Posgrado en Ciencias del Mar y Limnología

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# Filogeografía de *Zoogoneticus quitzeoensis, Xenotoca variata* y *Alloophorus robustus* (Cyprinodontiformes: Goodeidae) en el Centro de México: implicaciones taxonómicas y de conservación.

## T E S I S

Que para obtener el grado académico de

Doctor en Ciencias
**(Limnología)**

presenta

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ÍNDICE

## PRESENTACIÓN

El presente trabajo de tesis está conformado en su mayoría por una serie de publicaciones que se corresponden con los diferentes capítulos que forman parte del contenido de la misma. La estructura comprende tres grandes secciones: Introducción (con los objetivos), Resultados y Conclusiones Generales. En primera instancia la sección introductoria se compone de cuatro rubros principales: el primero se refiere al área de estudio, que comprende la denominada Mesa Central de México, el segundo al objeto de estudio, los Goodeidos, el tercero se refiere al marco teórico de la propuesta metodólogica adoptada en el presente estudio es decir, la filogeografía y, por último, se hace un planteamiento general del problema que se aborda en el presente trabajo para fundamentar los objetivos del mismo. Cabe señalar que la literatura citada correspondiente a la sección introductoria aparece inmediatamente después de ésta.

El segundo apartado, los resultados, están a su vez divididos en dos grandes rubros:
1) El primero está conformado por tres artículos que se encuentran en distinto estado de publicación. El primero se refiere al estudio filogeográfico de *Zoogoneticus quitzeoensis*, mismo que se encuentra aceptado en la revista BMC Evolutionary Biology. El segundo enfocado a la conservación de *Z. quitzeoensis* utilizando datos genéticos, ambientales, ecológicos, sociales y biológicos, mismo que ya fue publicado en la revista Conservation Biology. Por último, el tercer artículo incluye la descripción de una nueva especie del género *Zoogoneticus*, *Z. purepechus*, utilizando datos genéticos y morfológicos, el cual está ya aceptado para su publicación en la Revista Mexicana de Biodiversidad.
2) El segundo rubro de resultados se encuentra a su vez conformado por dos artículos, el primero trata la filogeografía comparada de dos especies de Goodeidos (*Xenotoca variata* y *Alloophorus robustus*) mientras que el segundo comprende la descripción de una nueva especie del género *Xenotoca*, los cuales se encuentran en proceso de preparación. Cabe señalar que no existe un rubro de metodología general, en virtud de que ésta es muy variada y está contenida dentro de cada artículo, de tal forma que los capítulos de resultados, estén ya publicados o en prensa o bien, aún no hayan sido enviados, conforman una estructura como artículo científico, con introducción, antecedentes, material y métodos, resultados, discusión y literatura citada.

Finalmente, el último apartado se refiere a la discusión general, en la cual se presenta una integración de los resultados obtenidos en los diferentes artículos, abordando los aspectos nodales del presente trabajo y que se refieren a las implicaciones taxonómicas, biogeográficas y de conservación de los goodeidos. En esta sección se hace referencia a los artículos del presente trabajo mediante los números romanos asignados a cada uno de ellos (ver índice).

FILOGEOGRAFÍA DE *Zoogoneticus quitzeoensis*, *Xenotoca variata* y *Alloophorus robustus* (Cyprinodontiformes: Goodeidae) EN EL CENTRO DE MÉXICO: IMPLICACIONES TAXONÓMICAS Y DE CONSERVACIÓN.

**RESUMEN**

Se utilizaron métodos filogeográficos, de genética de poblaciones, taxonomía tradicional y datos ambientales para estudiar la historia evolutiva de tres especies de goodeidos y su relación con eventos geológicos, climáticos, biológicos y ecológicos, determinar unidades de conservación y describir nuevos taxones. La historia evolutiva de ambas especie ha estado estrechamente ligada a la historia geológica y climática de la zona. Las especies estudiadas no presentan patrones filogeográficos concordantes, diferencias que son atribuibles a las características intrínsecas de las especies o eventos de deriva génica, influyendo en la diversidad genética observada. Las fuertes alteraciones en los cuerpos de agua en los últimos 200 años han impactado en la diversidad genética. Las zonas de manantiales son zonas de refugio, sin embargo, la viabilidad genética de estas poblaciones no está asegurada. Cada cuenca debe ser considerada como una Unidad de Conservación, sin embargo, el intercambio genético entre las poblaciones de un mismo linaje, es importante para mantener la variabilidad genética. Deben plantearse programas de restauración ecológica que recuperen de corredores biológicos entre dichas áreas. Basados en caracteres morfológicos y moleculares se describe *Zoogoneticus purepechus* que se distribuye en las cuencas de los ríos Ameca, Armería, Santiago, Bajo Lerma y Chapala y la especie *Xenotoca diblenii*, endémica de la cuenca del Lago de Cuitzeo.

Palabras clave: Filogeografía, Goodeinae, Conservación, Taxonomía, Evolución

# INTRODUCCIÓN

## 1. La Mesa Central de México

### *Geología de la Mesa Central de México*

La Mesa Central de México es uno de los altiplanos tropicales más extensos del mundo (Miller & Smith, 1986). Se encuentra entre los paralelos 21º00´-24º00´ de latitud norte y los meridianos 100º 00´-104º00´ de longitud oeste. La provincia abarca la mayor parte de Guanajuato, el occidente de Querétaro y San Luís Potosí, y el oriente de Jalisco, Zacatecas, Michoacán y Aguascalientes. Su área forma un paralelogramo orientado en sentido noroeste-sureste, con dimensiones de 135 km norte-sur y 225 km este-oeste y unos 85,300 $km^2$. Más de la mitad de la superficie que compone esta planicie elevada presenta cotas por arriba de los 2000 metros. Esta mesa es atravesada por un gran lineamiento cenozoico de dirección NW de más de 600 km de longitud (Nieto-Samaniego *et al.,* 2005). Se encuentra enmarcada casi en su totalidad por sistemas montañosos; al este colinda con la Sierra Madre Oriental, al Noroeste cor la Sierra Madre Occidental, al Oeste por las estrechas planicies costeras del Pacífico y al sur por los llamados Escarpados del Sur y la depresión del Balsas, por lo que está rodeada en todo su contorno, excepto el norte, por montañas (Tamayo & West, 1964). De acuerdo con Barbour (1973), el Altiplano Mexicano está dividido en dos regiones, la Mesa del Norte que se extiende del Río Bravo (Grande) hasta aproximadamente la latitud de las ciudades de Aguascalientes y San Luis Potosí (Fig. 1), siendo la continuación de la región de las cuencas de la provincia del oeste de los Estados Unidos, y la Mesa Central, siendo denominada por Miller & Smith (1986) como la región Sur del Altiplano Mexicano. Otros autores ubican la frontera Sur de la Mesa Central en el Cinturón Volcánico Transmexicano que atraviesa el centro del país (Mateos *et al.*, 2002).

El margen localizado al Este de la Mesa Central está representado por la Sierra Madre Oriental, estas cadenas montañosas plegadas son efecto de la orogenia Laramide (Eguiluz -de Antuñano *et al.,* 2000), que surgieron hace *ca*. 75 Ma como producto del cabalgamiento y transporte hacia el oriente. Aparentemente su mayor actividad se registro en el Eoceno (*ca*. 45 Ma), siendo la Sierra Madre Oriental la continuación de las Montañas Rocallosas, extendiéndose a través de los estados de Coahuila, Nuevo León, Tamaulipas, y al este de San Luís Potosí, Hidalgo y Puebla. Durante la orogenia del Laramidico los sedimentos del Geosyclinal mexicano fueron severamente plegados. Sin embargo, se

presentaron fallamientos y movimientos durante el Terciario en algunas áreas y el vulcanismo del Cenozoico ocurrió en la porción más sureña, principalmente en el estado de Veracruz. En nuestros días, el relieve está dado principalmente como resultado de la erosión de los plegamientos del Jurásico, Cretácico y las camas sedimentarias del Terciario Temprano.

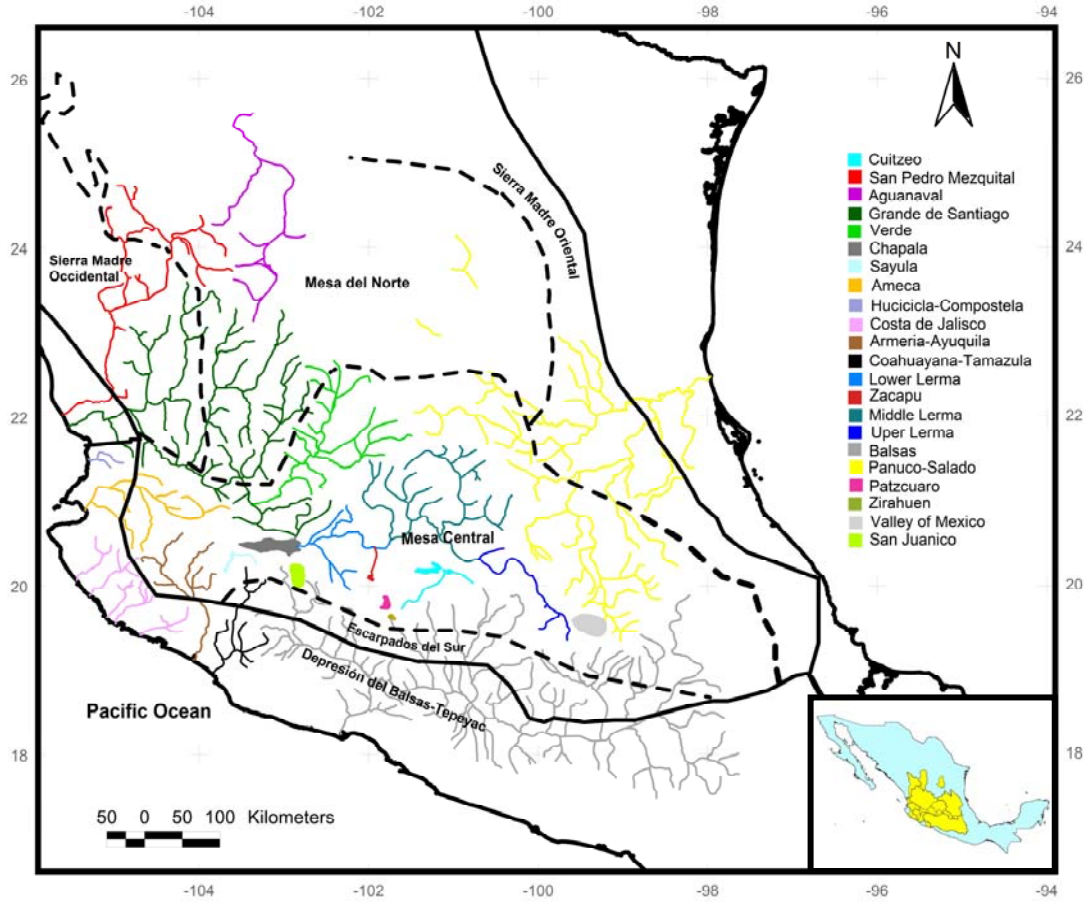


Figura 1. Regiones Fisiográficas de México de acuerdo con Tamayo & West (1964), la línea sólida se refiere al Altiplano Superficial, la línea punteada a las fronteras de la Mesa Central, Sierra Madre Oriental y Sierra Madre occidental (tomado de Domínguez-Domínguez & Pérez-Ponce de León, 2007).

El límite Noroeste de la Mesa Central (la Sierra Madre Occidental)(Fig. 1), está relacionada con la tectónica del "Basin and Range" en el Eoceno-Oligoceno (32 Ma) que conforman sistemas de cuencas y sierras alineadas en dirección Norte-Sur, misma que forma parte de un cinturón plegado en el margen Oeste de la Placa Norteamericana que abarca desde Alaska hasta el Sur de México, alcanzando sobre todo el margen noroccidente de México y teniendo como característica espesores importantes de sedimentos rellenando

cuencas aluviales. Estas montañas se extienden al sur por los estados de Sonora, Sinaloa, Chihuahua, Durango, Nayarit y el oeste de Jalisco. Asociada a esta actividad tectónica se constituye la provincia de la Sierra Madre Occidental hace aproximadamente 12 a 10 Ma a través de pulsos en la actividad de las placas (Aranda Gómez *et al.,* 2000). El Oeste de la Sierra Madre Occidental difiere de su contraparte del este ya que la primera ha sido una zona de fuerte actividad volcánica. La actividad tectónica cerca del Cretácico resulto en levantamientos e intenso plegamiento. Esta fase fue seguida de vulcanismo en el Oligoceno (*ca*. 30 Ma) y Mioceno (*ca.* 23 Ma), que provocó la formación de montañas en el Terciario Medio. La actividad volcánica moderada se extendió durante el Terciario Tardío y al final del Terciario se renovó la formación de montañas (Plioceno tardío-Pleistoceno). En el Pleistoceno medio las crestas de la Sierra Madre se elevaron fuertemente, formando la configuración actual de las cuencas y las profundas barrancas en la vertiente Oeste de las montañas (Aranda Gómez *et al.,* 2000).

