En este sentido, los estudios genéticos pueden aportar argumentos tangibles para la conservación de la diversidad biológica, en los últimos años, con la incorporación de estudios de genética de poblaciones, se han podido diagnosticar poblaciones distintas genéticamente, aunque los expertos no las consideren como especies diferentes, si pueden ser catalogadas como linajes diferenciados. Como el caso del charal prieto *Chirostoma attenuatum*, restringido a los lagos de Pátzcuaro y Zirahuén, que a partir de estudios genéticos realizados en el Laboratorio de Biología Acuática de la Facultad de Biología de la

UMSNH, se ha demostrado que cada población representa un linaje evolutivo independiente, por lo que, preservar la diversidad adaptativa y los procesos evolutivos de cada linaje es de gran importancia para la conservación de la diversidad biológica, lo cual debería ser tomado en cuenta en la toma de decisiones para su manejo y conservación.

Figura. Río verde cause principal en Tepatilán Jalisco. Sistema lotico hábitat de los charales Chirostoma arge y Chirostoma jordani. Imagen por Isaí Betancourt Resendes

Para Saber más:

Barbour C.D. 1973. The systematics and evolution of the genus *Chirostoma*, Swainson. Tul Stud Zool Bot 19:97-141.

Miller R.R. 2005. Peces dulceacuícolas de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad A. C., El colegio de la Frontera Sur y Consejo de los Peces del Desierto México-Estados Unidos. México, D.F. http://www.biodiversidad.gob.mx/pdf/libros/paguadulceA.pdf

Barriga-Sosa I. et al. 2005. Inter-Specific variation of the mitocondrial r16S gene among silversides, “Peces Blancos”, (Atherinopsidae: Menidiinae) and its utilization for species identification. Aquaculture 250. 637-651

***M.C. Isaí Betancourt Resendes** es estudiante del Programa Institucional de Doctorado en Ciencia Biológicas.*

***D.C. Omar Domínguez Domínguez** es profesor investigador del Laboratorio de Biología Acuática, ambos de la Facultad de Biología de la Universidad Michoacana de San Nicolás de Hidalgo.*