Por su parte, la porción Sur de la Mesa Central, se encuentra limitado por el Cinturón Volcánico Transmexicano (CVTM) y su frontera (los Escarpados del Sur) (Fig. 1), los cuales se extienden en dirección Este-Oeste entre los paralelos 19º y 20º, y cuya actividad está asociada a una tectónica transtensional producto de la subducción oblicua de la placa pacífica por debajo de la Norteamericana. El CVTM atraviesa el país de costa a costa e incluye el sur de Jalisco y Nayarit, la mayor parte de Michoacán, noroeste de Colima, occidente de Guerrero, Morelos, Distrito Federal y Estado de México, sur de Querétaro, Guanajuato, Tlaxcala e Hidalgo, norte de Puebla y las regiones adyacentes de Veracruz. Tiene cerca de 930 km de longitud y en promedio 120 km de anchura, cubre una extensión cercana a los 175,700 $km^2$. Altitudinalmente se encuentra entre 1000 y 5000 msnm, sin embargo la zona altitudinal dominante se encuentra entre los 1500 y 2500 msnm, lo que corresponde principalmente a la porción oriental (Ferrusquia-Villafranca, 1998). Esta región se caracteriza por una inclusión oceánica en el Cretácico Medio, dado por el Portal del Balsas, que conectaba el Altiplano Mexicano con el Océano Pacífico (Maldonado-Koerdell, 1964). A esto le siguió el vulcanismo durante el Cretácico, sin embargo, la actividad que ha contribuido en mayor medida a moldear la fisiografía de la zona, y en general de la Mesa Central, inició en el Mioceno (12-7 Ma), presentando un pico

de intensidad en el Plioceno-Pleistoceno, y continúa de manera intermitente hasta nuestros días (Tamayo & West, 1964).

La Mesa Central se formó en parte por la acumulación de sedimentos vulcanoclásticos producidos por eventos subsecuentes de vulcanismo desde el Mioceno (Tamayo & West, 1964), esta región incluye los volcanes Pico de Orizaba, Cofre de Perote, Malinche, Popocatepetl, Ixtlacihuatl, Nevado de Toluca, Tancítaro y Volcán de Colima, entre muchos otros. Entre o adyacente a los grandes volcanes se encuentras montañas más pequeñas surgidas de erupciones volcánica de menor magnitud.

Los materiales que constituyen el Mioceno tardío de la Mesa Central están constituidos por aparatos centrales y derrames fisurales de basalto donde se obtuvieron edades de 13.5 Ma. (Aguirre Diaz *et al.,* 1997), 12 Ma (Mc Dowell & Keizer, 1977), 10.6-13.6 Ma (Luhr *et al.,* 1995), cubriendo también varios sectores del CVTM.

Entre 11 y 6 Ma es cuando se emplazan grandes volúmenes de vulcanismo que emerge a través de fallas extensionales cuyas edades son progresivamente más jóvenes de Oeste a Este, dicho emplazamiento se ha asociado a la fase final de la subducción de la microplaca Magdalena hace 12.5 Ma (Ferrari *et al.,* 2000), sin embargo la actividad del Mioceno superior-Plioceno está distribuida principalmente en las porciones Sur de Celaya y Oeste de Durango.

A continuación se presentan las principales fallas que dan lugar a la configuración de escarpes de la Mesa Centra, mismas que se generan en la región a partir del Oligoceno:

- La falla normal de dirección *ca.* E-O de Celaya Irapuato. Los niveles de los escarpes de la Sierra son de entre 150 y 250 m.
- La falla de la sierra de Guanajuato- de Irapuato a León 80 km largo con desplazamiento de *ca*. 1200 m, formada a partir del Oligoceno pero reactivada seguramente hasta el mioceno Tardío.
- Al oriente es característico el Sistema de fallas normales de Taxco-San Miguel de Allende producidas durante el Oligoceno Mioceno-medio con saltos de 450 m y la de Querétaro con edades de actividad del Mioceno tardío y con saltos de 100 m.

Sin embargo, cabe destacar que los diversos trabajos en relación a la geología de la región, no dejan claro un límite fisiográfico bien definido entre las Sierra Madre Oriental, Occidental y Mesa Central con el Cinturón Volcánico Transmexicano ya que la actividad

vulcanotectónica del periodo Neógeno-Cuaternario esta sobrepuesta en la región Sur de la Mesa Central (Mioceno superior Plioceno), época que es considerada como aquella de mayor diferenciación de cuencas lacustres y sus sistemas fluviales (Isarde-Alcantara *et al.*, 2008).

***Composición y evolución de los componentes hidrológicos de la Mesa Central***

Desde el punto de vista hidrográfico, el sistema más representativo de la Mesa Central es el Río Lerma, el cual es incluido en su totalidad. Otras cuencas incluidas totalmente dentro de la Mesa Central son las del Valle de México, Chapala, Cuitzeo, Pátzcuaro, Zirahuen, Zacapu y Cotija; así mismo incluye las partes altas y algunos tributarios de los ríos Pánuco, Balsas, Coahuayana, Armería y Ameca, así como el Río Verde, tributario del Río Santiago (Fig. 2).

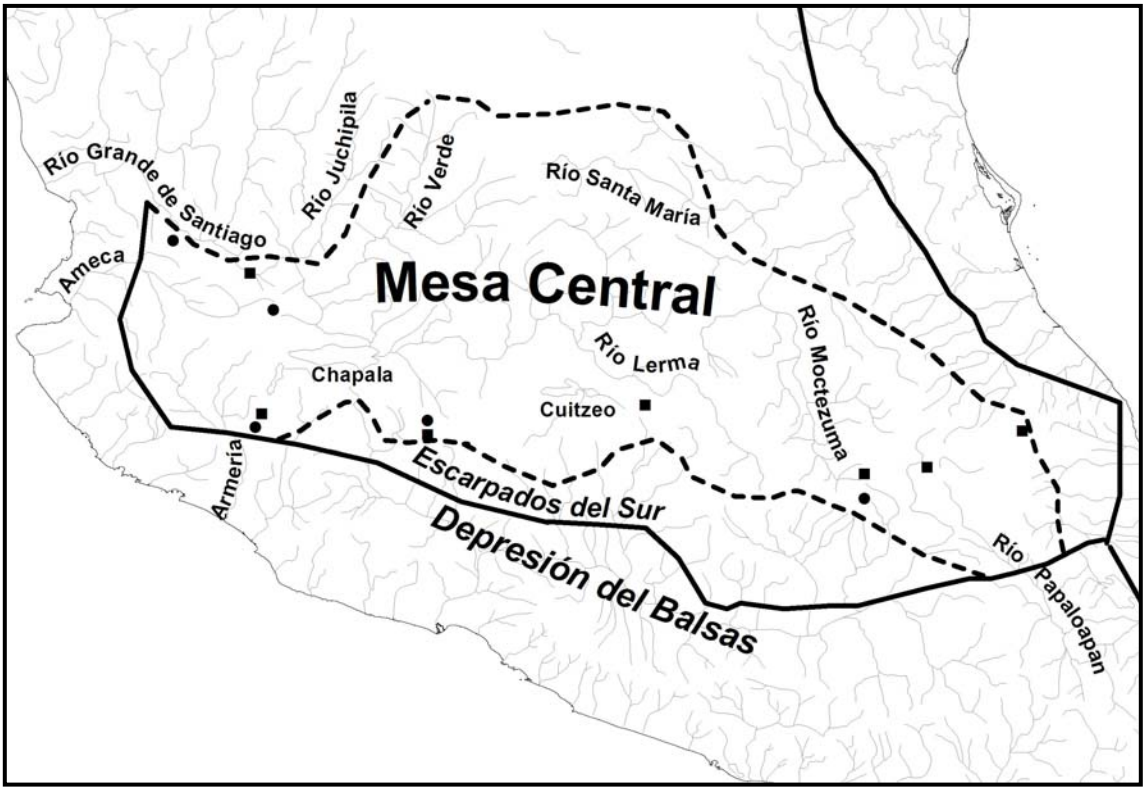


Figura 2. La Mesa Central de México y cuerpos de agua comprendidos dentro de la misma. En líneas punteadas los límites de la Mesa Central. En círculos se representan volcanes activos en tiempos históricos, en cuadros se representan los volcanes extintos (Tomado de West, 1964)

Sin embargo, se observa claramente que las tendencias de los principales drenajes en la región central de México están asociadas a los sistemas estructurales, por lo que la conformación hidrográfica de la Mesa Central ha variado de manera importante debido al intenso fallamiento, vulcanismo y constantes levantamientos de la zona.

La Mesa Central de México es una de las regiones lacustres más importantes de México; en esta zona encontramos un gran número de cuencas y cuerpos de agua de diversos tamaños (tanto endorreicos como exorreicos), formados en su mayoría por taponamiento de ríos debido a la actividad volcánica (v. gr. Zirahuén). Dichos lagos varían en altitud entre los 1400 a los 2600 msnm (Fig. 3).


Figura 3. Cuencas endorreicas de la Mesa Central de México (Tomado de Rojas-Rabiela, 2004)

Así mismo, uno de los rasgos representativos de ésta zona son los sedimentos lacustres entre las colinas volcánicas y sierras que varían en extensión y profundidad, lo que hace suponer la existencia de un gran número de lagos. Durante gran parte del Pleistoceno, estos lagos fueron formados por la interrupción de drenajes debido al vulcanismo, muchos de los cuales fueron abiertos por erosión de cabeceras de ríos y desecación por acumulación de sedimentos, resultando en vasos lacustres planos. Muchas de las cuencas interiores, particularmente en el sur, aun contienen remanentes de antiguos grandes cuerpos de agua, mientras que en el norte la mayoría se encuentran secos (Barbour, 1973).

Dichos lagos son reconocidos en el Valle de México, Chapala, San Marcos y Santa Rosa (Miller & Smith, 1986), así como algunos otros mencionados por diferentes autores

(Israde-Alcántara, 1999; Mateos *et al.,* 2002). Se ha inferido, de acuerdo a depósitos en la denominada Formación de Chapala, que el lago de Chapala tenía una extensión mucho mayor a la que actualmente tiene. Los depósitos sugieren una prolongada uniformidad de condiciones ambientales, a pesar de la fuerte actividad volcánica sugerida por estos mismos depósitos. Los depósitos lacustres de Jocotepec indican que en este lugar se encontraba un arroyo que fluía al Oeste, lo que sugiere un efluente del Lago de Chapala que posiblemente lo interconectaba con otras regiones (v. gr. Ameca).

San Marcos es una cuenca endorreica que contenía varios lagos someros que presentaban una fuerte fluctuación temporal (la mayoría de ellos están actualmente secos). El hallazgo de sedimentos lacustres demuestran la conexión entre el antiguo Lago de Chapala y los Lagos San Marcos, Zacoalco y Atotonilco. Esto sugiere la posibilidad que la antigua salida del Lago de Chapala fluía de Jocotepec a la cuenca de San Marcos (Miller & Smith, 1986). Por su parte Barbour (1973), con base en depósitos aluviales en la cuenca del Río Ameca, sugiere que los lagos del centro de México fluían a la cuenca de Chapala, para posteriormente fluir a la cuenca de San Marcos y por último encontrar su salida por el Río Ameca, representando este río la salida al mar del Río Lerma, lo cual es mencionado también por Tamayo & West (1964). Aunque es poco probable que el drenaje del Río Lerma fluyera directamente hacia el Río Ameca, la conexión e intercambio de fauna entre estas dos zonas, o con respecto al lago de Chapala ha sido ampliamente discutida y corroborada por diversos estudios realizados en organismos acuáticos de la zona (Mateos *et al.,* 2002; Doadrio & Domínguez, 2004; Webb *et al.,* 2004; Domínguez-Domínguez *et al.,* 2006a).

Tamayo & West (1964) mencionan que existe evidencia para aseverar que el Río Lerma y el Río Santiago eran cuencas independientes en un pasado geológico, hasta la captura del Río Lerma por el Río Santiago. Barbour (1973) menciona que el Río Lerma y el Santiago pudieron estar unidos en el pasado, sin embargo, una separación en el Pleistoceno Temprano es evidenciada por el vulcanismo de la zona entre Tepic y Guadalajara, por lo que periodos de interconexión y separación pudieron haber ocurrido hasta el presente. Esto ha sido recientemente corroborada con estudios moleculares de especies con poblaciones tanto en el Río Lerma como en el Río Verde (tributario este del Río Santiago) en Goodeidos (Domínguez-Domínguez *et al.,* 2008a) y Ciprinidos (Domínguez-Domínguez *et*

*al.*, 2007a, 2008b), arrojando divergencias menores a 1% entre las poblaciones de ambos Ríos, lo que sugiere que existió una conexión e intercambio de fauna menor a 1 Ma. Sin embargo las divergencias encontradas en algunas especies dentro de los género *Algansea*, *Ictaluridae* (datos no publicados) y Goodeidos (Domínguez-Domínguez *et al.*, 2008a) arrojan divergencias que van de 5 a 9%, lo que sugiere que el Río Santiago presenta al menos dos eventos e historias independientes en espacio y tiempo, una de ellas relacionada con la captura del Río Verde por el Río Santiago en fechas geológicas recientes y otra por la región oeste del Río Santiago y sus tributarios (vr. g. Ríos Juchipila, Clavillo, Bolaños), en el Mioceno-Plioceno.

Tamayo & West (1964) mencionan que en el Pleistoceno el Río Lerma estaba constituido por una sucesión de lagos orientados de Este a Oeste. Estos lagos fueron formados por el represamiento de sistemas de ríos bloqueados por flujos de lava. Mencionan también la posible conexión entre las cuencas endorreicas de Pátzcuaro, Zacoalco, Magdalena, Sayula, Cotija, Zirahuén, Cuitzeo y Valle de México, formando parte de la cuenca del Lerma en el Terciario. Esto es deducido por evidencia biológica más que geológica. Por su parte, De Buen (1943) sugiere que los lagos de Pátzcuaro, Zirahuén y Cuitzeo fueron sucesivamente separados del Río Lerma por algún tipo de actividad volcánica, siendo el Lago de Zirahuen el más joven, seguido de Pátzcuaro y por último, Cuitzeo. Lo anterior fue utilizado como argumento para sugerir una posible conexión fluvial que corría de Zirahuén a Pátzcuaro y posteriormente a Cuitzeo, a través del Río Grande de Morelia y señala una considerable diferencia en la diversidad de hábitats que cada uno presenta. Esta conexión fue corroborada con datos moleculares en Goodeidos (Doadrio y Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008a), fechando la conexión de estos cuerpos de agua en menos de 1 Ma.

Waitz (1943) menciona que la parte alta del Río Lerma estaba ocupada por un gran lago que se extendía hasta el Noroeste de Atlacomulco, lugar donde el río fue taponado por una intrusión basáltica presumiblemente en el Terciario Medio. Este taponamiento fue posteriormente erosionado, formando los rápidos que vemos en la actualidad, en áreas cercanas al Valle de Atlacomulco. En su curso, el Río Lerma es cortado por mas intrusiones basálticas, una entre Maravatío y Ziritzícuaro y, por último, cerca de la Presa de Solis (Acámbaro), además de presentarse una cascada de aproximadamente seis metros de altura

cerca de la presa Tepuxtepec (Barbour, 1973). La cuenca de Maravatío es la zona que ha sido mayormente afectada por el vulcanismo, que probablemente ha causado la formación y desecación de lagos en el pasado (Barbour, 1973). Para la región del Bajío, el Río Lerma se entrelaza con depósitos aluviales, flujos de lava y cráteres de volcanes, lo cual sugiere que un gran número de lagos se formaron en esta zona, sin que sus relaciones sean aún claras (Barbour, 1973).

Aguilera (1909) y Jaeger (1926) mencionan la posible conexión del Valle de México con el Pánuco vía el Río Tula, mencionando que un gran lago llenaba la mayor parte del Valle de México y que pudo haberse unido en la región más baja que separa el Valle con el Río Tula. Hibbard (1955) menciona una posible conexión del Valle de México con los Llanos de Puebla en el Pleistoceno Tardío, lo que sugiere que el drenado del Valle se llevaría a cabo por el Noreste y no hacia el Río Balsas.

Barbour (1973) menciona que el origen del Valle de México puede ser trazado en el Terciario Medio, en el tiempo que comenzó el fallamiento, cuando el Valle drenaba hacia el sur, hacia el actual Río Balsas. A partir del Mioceno y hasta el Plioceno Temprano, una fuerte actividad volcánica afectó al Valle de México, lo que provocó el cierre del dren hacia el Balsas en el Mioceno. En el Pleistoceno Temprano comenzó un nuevo ciclo de vulcanismo, lo que, combinado con factores climáticos, formó dos sistemas de cuencas, la primera se origina en Zumpango y Pachuca, drenando la regiones de Xochimilco, donde actualmente se encuentra la ciudad de México. Mientras que la otra cuenca drenaba la zona de Amecameca y Chalco hacia el Río Balsas. En el Pleistoceno Medio, nuevamente la actividad volcánica cerró la salida de estos drenes y se formó una cuenca cerrada en el Valle de México, que rápidamente se llenó de sedimentos aluviales. Alvarez (1963) menciona que del Valle de Tocumbo corría un tributario del lago de Chapala, el cual fue posteriormente taponado por un flujo de lava, formando así el Lago de San Juanico. Este lago y el antiguo Lago Magdalena fueron posteriormente drenados hacia el Río Balsas con fines agrícolas.

La captura de ríos de la Mesa Central por parte de cabeceras de ríos como el Pánuco o el Balsas es considerado como un fenómeno importante en la zona (Miller & Smith, 1986; Domínguez-Domínguez *et al.*, 2006a, 2008a), teniendo como ejemplo la captura de

tributarios por el Río Balsas (Tuxpan, Cupatitzio, Ario de Rosales), los cuales formaban parte de los ríos de la Mesa Central (Tamayo & West, 1964).

Por su parte, tributarios del Río Pánuco son mencionados como antiguos tributarios del Río Lerma, como lo es el Río San Juan, o bien que otros como el Moctezuma han erosionado fuertemente la Mesa Central incluso en nuestros días (Tamayo & West, 1964). El intercambio de ictiofauna entre las región del Río Lerma y la parte alta de algunos tributarios del Río Panuco parece haber ocurrido en al menos dos ocasiones, la primera es evidenciada por especies altamente divergentes con respecto a sus grupos más cercanos (± 6%), como lo son las especies *Xenoophorus captivus* y *Ataeniobius toweri* (Doadrio & Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008a). Una segunda conexión, posiblemente debida a captura de cabeceras de tributarios del Río Lerma por parte de tributarios del Río Panuco, se ha sugerido que ocurrió hace menos de 1Ma en base a dataciones del reloj molecular realizadas para poblaciones distribuidas en ambas cuencas de; *Poeciliopsis infans* (Mateos *et al.*, 2002), *Yutiria alta* (Domínguez-Domínguez *et al.*, 2007a) y *Notropis sallaei* (Schönhuth & Doadrio, 2003). La cuenca del Pánuco actualmente drena las aguas del Valle de México, a partir de la conexión artificial del mencionado valle con este río llevada a cabo entre los siglos XVI y XX, antes de lo cual, el Valle de México era una cuenca cerrada.

La composición y distribución de la fauna íctica también sugiere una posible captura de tributarios que fluían a las cuencas de la Mesa Central por parte de los Ríos Armería-Ayuquila y Compostela (Domínguez-Domínguez *et al.*, 2008a). En el caso de Compostela, la presencia de poblaciones de las especies *Xenotoca eiseni* y *Algansea avia*, las cuales están estrechamente relacionadas, con divergencias menores a 0.7%, con poblaciones distribuidas en la parte baja de la cuenca del Río Santiago, lo que sugiere la existencia de una conexión menor a 1Ma entre ambas cuencas (Domínguez-Domínguez *et al.*, 2008a; Perez-Rodríguez *et al.*, en preparación). Para la cuenca de Ayuquila-Armeria existe evidencia que de al menos dos eventos de conexión con cuencas de la Mesa Central. La primera de ellas es data en aproximadamente 7 Ma, de acuerdo a la presencia de la dos especies dentro de la tribu Ilyodontini (Webb, 2002; Doadrio y Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008a) y *Algansea aphanea* (Pérez-Rodríguez *et al.*, en prep). Mientras que por otro lado, la presencia de poblaciones de las especies *Goodea*

*atripinnis* y *Zoogoneticus quitzeoensis* sugieren un intercambio reciente durante el Plesitoceno (Domínguez-Domínguez *et al.,* 2008a), y las especies *Ictalurus dugesi* y *Scartomyzon austrinum*. De igual forma se ha establecido que diferentes conexiones en espacio y tiempo tuvieron que ocurrir entre esta cuenca y cuencas contiguas, como lo son las cuencas de los ríos Coahuayana y Ameca, como lo sugieren poblaciones de las especies *Cichlasoma istlanum, Astyanax aeneus,*e *Ilyodon furcidens.*

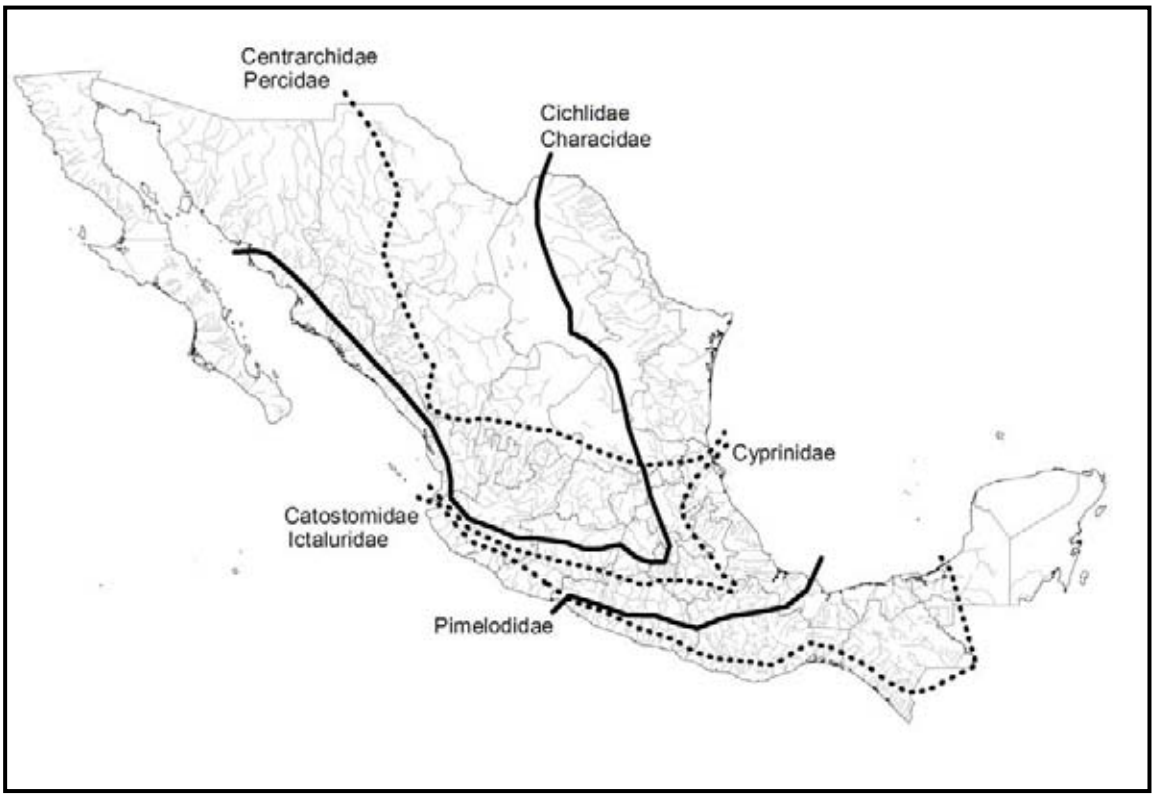


Figura 4. Patrones generales de los límites norte y sur de varias familias de peces de origen neártico (líneas punteadas) y neotropical (líneas sólidas). (Modificado de Stuart, 1964)

La variada y accidentada fisiografía derivada de la intensa actividad tectónica y volcánica ha generado la compleja historia paleohidrológica de la Mesa Central de México. Aunado a esto existen otros factores que han determinado la actual configuración de los sistemas hidrograficos de esta región del país: 1) la historia climática de la zona, donde se reportan al menos dos episodios secos y uno húmedo en los últimos nueve millones de años, los cuales afectaron de manera importante dicha paleohidrología y por ende tuvieron un efecto en la distribución de la ictiofauna (Domínguez-Domínguez *et al.,* 2008a), 2) la posición geográfica de México, lo que genera que en su región central, donde está

comprendida la Mesa Central, confluyan dos regiones biogeográficas distintas, la Neártica y la Neotropical, y por ende la presencia de elementos faunísticos con orígenes evolutivos distintos (Fig. 4) y 3) la propia adaptación de elementos ictiofaunísticos marinos a ambientes dulceacuícola (v. gr. Atherinopsidae). Lo anterior ha generado un complejo laboratorio natural, en el cual las especies han sido afectadas no solo por fenómenos físicos, sino también por diversas presiones ecológicas y biológicas, lo que ha moldeado la gran riqueza de especies y el elevado índice de endemismos entre los vertebrados de la Mesa Central.

De manera particular, en la Mesa Central de México (*sensu* Barbour, 1973) se han registrado aproximadamente 100 especies nativas de peces dulceacuícolas de las cuales el 70% son endémicas de la región (Guzmán-Arroyo, 1994). Mientras que, tan solo para la cuenca del Río Lerma-Santiago, se alcanza un nivel de endemismo del 66% (Miller & Smith, 1986, Lyons *et al.*, 1998), encontrando especies representativas de la fauna neártica (Ictaluridae y Cyprinidae), neotropical (Poecilidae y Cichlidae) y endémicas (Atherinopsidae y Goodeinae).

## 2. Los Goodeidos

### *Características generales*

Dentro de la icitiofauna más representativa de la Mesa Central encontramos a la familia Goodeidae, conformada por aproximadamente 21 géneros y 43 especies. La familia Goodeidae está dividida en dos subfamilias, Empetrichthynae y Goodeinae (Parenti, 1981; Doadrio & Domínguez, 2004), la primera está conformada por dos géneros, *Empetrichthys* y *Crenichthys* y cuatro especies distribuidas en la región del Valle de la Muerte y Nevada del Este en los Estados Unidos. Estos organismos presentan fertilización externa (los gametos son liberados al agua donde se lleva a cabo la fertilización), ovíparidad, y lecitotrofia (los embriones se nutren por medio del vitelo contenido en el huevo).

Por su parte, la subfamilia Goodeinae está representada por aproximadamente 41 especies distribuidas en 19 géneros (Domínguez-Domínguez *et al.*, 2005). Este grupo de peces está restringido a las cuencas hidrológicas de la Mesa Central de México y algunas regiones adyacentes, extendiéndose hacia el norte a la cuenca de los ríos Aguanaval y San

Pedro-Mezquital y al sur a la cuenca del Río Balsas por el Pacífico y a las partes altas de las Cuencas del Río Pánuco y Salado por el Golfo de México (Fig. 5).

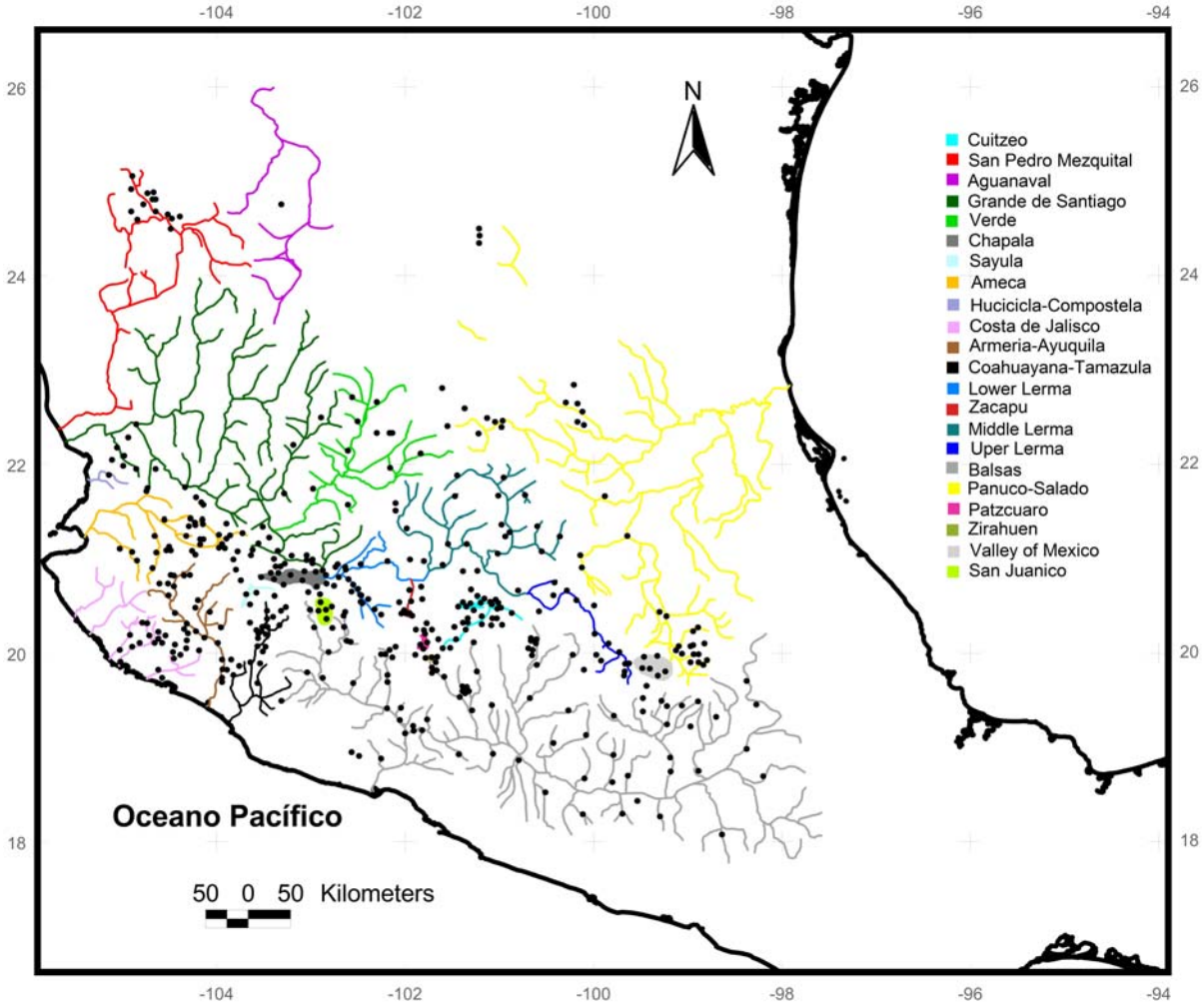


Figura 5. Distribución de la subfamilia Goodeinae en la Mesa Central de México y regiones adyacentes. Cada punto representa una localidad de colecta de al menos una especie de Goodeidos registrada en bases de datos ictiológicas (Tomado de Domínguez-Domínguez & Pérez-Ponce de León, 2007).

Una de las principales características de los Goodeinae, lo que los hacen que sean considerados como un tesoro natural, son sus adaptaciones únicas asociadas a una peculiar estrategia de reproducción y desarrollo embrionario (Domínguez-Domínguez & Pérez-Ponce de León, 2007). La primera de ellas es la fertilización interna, para lo cual, los machos presentan una modficación en la aleta anal a manera de lóbulo copulatorio; este lóbulo está formado por los primeros 5 a 7 radios anales, los cuales están reducidos y forman un pequeño abultamiento llamado espermatopodio (Fig 6). Esta modificación juega un papel crucial en la transferencia del paquete espermático (espermatozeugmata) al formar

una pequeña bolsa cuando el espermatopodio es presionado contra la abertura genital de la hembra, en este punto un órgano muscular interno en forma de anillo se contrae y expulsa el paquete espermático, siendo introducido en la apertura genital de la hembra, produciéndose así la fertilización interna.


Figura 6.- Macho y hembra de *Xenoophorus captivus* de la localidad de Jesús María en la región del Río Pánuco. La flecha indica el espermatopodio del macho (Tomado de Domínguez-Domínguez & Pérez-Ponce de León, 2007).

El estrecho contacto que es necesario para lograr de forma exitosa la fertilización en los goodeidos ha llevado a la aparición de estrategias de selección sexual entre los miembros de esta familia, las cuales han sido ampliamente discutidas como una de las posibles causas que han llevado a la gran radiación evolutiva de este grupo (Macias *et al.*, 1994, 1998; Bisazza, 1997; Macias & Ramirez, 2005; Ritchie *et al.*, 2005, 2007). Se ha mencionada que estos procesos de selección sexual han llevado a la aparición de un marcado dimorfismo sexual entre hembras y machos de algunas de las especies (Fig. 7), así como al elaborado cortejo que los machos de algunas especies adoptan para atraer la atención de la hembra (Domínguez-Domínguez & Pérez-Ponce de León, 2007).


Figura 7.- Macho y hembra de *Xenotoca eiseni* de la localidad de Tamazula en la cuenca del Río Coahuayana (Tomado de Domínguez-Domínguez & Pérez-Ponce de León, 2007).

Otra característica del grupo, quizá la más distintiva de los goodeinos, es su forma de nutrición embrionaria, llamada matrotrofia. Para ello, los embriones presentan una estructura especializada para la obtención de nutrientes y macromoléculas (lípidos y proteínas) y el intercambio gaseoso con la madre. Una vez que el ovulo fecundado en el folículo es expulsado al lumen del ovario (en teleósteos vivíparos los dos ovarios se fusionan para formar uno solo) el embrión completa su desarrollo (gestación intraluminar), y ya que el saco vitelino es absorbido por completo, el embrión comienza a desarrollar una estructura a partir de la parte posterior del intestino, en la parte ventral a la altura del ano, la cual es conocida como trofotenia (Fig. 8).


Figura 8.- Cría recién nacida de *Characodon lateralis* de la localidad de Los Berros en la región del Río Mezquital mostrando el proceso trofoténico en forma de listón (Tomado de Domínguez-Domínguez & Pérez-Ponce de León, 2007).

La trofotenia presenta una superficie altamente vascularizada, y le sirve al embrión como una estructura para la obtención de nutrientes directamente del fluido contenido en el lumen ovárico (histotrofia), el cual es rico y abundante en este tipo de substancias (Wourms, 1981; Uribe *et al.*, 2004). La trofotenia tiene diferentes formas y tamaños dependiendo de la especie, e incluso algunas pueden carecer de ella, al menos de forma aparente, tal es el caso de *Ataenobius toweri*, la cual, más que carecer de trofotenia, se ha visto que está muy reducida y solo escaso tejido trofoténico a sido detectado (Wourms, 2005). Se han reportado otros tipos de nutrición embrionaria en Goodeidos, sin embargo la trofotenia es la más característica y la que aporta la mayor cantidad de nutrientes al embrión.

La viviparidad confiere importantes ventajas a los goodeinos, como lo es el nacimiento de crías en un estado más avanzado de desarrollo, de mayor talla y con la posibilidad de acceder a una gama mayor de artículos alimentarios, por lo que sus probabilidades de sobrevivencia son más altas; sin embargo presentan la desventaja de en el número de descendientes, el cual es mucho menor que en los peces ovíparos.

***Estado de conservación de los goodeidos en el centro de México***

Desgraciadamente, esta subfamilia de peces endémicos del Centro de México, al igual que la mayor parte de la fauna acuática de esta región, está en grave riesgo de desaparecer. Aunque los Goodeinae como grupo están distribuidos en gran parte de la Mesa Central, incluyendo 15 estados de la República Mexicana, la mayoría de las especies presentan intervalos de distribución muy restringidos y varias de ellas viven en pequeñas porciones dentro de las cuencas donde se distribuyen, llegando a encontrar especies microendémicas que viven en pequeños manantiales, o en tramos de pequeños arroyos montañosos (v. gr. *Chapalichthys pardalis, Zoogoneticus tequila* y *Allotoca zacapuensis*). Más aun, estudios genéticos han revelado la fuerte estructuración y diferenciación genética que existe entre las poblaciones de algunas especies de amplia distribución, como es el caso de *Zoogoneticus quitzeoensis, Xenotoca variata, X. eiseni* y *Alloophorus robustus* (Doadrio & Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008a).

Esta marcada endemicidad y estructuración genética se enfrenta a la grave problemática ambiental que aqueja a los hábitats dulceacuícolas de la Mesa Central, región

donde se concentra la mayor densidad poblacional, como lo son el Estado de México, Distrito Federal y Jalisco (INEGI, 2006). Esto hace que la presión ejercida por las actividades humanas hacia los servicios ambientales que los ecosistemas acuáticos proporcionan sea de gran magnitud. Más aun, la cuenca más representativa de la Mesa Central, el sistema hidrológico Lerma-Chapala, enclavado en el Cinturón Volcánico Transmexicano, presenta uno de los mayores volúmenes de extracción de agua del país, tanto de aguas subterráneas como superficiales (SEMARNAT, 2006), así mismo, la cuenca del Lerma sustenta una de la economías más significativas del país, con una importante cantidad de industrias y zonas agropecuarias en sus márgenes, por lo que es considerada como una de las cuencas más contaminadas e impactadas del mundo (Soto-Galera *et al.,* 1998; Mercado-Silva *et al.,* 2006). Paradójicamente, este sistema hidrológico es uno de los más importantes para la diversidad ictiológica del país y en especial para la subfamilia Goodeinae, albergando 17 especies (Domíguez-Domínguez *et al.,* 2005). Además de la sobreexplotación de los cuerpos de agua existen otros factores que contribuyen al deterioro de los ecosistemas acuáticos e impactan de manera negativa a su flora y fauna, tal es el caso de la contaminación del agua, la deforestación de las cuencas, la modificación del hábitat y la introducción de especies exóticas y sus parásitos (Soto-Galera *et al.,* 1998, 1999; Chacón-Torres & Rosas-Monge, 1998; Orbe-Mendoza *et al.,* 2002; Mercado-Silva *et al.,* 2002, 2006).

De manera específica son varios los trabajos que abordan la problemática ambiental en torno a las especies de Goodeinos (De la Vega-Salazar *et al.*, 2003; Domínguez-Domíguez *et al.,* 2006b; Escalera-Vázquez *et al.,* 2006; Domínguez-Domínguez *et al.,* 2008c). Desafortunadamente, en algunos casos dichos impactos han llevado a la extinción de algunas especies (v. gr. *Characodon garmani* y *Skiffia franceasae*), aunque de esta última aun se mantienen organismos en cautiverio. Asimismo, existe la posibilidad de que otra esté ya extinta (*Zoogoneticus tequila*), y el riesgo de que se extingan otras tantas (v. gr. *Ameca splendens, Allotoca goslinae, Allotoca maculata* y *Allodontichthys polylepis*), las cinco últimas pertenecientes a la cuenca del Río Ameca en el estado de Jalisco, donde la contaminación del agua por fertilizantes agrícolas, la desecación, la introducción de especies exóticas y la fragmentación del hábitat por la construcción de embalses ha sido la

causa principal del deterioro en su estado de conservación (De la Vega-Salazar *et al.*, 2003; Escalera-Vázquez *et al.*, 2006; Domínguez-Domínguez *et al.*, 2005, 2006b; 2008c).

De manera oficial, de acuerdo con el Diario Oficial de la Federación (DOF, 2001), del total de las 185 especies de peces enlistadas en alguna de las categorías de conservación, 14 pertenecen a la familia Goodeidae, de las cuales dos son consideradas especies extintas en la naturaleza, ocho en peligro y cuatro amenazadas. A pesar de este esfuerzo por catalogar las especies de peces del país en alguna categoría de conservación, datos obtenidos por distintas instituciones de investigación en el país, han puesto de manifiesto que la Norma Oficial Mexicana-059 (DOF, 2001) está muy lejos de reflejar la magnitud del problema ambiental de los cuerpos de agua de México. Por su parte, Domínguez-Domínguez *et al.* (2005), tomando en cuenta una gran cantidad de datos históricos y actuales de los peces de la subfamilia Goodeinae, y siguiendo los criterios de clasificación de la Unión Internacional para la Conservación de la Naturaleza (UICN), concluyen que 2 especies están extintas en la naturaleza (una con poblaciones en cautiverio), 17 en peligro crítico (una posiblemente extinta), cinco en peligro, dos amenazadas, 11 vulnerables y tres en bajo riesgo. Así mismo, autores como Duncan & Lockwood (2001) consideran a los Goodeidos como un grupo de peces con uno de los mayores riesgos de extinción en el mundo. Además de que se ha puesto de manifiesto la incapacidad del actual sistema nacional de áreas naturales protegidas para resguardar áreas con una alta riqueza de especies de Goodeidos y más aun, de la riqueza genética de muchas de sus poblaciones (Domínguez-Domínguez *et al.*, 2006a, 2007b).

## 3. La filogeografía

La combinación de los análisis filogenéticos con la genética de poblaciones y la biogeografía ha tenido fuertes repercusiones en años recientes en las áreas de le biología evolutiva, la ecología y la conservación. En síntesis, la filogeografía estudia los principios y procesos que gobiernan la distribución geográfica de los linajes genealógicos (Avise *et al.*, 1987, Avise, 2000). Debido a que la filogeografía es el estudio de los aspectos históricos de la actual distribución de los linajes genealógicos, es considerada como una subdisciplina de la biogeografía histórica, la cual integra conceptos y técnicas de biología molecular, genética de poblaciones, demografía, sistemática filogenética, etología y paleontología

(Avise, 2000), o bien es mencionada como parte de la biología evolutiva, siendo el parte aguas entre la microevolucion (relaciones tocogenéticas) y la macroevolución (relaciones jerárquicas), recayendo en la frontera de la especiación.

La filogeografía aplica el análisis de genealogías genéticas al estudio de la evolución de las poblaciones. Es capaz de determinar el impacto de los eventos históricos en la composición genética y en la estructura de las poblaciones modernas, y por ello ha venido a revolucionar la ecología, la biogeografía y la genética de poblaciones (Avise et *al.*, 1987). A pesar de que esta disciplina se ha utilizado comúnmente como una herramienta para establecer patrones evolutivos entre las poblaciones de una misma especie, las aproximaciones filogeográficas también pueden ser útiles para inferir procesos demográficos históricos como el flujo génico, el tamaño efectivo de la población, la secuencia de colonización, cuellos de botella, las trayectorias evolutivas, fronteras entre especies e identificar unidades de conservación, siendo también considerada como una herramienta importante en los métodos actuales de biogeografía histórica a nivel intraespecífico.

De esta forma, el uso de la distribución geográfica de datos filogenéticos ha sido ampliamente usada para descubrir eventos históricos, como la fragmentación de hábitats o expansión en el rango de distribución, eventos de migración, extinción de los linajes génicos en determinadas áreas, así como muchos otros procesos que afectan la estructura de las poblaciones o que causan especiación en un contexto espacial y temporal (Hardy *et al.*, 2002). Además, el estudio comparado de los patrones filogeográficos de varias especies o poblaciones co-distribuidas contribuye a plantear hipótesis sobre posibles eventos comunes de vicarianza o dispersión y a identificar las causas geológicas, ecológicas o etológicas que pudieron haber influido en ellos (Lanteri & Confalonieri, 2003).

En esencia, la filogeografía parte de la idea de que la gran mayoría de las especies en la naturaleza exhiben cierto grado de estructura genética asociado con la geografía. Esta estructura puede ser muy compleja, como en especies que habitan áreas de fuerte actividad tecto-volcánica o paleoclimatica, o de menor complejidad, como el caso de especies que experimentas migraciones de individuos frecuentemente o que su aislamiento, hablando en tiempos geológicos, es relativamente reciente (v. gr. última glaciación). De esta forma, cuando una dimensión genealógica es analizada a la par de los patrones geográficos, la

estructura filogeográfica se pone en evidencia. Es decir, la estructura filogeográfica refleja la interacción entre los procesos demográficos y genealógicos y la dinámica de los procesos de la tierra (geológicos o climáticos).

Actualmente los estudios filogeográficos en especies animales se basan principalmente en secuencias de ADN mitocondrial, debido a que, en general, el ADNmt presenta una elevada tasa de mutación, no recombina y su herencia es casi exclusivamente materna, es decir va acorde con la teoría de la neutralidad y la coalesencia (Lanteri & Confalonieri, 2003). La coalescencia es un proceso estocástico que describe como los eventos genéticos poblacionales determinan la forma de la genealogía de las secuencias de genes muestreadas. Dado que los haplotipos o variantes del ADNmt registran una historia matrilineal de eventos mutacionales, es posible conectarlos de un modo filogenéticamente inteligible en un filograma, el cual se superpone con la distribución geográfica del grupo de estudio, conllevando a la reconstrucción filogeográfica (Avise, 2000). Otra alternativa es hacer uso de los múltiples análisis estadísticos que se han desarrollado en los últimos años, los cuales incorporan los filogramas, la coalescencia y valores estadísticos de probabilidad. Sin embargo, el uso exclusivo del ADN mitocondrial en algunos casos puede ser riesgoso, ya que está implicado un único locus que puede estar ligado a selección, ser afectados por deriva génica, presentar introgresión o que su dispersión entre las poblaciones estudiadas este obscurecida por diferencias etológicas o ecológicas entre hembras y machos, todo esto puede implicar limitaciones en el momento de hacer la reconstrucción de historias poblacionales (Vázquez-Domínguez,  2007). Por ello, en los últimos años se ha experimentado un aumento en el uso combinado y compartido de genealogías obtenidas de datos de ADN mitocondrial y ADN nuclear, lo cual conlleva los problemas inherentes de la recombinación y la posibilidad que cada uno de los locus (que en algunas especies suelen ser mas de dos) arrojen historias evolutivas independientes (Vázquez-Domínguez, 2007). Para enfrentar este problema, existen dos aproximaciones; 1) hacer reconstrucciones y comparaciones de más de un gen, con lo cual, en caso de existir congruencia en las historias filogeográficas tratadas, aumentaríamos la probabilidad de que la historia filogeográfica que estamos obteniendo sea de la especie y no de un gen, y 2) realizar comparaciones filogeográficas entre más de una especie co-distribuidas, con lo cual se intentaría encontrar patrones filogeográficos recíprocamente apoyados.

En general la filogeografía es dividida en dos escuelas que difieren en sus bases y análisis. La primera se basa fundamentalmente en una idea grafico-descriptiva de las ramificaciones de un árbol para convertirla en una hipótesis de la historia biogeográfica de los organismos, lo cual es sustentado en métodos de reconstrucción filogenética de árboles de genes, o bien, en la construcción de redes de haplotípos, las cuales se basan en la reconstrucción de arboles multibifurcados arreglados a manera de redes de haplotípos bajo métodos filogenéticos o métodos de coalescencia. La segunda escuela se basa en fundamentos estadísticos y matemáticos de demografía y estructura poblacional (Hey & Machado, 2003). Sin embargo, los límites de cada una están aún en discusión y los métodos pueden enmarcarse en una escuela u otra de acuerdo con el autor que sea revisado. A continuación se presentan los argumentos en los cuales se basa la fundamentación del uso de la filogeografía en dos aspectos nodales del presente trabajo, que son la taxonomía, básicamente en el uso de la filogeografía en el reconocimiento y establecimiento de límites entre especies y por otro lado, su uso en la conservación.

***La filogeografía en el ámbito de la taxonomía***

La filogeografía puede también ser usada como una herramienta en estudios taxonómicos. En sistemática, los taxa son el punto de partida para la clasificación biológica y los estudios filogenéticos. Sin embargo, cuando el taxón se convierte en la herramienta para el estudio de entidades evolutivas, el encontrar las fronteras y atributos de una unidad evolutiva (taxón) se vuelve complicado, mas cuando la respuesta puede variar en cada grupo de organismos (Hey & Machado, 2003). Los conceptos de especie acuñados en los últimos 40 años son conflictivos y en ocasiones contradictorios, lo que hace que aun no exista un acuerdo general. En este sentido, la filogeografía, combinada con otros métodos usados en la taxonomía tradicional, puede aportar información de las fronteras entre especies o poblaciones. El uso de caracteres moleculares en la delimitación de especies inicia cuando se acuña el concepto filogenético de especie, el cual es descrito como el agrupamiento mínimo de individuos, de poblaciones o grupos de poblaciones que son diagnosticables por un número dado de caracteres compartidos dentro de los cuales hay un patrón claro de ancestaría-descendencia (Cracraft, 1983; McKitrick & Zink, 1988; Nixon &

Wheler, 1990; Davis & Nixon, 1992), es decir la unidad taxonómica mínima que puede ser analizada desde un punto de vista filogenético.

Al delimitar una especie bajo el concepto filogenético se debe poder identificar las fronteras por arriba de las cuales el arreglo filogenético representa entidades biológicas independientes (relaciones jerárquicas), y por debajo de ellas, esta jerarquización es inadecuada (relaciones tocogenéticas o genealógicas) (Goldstein *et al.,* 2000). El uso de caracteres moleculares como una herramienta importante (aunque no exclusiva) en la delimitación de especies ha venido creciendo en la literatura taxonómica. Sin embargo este concepto filogenético de especie ha sido ampliamente discutido y criticado por la problemática de distinguir la historia de los caracteres de la historia de las especies (Sites & Crandall, 1997) y el hecho de que la mayoría de las reconstrucciones filogenéticas usan un solo locus, por lo que eventos como el polimorfismo dentro de la especie puede arrojar reconstrucciones filogenéticas erróneas (Templeton, 2001). Por ello el uso de diferentes caracteres en muchas ocasiones resulta en el diagnostico de unidades evolutivas diferentes dentro de una misma especie (ver Crother, 1990; Smith, 1992; Moore, 1995).

El conceptos de especie delimitado por caracteres moleculares es definido como un grupo de organismos o poblaciones que presentan monofilia reciproca, los cuales son candidatos a ser identificados como unidades evolutivas independientes. Este concepto es prácticamente igual al término acuñado por Donoghue (1985) con el concepto monofilético de especie. Sin embargo, la monofilia reciproca (entendida en el contexto de árboles de genes) no es el único concepto implícito. El problema persiste en cuanto a qué nivel jerárquico la monofília reciproca es aplicable a la delimitación de especie o es una mera representación de unidades dentro de una especie, por lo que el uso de relaciones filogenéticas intraespecificas se ha mencionado como inapropiadas en la delimitación de especies (Golstein *et al.,* 2000). Es decir, una de sus mayores dificultades es el encontrar la frontera entre las relaciones tocogenéticas y jerárquicas, lo cual es un tema debatido en todos los ámbitos donde la filogeografía es aplicable.

Por otra parte, Templeton (2001) menciona que los arboles de genes tienen el potencial de encontrar la interface entre la evolución intra e interespecifica, por lo que es capaz de delimitar especies, de manera que acuña el termino de especie “cohesiva”. Este concepto es descrito como un linaje evolutivo cuyas fronteras pueden ubicarse a partir de

las fuerzas genéticas y evolutivas que crean una cohesión reproductiva en la comunidad (Templeton, 1999). Templeton (2001) denomina un linaje evolutivo como una población o grupo de poblaciones reproductivas con suficiente historia de relaciones de ancestria-decendencia, la cual presenta sus propias trayectorias y tendencias evolutivas. Por ello una especie es definida como un linaje o un grupo de linajes evolutivos que pueden presentar intercambio genético y/o presentar cohesion ecológica. Templeton (2001) propone el uso del Análisis de Clados Anidados (NCA por sus siglas en inglés) como un método de análisis riguroso y con los criterios para prevenir la confusión entre árboles de haplotípos y aquellos de poblaciones o especies, ya que es capaz de identificar los efectos del flujo genético recurrente y los eventos históricos que han afectado a toda la población, los cuales pueden causar asociación geográfica en los arboles de genes. La prueba más fehaciente que arroja el NCA para inferir que las muestras en cuestión se derivan de linajes evolutivos independientes, es cuando uno o más eventos de fragmentación son inferidos. Otro atributo que Templeton (2001) menciona del NCA es el incorporar el polimorfismo interespecífico, sorteo de linajes y eventos de hibridación, representando una ventaja a la hora de delimitar especies. En el NCA los linajes evolutivos son inferidos por patrones filogenéticos que tienen un valor estadístico y no como un patrón absoluto (Templeton, 2001).Una crítica en el uso exclusivo de análisis filogeográficos para ubicar las fronteras entre especies está relacionado con uno de los conceptos básicos en la teoría de coalescencia que es la herencia uniparental y la no recombinación del gen utilizado (en caso de estar representado exclusivamente por ADN mitocondrial). Por ello, las relaciones de los árboles mitocondriales pueden representar de manera errónea las relaciones jerárquicas entre poblaciones o especies. Se ha sugerido que genes mitocondriales por sí solos no son suficientes para diagnosticar especies, ya que los caracteres se fijaran más rápido que los nucleares, por lo que estos pueden no reflejar la “verdadera” frontera entre especies (Avise & Ball, 1990). Con base en lo anterior, se recomienda el uso de datos nucleares, los cuales corroborarían o rechazarían la hipótesis obtenida con genes mitocondriales, o bien, la utilización de otro tipo de caracteres (v. gr. morfológicos o etológicos).

***La filogeografía en el ámbito de la conservación***

Al igual que en la taxonomía, la filogeografía está resultando una herramienta importante en la biología de la conservación. La idea de proponer políticas de conservación en unidades por debajo del nivel de especie utilizando datos moleculares cobró mayor importancia cuando el concepto de Unidades Evolutivas Significativas (ESUs por sus siglas en inglés), fue mencionado por primera vez por Ryder (1986). Sin embargo, a la fecha, analizar la diversidad genética por arriba del nivel de especie está parcialmente bien definido con métodos filogenéticos, sin embargo, la manera de representar de mejor manera la diversidad genética por abajo del nivel de especie es una tarea aun no resuelta, y es precisamente aquí donde la filogeografía puede ser una herramienta importante.

Esta idea de conservación a nivel infraespecífico pretende identificar de manera precisa unidades de manejo que reflejen la importancia evolutiva de los linajes dentro de las especies y con ellos crear programas efectivos para la conservación de especies en riesgo (Avise, 1994). Por ello, la información genética heredable ofrece una forma de delinear dichas unidades de conservación, y provee un contexto evolutivo a partir del cual desarrollar estrategias y definir prioridades de conservación (King & Burke, 1999). En este sentido, desde que se reconoció a la diversidad genética como el nivel basal de la biodiversidad (como fue recomendado en la Convención Sobre la Diversidad Biológica en Brasil, 1992), la genética de la conservación se ha desarrollado de manera exponencial, y en la actualidad es común encontrar trabajos que usan caracteres moleculares para priorizar especies o poblaciones para su conservación.

La genética de la conservación, de forma general, trata de hacer inferencias de eventos genéticos que son relevantes en el conocimiento y conservación de la diversidad. Esto inicia desde la base de un conocimiento amplio de la diversidad biológica (incluyendo el reconocimiento de especies o unidades evolutivas independientes), hasta el conocimiento del tamaño efectivo de las poblaciones (el cual difiere del concepto de un tamaño poblacional censal usado en ecología), la depresión genética por endogamia, por exogamia, o cuellos de botella, el efecto de la fragmentación y el flujo genético en las poblaciones y la reducción en la adecuación de las especies para poder sobrevivir a las condiciones cambiantes de un entorno dinámico. En esencia, la genética de la conservación pretende no solo identificar aquellas especies en grave peligro de extinción, sino los eventos que han

podido afectarlas y como revertirlos, pero sobre todo intenta aportar las bases para conservar no solo las especies (con la problemática que implica su "correcta" identificación), sino las unidades evolutivas dentro de ellas y preservar sus procesos evolutivos, asegurando la permanencia de las especies a largo plazo y las implicaciones evolutivas relacionadas.

Sin embargo, ha existido una importante discusión en relación a cómo usar la información genética en la identificación de "grupos operativos" en conservación, la cual, de forma práctica, se enmarca dentro de tres grandes rubros: la viabilidad de las poblaciones a largo plazo (Loeschecke *et al.*, 1994); la identificación de unidades biológicas para su protección (Moritz, 1994; Amato *et al.*, 1995); y la identificación de las relaciones históricas entre poblaciones (Avise & Hamrick, 1996).

En este sentido, un gran número de definiciones han sido acuñadas para nombrar estos "grupos operativos". Dentro de todos estos éstos términos, el más usado en estudios filogeográficos que son enmarcados en un contexto de conservación es el concepto de ESUs, el cual incluso ha sido incorporado a la legislación ambiental en un importante número de países como una forma de identificar poblaciones distintas con fines de conservación, sin embargo esto no es del todo aceptado (Pennock & Dimmick, 1997).

El primer concepto de ESU fue acuñado por Ryder (1986) que lo definía como un grupo de organismos que han estado asilados de otros grupos de la misma especie por un periodo de tiempo suficiente para haber desarrollado divergencias genéticas significativas entre ellos. Más tarde, Moritz (1994, 2002) define una ESU como un grupo de individuos o poblaciones que presentan monofilia recíproca para marcadores mitocondriales y divergencias significativas en frecuencias alelicas en loci nucleares, pudiéndose referir a poblaciones, especies o subespecies, considerando también el tiempo que dichas poblaciones han estado aisladas (Avise & Ball, 1990).

Algunas de las principales críticas mencionadas a estas aproximaciones es que ningún método filogenético es tan poderoso como para poder inferir una filogenia correcta, más cuando se trata de poblaciones dentro de una misma especie, por el contrario lo que se genera es una hipótesis con una probabilidad de que lo sea. De igual forma, las variaciones estocásticas pueden generar arreglos erróneos en un árbol de poblaciones, por lo que la monofília reciproca no siempre denota aislamiento histórico (Crandall *et al.*, 2000). En este

sentido, el tamaño poblacional es un factor importante para que se de la monofilia reciproca, de esta forma, imaginando dos casos en que una especie es dividida en dos poblaciones por una barrera, cuando una de las poblaciones que se ha aislado es pequeña, el tiempo que tiene que transcurrir para que califique como una ESU es mucho menor que en el caso de que la población aislada sea mucho mayor (Neigel & Avise, 1986). Esto es aun más marcado en la genética de la conservación, ya que la mayoría de las especies o poblaciones con importancia para la conservación son naturalmente pequeñas, o bien han sufrido un declive en su tamaño poblacional, experimentado fragmentación o simplemente perturbadas (Perase & Crandall, 2004).

También se hace mención que el concepto no hace suficiente énfasis en el potencial de las especies para maximizar el éxito evolutivo mediante el mantenimiento de la diversidad adaptativa (Lynch *et al.,* 1999), y que un planteamiento exclusivamente genético tendría graves riesgos en el reconocimiento del potencial adaptativo y la adecuación. Finalmente, los proponentes del concepto filogenético de especie mencionan que si cualquier linaje evolutivo perfectamente diagnosticable tiene que ser elevado al nivel de especie, entonces el concepto de ESU está de más (Vogler y De Salle, 1994).

El uso de marcadores moleculares altamente variables en la conservación de especies en peligro y su uso en los planes de manejo ha llevado a la implementación de nuevos conceptos en biología de la conservación, las Unidades de Manejo (MU's por sus siglas en inglés). Estas unidades intentan integrar la diversidad genética y la demografía de distintas poblaciones, las cuales tienen que ser manejadas de manera independiente para asegurar la viabilidad de una ESU (Moritz, 2002). Por otor lado, uno de los últimos métodos que se han desarrollado en el uso de la genética de poblaciones para fines de conservación es la combinación de análisis filogeográficos, la genética del paisaje (landscape genetics) y las aproximaciones estadísticas, cuya combinación es capaz de definir la estructura poblacional a través del terreno y la historia demográfica de las poblaciones, identificando las poblaciones a ser conservadas y su distribución geográfica (Masta *et al.,* 2003).

La diversidad biológica incluye la variación genética entre especies y dentro de las especies, tanto en poblaciones geográfica y genéticamente separadas como a nivel de individuos dentro de cada población. Sin embrago, el encontrar un método que lleve a la

correcta identificación de dichas unidades es prácticamente imposible dada la gran diversidad de posibilidades encontradas en la naturaleza, e incluso, dada la carencia de métodos adecuados o la correcta aplicación de los existentes. Por ello, se debe tener claro en el proceso de la identificación de unidades de conservación la división de la diversidad biológica en dos componentes: aquella resultante del aislamiento histórico y aquella que tiene que ver con la evolución adaptativa (Moritz, 2002).

A pesar de todo lo anterior, el uso exclusivo de datos moleculares para definir las estrategias de conservación dentro de una especie puede ser altamente riesgoso. Para que todos estos conceptos y aplicaciones de la genética de la conservación puedan ser de utilidad en los planes de conservación, los conceptos teóricos tienen que ser prácticos en su aplicación. Los datos obtenidos de esta manera, deben ser cuidadosamente evaluados junto con datos históricos, ecológicos, sociales y de distribución, con la finalidad de obtener una perspectiva más acertada y realista (Crandall *et al.,* 2000). Recientemente otras fuentes de información han sido incorporadas a la identificación de grupos operacionales para conservación, como lo es la distribución espacial de la diversidad genética, datos taxonómicos y fenotípicos, los servicios ecológicos y ambientales, datos biogeográficos, aspectos socioeconómicos y datos etológicos (Doadrio *et al.*, 1996; Dodson *et al.*, 1998; Luck *et al.*, 2003; Manel *et al.*, 2003; Green, 2005).

## 4. Planteamiento del problema

La evolución y dispersión de los peces de agua dulce están estrechamente relacionadas con la paleogeografía y especialmente con la historia de conexión, captura y separación de cuerpos de agua como consecuencia de la actividad tectónica, volcánica y/o climática (Durand *et al.,* 1999). Por esta razón, la evolución de los sistemas hidrográficos es la principal causa de dispersión, diversificación y especiación en peces de agua dulce (Bermingham & Martín, 1998). Sin embargo, en los últimos años existe una amplia discusión que incorpora otros elementos como motores de la evolución de los sistemas bióticos, como lo son las características intrínsecas de los organismos relacionadas con la etología, ecología y biología (Bisazza, 1997; Panhuis *et al.,* 2001).

La historia biogeográfica de la ictiofauna de la Mesa Central de México, ha sido materia de una amplia discusión por muchos años, como lo muestran los trabajos pioneros

de De Buen (1943), Barbour (1973) y Álvarez del Villar (1972), así como los de Miller & Smith (1986), Parenti (1981), Moncayo-Estrada *et al.* (2001), Pérez Ponce de León (2003), Webb *et al.* (2004) y Doadrio & Domínguez (2004). En la mayoría de estos trabajos se estudió la distribución de las especies desde un punto de vista histórico y descriptivo, sin utilizar ningún método reconocido en biogeografía histórica. El primer trabajo en presentar un análisis biogeográfico de la ictiofauna de la Mesa Central (Goodeinae) utilizando métodos en biogeografía histórica es el presentado por Domínguez-Domínguez (2004), posteriormente se realizaron otros trabajos usando este tipo de métodos y los goodeines como grupo de estudio (Gesundheit & Macías, 2005; Domínguez-Domínguez *et al.*, 2006). La mayoría de estos trabajos concuerdan con que la historia biogeográfica de la Mesa Central y su biota, ha estado ligada a la compleja historia tectónica, volcánica y climática desde el Mioceno temprano, acentuándose con el aumento en el vulcanismo desde el Plioceno, mismo que continúa hasta nuestros días (West, 1964; Ferrari, *et al*., 2000). Esta intensa actividad volcánica, tectónica y climática ha generado un sistema hidrológico cuya interpretación histórica es compleja. Dicha historia responde a patrones espacio-temporales de interconexiones de ríos, captura de cuerpos de agua, compartamentalización de cuencas y aislamiento por barreras, resultando en la configuración hidrológica observada en tiempos recientes. Por otra parte, estos patrones espacio-temporales han tenido una influencia directa en la historia evolutiva y biogeográfica de los componentes faunísticos dulceacuícolas, como lo son la dispersión de las poblaciones de peces por los eventos de interconexión entre cuerpos de agua y la captura de cabeceras de ríos, aislamiento ancestral entre poblaciones por fenómenos de vicarianza inducidos por la formación de barreras biogeográficas, así como la influencia del cambio climático durante el pleistoceno en el aislamiento poblacional y con ello en la radiación inter e intrapoblacional (Doadrio & Domínguez, 2004; Domínguez-Domínguez *et al.*, 2006, 2008a).

Sin embargo, los trabajos antes mencionados no arrojan datos concretos a una escala más fina de los procesos que han gobernado la historia evolutiva y biogeográfica de la Mesa Central y su biota, y más aun, no llegan a conclusiones de las implicaciones que dichos eventos y procesos biogeográficos han tenido en la diversidad, taxonomía y demografía de los grupos de peces y sus implicaciones de conservación. Por lo que la presente tesis plantea los siguientes objetivos. 1) El uso de herramientas moleculares

utilizando como modelo de estudio a tres especies de goodeidos con amplia distribución en la región Central de México, para el estudio y entendimiento de los procesos evolutivos y demográficos, tanto entre especies como entre poblaciones, y su relación en espacio y tiempo con eventos tectónicos, volcánico y climáticos en el contexto de la compleja historia geológica y climática de la región. 2) Mediante la comparación de los diferentes análisis de datos moleculares, se pretende indagar en la posible influencia que ha tenido las diferencias ecológicas, biológicas y etológicas de cada una de las especies en su historia filogeográfica y demográfica, y más aun, mediante el uso de dos grupos de marcadores moleculares (Citocromo *b* y microsatellites), se pretende establecer la influencia que han tenido los eventos históricos (barreras biogeográficas) y recientes (procesos de deriva génica) en la estructuración genética y la historia filogeográfica. 3) Utilizar los datos moleculares como una herramienta en el reconocimiento de linages evolutivos independientes, y en combinación con datos morfológicos, merísticos y biogeográficos impactar en la taxonomía del grupo mediante la delimitación y descripción de especies. 4) La información en torno a la evolución de la diversidad biológica de la Mesa Central obtenida mediante el análisis de los datos moleculares, así como la integración de dicha información con los datos taxonómicos y aquellos derivados de las salidas de campo (ecológicos y de hábitat) será usada para proponer unidades de conservación dentro de las tres especies estudiadas, además de proponer medidas de manejo que apoyen y promuevan la toma de decisiones en torno a la conservación de la diversidad biológica de los sistemas acuáticos del Centro de México.

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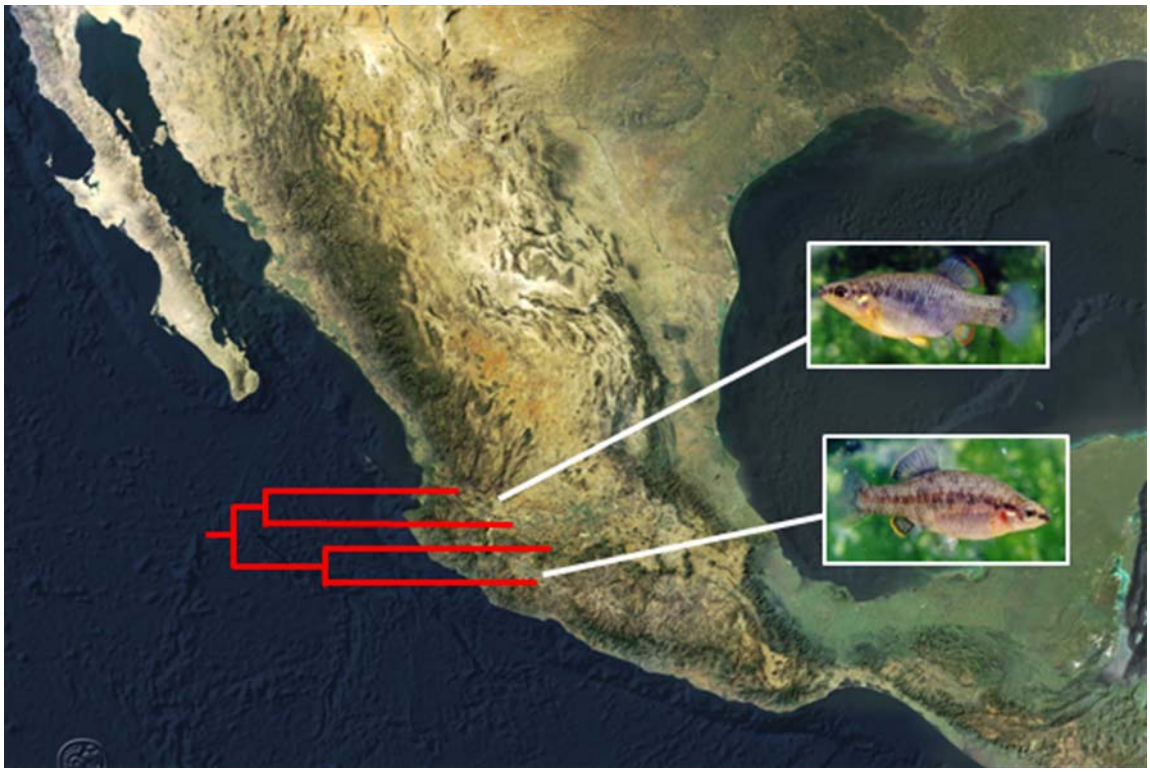
# CAPITULO 1

## *Zoogoneticus quitzeoensis*

# FILOGEOGRAFÍA

## ARTICULO I



Domínguez-Domínguez Omar

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**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**

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## Abstract

### Background

Tectonic, volcanic and climatic events that produce changes in hydrographic systems are the main causes of diversification and speciation of freshwater fishes. Elucidate the evolutionary history of freshwater fishes permits to infer theories on the biotic and geological evolution of a region, which can further be applied to understand processes of population divergence, speciation and for conservation purposes. The freshwater ecosystems in Central Mexico are characterized by their genesis dynamism, destruction, and compartmentalization induced by intense geologic activity and climatic changes since the early Miocene. The endangered goodeid *Zoogoneticus quitzeoensis* is widely distributed across Central México, thus making it a good model for phylogeographic analyses in this area.

### Results

We addressed the phylogeography, evolutionary history and genetic structure of populations of *Z. quitzeoensis* through a sequential approach, based on both microsatellite and mitochondrial cytochrome *b* sequences. Most haplotypes were private to particular locations. All the populations analysed showed a remarkable number of haplotypes. The level of gene diversity within populations was $\bar{H}_d$ = 0.987 (0.714 - 1.00). However, in general the nucleotide diversity was low, $\pi$ = 0.0173 (0.0015 - 0.0049). Significant genetic structure was found among populations at the mitochondrial and nuclear level ($\Phi_{ST}$ = 0.836 and $F_{ST}$ = 0.262, respectively). We distinguished two well-defined mitochondrial lineages that were separated *ca.* 3.3 million years ago (Mya). The time since expansion was *ca.* 1.5 x $10^6$ years ago for Lineage I and *ca.* 860,000 years ago for Lineage II. Also, genetic patterns of differentiation, between and within lineages, are described at different historical timescales.

### Conclusions

Our mtDNA data indicates that the evolution of the different genetic groups is more related to ancient geological and climatic events (Middle Pliocene, *ca.* 3.3 Mya) than to the current hydrographic configuration of the basins. In general, mitochondrial and nuclear data

supported the same relationships between populations, with the exception of some reduced populations in highly polluted basins (Lower Lerma River), where the effects of genetic drift are suggested by the different analyses at the nuclear and mitochondrial level. Further, our findings are of special interest for the conservation of this endangered species.

## Background

Primary freshwater fishes are strictly confined to freshwater basins, limiting their dispersal capacity. The evolution and dispersal of primary freshwater fishes are closely tied to the palaeogeography and history of connections, captures or separation of the water bodies they inhabit [1]. Accordingly, tectonic, volcanic and climatic events that produce changes in hydrographic systems are the main causes of diversification and speciation of freshwater fishes [2]. As these events reflect the geological development of landscapes, the phylogeographic studies of freshwater fishes permit to infer the biotic and geological evolution of a region [3].

The freshwater ecosystems of Central Mexico are characterized by their genesis dynamism, destruction, and compartmentalization induced by intense tectonic and volcanic activity. Its major physiographic feature is the Mesa Central, a large and isolated tropical highland, which includes the geological active Transmexican Volcanic Belt (TMVB), defined as the southern limit of the massive uplifted and as the transition area between the Nearctic and Neotropical provinces [4]. The tectonic activity of the Mesa Central started in the Miocene and reached its climax during the Pliocene-Pleistocene and has continued intermittently to the present, mainly in the TMVB region [5]. This intense geologic activity has generated a complex hydrologic system, which is the promoter of continuous processes of dispersion and vicariance. It has been suggested as the main cause for the high freshwater fish species richness (around 100 species) and unusual high levels of endemicity (around 70%) of the Mesa Central. Thus, this region is an interesting model for the understanding of the evolution and development of the biotic components of complex areas [6].

Many studies have discussed the biogeography of the Mesa Central, and have described the vicariant events that have resulted in subsequent differentiation in beetles [7], salamanders [8], toads [9], fishes [10] and mammals [11]. Most of the works centred in central Mexico have involved terrestrial taxa, but studies dealing with freshwater taxa are scarce [12]. More specifically, in the last years, the historical biogeography of the ichthyofauna of the Mesa Central of Mexico has been studied based on historical and descriptive methods of analysis [6, 13-15]. These studies corroborated the pioneer works of several authors, who

described general patterns of distribution of the freshwater fish fauna of the region, using occurrence data and detailed morphological comparisons (e.g. [16-18]). These contributions discussed diverse hypotheses, such as repeated events of connection and isolation of water bodies, river piracy, centers of origin, ancestral isolations between populations, and the effect of Pleistocene glaciations. However, these hypotheses have been widely debated and poorly understood [6, 14, 19].

Recent molecular studies have demonstrated the genetic signatures these volcanic, tectonic, and climatic events have left in some freshwater fish species of the Mesa Central, such as the Poecilids [10], Cyprinids [20] and Goodeines [21]. These studies have investigated the phylogenetic relationships at higher taxonomic levels and mainly evoke processes of isolation and vicariance. Nevertheless, to date, no specific evolutionary scenario of any freshwater organism has been proposed in the context of a phylogeographical approach with reference to climatic and geological events.

Within the endemic freshwater fish fauna of the Mesa Central, the Goodeinae is one of the most diverse groups (around 41 species), characterized by its particular life history, including internal fertilization, matrotrophy and viviparity, and a high degree of genetic divergence [15, 21, 22]. Within the Goodeinae, the genus *Zoogoneticus* is represented by two species, *Z. tequila* (Webb and Miller, 1998) and *Z. quitzeoensis* (Bean, 1898). The former is a microendemic species of the upper Ameca River basin, while the latter is widely distributed across the hydrological basins that drain the TMVB. Previous works have demonstrated that the populations of *Z. quitzeoensis* from Cuitzeo and Zacapu are significantly divergent when compared with populations from Lower Lerma [23] and Ameca basins [21]. However, no information about its evolutionary and demographic history has been yet provided. Further, the distribution range of this genus has been dramatically reduced due to habitat fragmentation and anthropogenic perturbations [24-26]. Because of these, *Zoogoneticus quitzeoensis* is considered an endangered species by the Mexican Official Norm of Ecology, and its sister species, *Z. tequila*, is now reported as extinct in the wild [27]. Thus, *Z. quitzeoensis* provides an interesting case-study for examining various features of the evolutionary and demographic history of the geologically

active TMVB and its biota. Also, it can serve as a model to understand the processes and events that rule the biodiversity assemblages of the area and to promote its conservation.

In this sense, phylogeographical approaches have generally served to establish patterns of evolutionary history in distinct geographical populations. However, they have also been successfully used to infer historical demographic processes such as gene flow, effective population sizes or evolutionary trajectories [28]. Elucidating the evolutionary history of a species is important to understand population divergence and speciation and to provide more specific and accurate information of the processes and events that influence the evolutionary and demographic history of a region and its biota. Further, this information can be applied to conservation biology, as historical contingencies have been largely responsible for creating important genetic subdivisions in most extant taxa [29].

Mitochondrial DNA is preferentially and commonly used in most phylogeographic studies [30], although markers showing a faster evolution rate can uncover patterns on a more recent temporal scale [31]. Thus, the combined use of mtDNA and microsatellites has proved to be particularly effective for exploring both contemporary and historical events [32]. In this way, the sequential approach to phylogeography is recommended, as it examines both haplotype relatedness and demographic history [29, 33]. This approach supports the idea that there is not one single and most powerful or informative analysis, but a combination of them [33]. Hence, the use of the sequential approach in phylogeography and different molecular markers, gives the opportunity to elucidate not only the spatial and temporal distribution of genealogical lineages, but the evolutionary and demographic history at different timescales.

Herein we describe the phylogeography, evolutionary and demographic history of *Zoogoneticus quitzeoensis* across its whole distribution range. Based on our results, we then infer the historical biogeographical scenario of its populations and propose future strategies for its conservation.

## Results

### Sequence variation and phylogenetic reconstruction

By sequencing 1140 bp of the entire mitochondrial cytochrome *b* gene in 80 individual *Z. quitzeoensis* specimens from 12 populations (Figure 1 and Additional file 1), 65 haplotypes were detected (Table 1). Fifty four sites were variable (4.73%), 37 were non-synonymous, and 17 were parsimony informative. As expected for a protein-coding gene, third codon positions were the most variable (10) followed by first (5) and second (2). The average ratio of non-synonymous/synonymous substitutions was $d_N/d_S = 0.45$ (CI: 0.35-0.56) and no evidence of positively selected sites was detected with any of the two methods used. Full details of substitution parameters and evolutionary models are given in Additional file 2.

Weighted parsimony analyses generated 95 equally parsimonious trees (length = 272, CI = 0.871, RI = 0.967). The three methods (NJ, MP and Bayesian) produced largely congruent tree topologies, with proportionally similar bootstrap and posterior probabilities supporting major lineage and population divergences (Figure 2).

Phylogenetic analyses identified two distinct lineages (Figure 2). Lineage I comprised 40 haplotypes distributed in the Lower Lerma and Ameca basins and in Lake Chapala, while Lineage II was composed of 25 haplotypes from the Middle Lerma Basin and from the Cuitzeo and Zacapu Lakes (Figure 2). Mean maximum likelihood and uncorrected *p* distance between the two lineages were $\bar{D}_{ML} = 3.05\% \pm 0.23$ and $\bar{D}_p = 2.81\% \pm 0.21$. Both lineages showed a well-defined internal structure (Table 2).

Within Lineage I, we found two main clades. One clade (Ameca Clade) included 18 haplotypes (*Hn*) from the 3 sampled sites in the Ameca River Basin (Los Veneros, Magdalena and Moloya), and the other clade (Chapala-Lower Lerma Clade: *Hn* = 22) contained individuals from the localities sampled in the Lower Lerma Basin and Lake Chapala. Distances between Ameca and Chapala-Lower Lerma clades were $\bar{D}_{ML} = 0.93\% \pm 0.2$ and $\bar{D}_p = 0.92\% \pm 0.2$.

In Lineage II, the first clade comprises the Lake Cuitzeo and Zacapu Lake Basin populations (Cuitzeo-Zacapu Clade: *Hn* = 20), and the second one, the San Francisco del Rincón population (Middle Lerma Clade: *Hn* = 5). The $\bar{D}_{ML}$ between the two clades was 1.17% and $\bar{D}_p$ = 1.21 (Table 2).

**Nested clade analysis**

The statistical parsimony haplotype network for *Z. quitzeoensis* indicated a similar pattern to that revealed by the phylogenetic analysis and showed the existence of two well-defined evolutionary lineages (Figure 3). The number of mutational steps (21) between lineages exceeded the maximum number of mutational connections justified by the 95% parsimony criterion (14 mutational steps). Thus, each lineage was treated independently in subsequent analyses.

The null hypothesis of the nested contingency analysis of no association between haplotype positions in the cladogram and their geographical locations was rejected in six of the tests performed ($P < 0.05$) (Figure 3 and Additional file 3).

Allopatric fragmentation could have rendered the geographical pattern of the full cladogram of *Z. quitzeoensis.* In Lineage I, the five-step clade showed significant values; there was insufficient evidence, however, to discriminate between range expansion, colonization and restricted dispersal or gene flow. Within this lineage, long distance colonization and/or past fragmentation was inferred for Clade 3.6 (Clade Ameca) and long distance colonization associated with subsequent fragmentation followed by range expansion for clades 3.5 (Clade La Luz-Orandino) and 4.2 (Clade Chapala-Lower Lerma) [34].

In Lineage II, the inference key [34] suggested long distance colonization and/or past fragmentation for Clade 4.1 (Lineage II).

**Genetic structure**

The mtDNA $\Phi_{ST}$ values obtained for the comparisons of all the populations ranged from 0.006 to 0.940 (Table 3). Highest significant values were obtained for most comparisons involving the Zacapu population ($\Phi_{ST}$ = 0.518 - 0.940), except for comparisons with two populations from Lake Cuitzeo (Belisario and San Cristóbal). Significant $\Phi_{ST}$ values were also obtained for all the comparisons of Los Veneros with the rest of the populations, except for those involving populations within the same basin (Magdalena and Moloya). The only significant $\Phi_{ST}$ values between samples within the same basin were found in the Lower Lerma River, except for Orandino and La Platanera (Table 3).

Pairwise $F_{ST}$ values based on microsatellite data were significant for most of the comparisons, except for Moloya-Magdalena in the Ameca basin and for Belisario-San Cristóbal in the Cuitzeo Lake basin (Table 3). Highest $F_{ST}$ values were observed for the comparisons between La Luz and San Francisco del Rincón, and La Platanera with La Mintzita ($F_{ST}$ = 0.455, 0.408 and 0.406 respectively). Although the La Luz and La Platanera populations occur in the same basin (Lower Lerma), their $F_{ST}$ values were among the highest. Notwithstanding, the other population inhabiting the Lower Lerma Basin (i.e. Orandino) showed significant yet much lower $F_{ST}$ values when compared with La Luz and La Platanera ($F_{ST}$ = 0.271 and 0.224 respectively).

The AMOVA performed for the mitochondrial and nuclear data, revealed significant structure among populations ($\Phi_{ST}$ = 0.836, $P < 0.001$ and $F_{ST}$ = 0.262, $P < 0.001$ respectively). For the subsequent analyses, populations were grouped in different hierarchical arrangements according to previous information and to uncover groupings obtained in the previous analyses (e. g. phylogenetic analysis and NCA) and by their biogeographical arrangement (Table 4). Significant values were obtained when the biogeographic arrangement of basins [6] was considered ($\Phi_{CT}$ = 0.787, $P < 0.001$, $F_{CT}$ = 0.107, $P < 0.05$). When the basins where each lineage was found were considered separately, only Lineage I showed significant structure for mtDNA (Table 5). When two gene pools corresponding to the two lineages found were considered, 74.8% ($P < 0.001$) of the total variance was explained as differences among groups for mtDNA and 11.18% in

the case of microsatellite data ($P < 0.01$). Division of the populations into the groups suggested by the phylogenetic and NCA analysis maximised among-group variance for the mtDNA data ($\Phi_{CT} = 0.823$, $P < 0.001$). Thus, the structure previously obtained in the phylogenetic and NCA was statistically supported for the mtDNA, but not for the microsatellite data.

The Mantel test revealed a significant correlation between geographical and mitochondrial genetic distances ($r = 0.4123$, $P = 0.01$). Otherwise, non significant correlation was found considering the genetic distances obtained using the microsatellite data ($r = 0.1894$, $P = 0.179$) (Figure 4).

The Bayesian structure analysis for the microsatellite data clearly revealed a genetic structure among the specimens analysed. According to the maximum likelihood value, we estimated a number of genetic clusters of $K = 6$. Estimates of $\ln\Pr(X|K)$ increased rapidly between $K = 1$ and $K \leq 6$, whereas beyond $K > 6$ $\ln\Pr(X|K)$ started to oscillate. But when we considered $\Delta K$, we obtained clear peak at $K = 5$ (Figure 5). Conversely, the highest global $F_{ST}$ value was found for $K = 6$, indicating that these clusters explained the maximum level of structure in our sample (Additional file 4). However, for the following discussion we adopted the more conservative measure of $K = 5$, obtained with the correction of Evanno *et al.* [35]. All populations were assigned with high probability ($Q = 0.844$ - $0.962$) to their inferred cluster.

The results derived from the microsatellite NJ tree were highly congruent with those from the Bayesian clustering analysis and most of the phylogenetic mtDNA tree, but with some differences within Linage I (Figure 5). Two well-supported lineages were obtained. In Lineage II, all samples from the Cuitzeo and Zacapu basins grouped together, and San Francisco del Rincón appeared differentiated from these. Otherwise, Lineage I showed a higher degree of structure, with its populations distributed in three different clusters (Figure 5). When considering $K = 6$, Orandino represented a separate cluster. Slightly different results, regarding the position of the Lower Lerma populations, were rendered by the two types of marker for population relationships (Figure 5). The population assignment test

correctly assigned 80.59% of the individuals to their original population. While for the Lineage I populations, 91.5% correct assignments were obtained, in Lineage II, gene flow was found among all populations (72% of the individuals were assigned correctly) except for the site San Francisco del Rincón, whose individuals were all unequivocally assigned.

**Mitochondrial DNA variation and demographic patterns**

Overall gene diversity was $H_d$ = 0.987 and nucleotide diversity $\pi$ = 0.0172. Most haplotypes were found at single sites. Only two haplotypes were shared among individuals from different localities. One of them was also the most common haplotype overall, found in 8 individuals from the sites Zacapu, San Cristóbal and Belisario (ZAC-SCR-BEL 59), and the other one was found in 3 individuals from Magdalena and Moloya (MAG-MOL 2) (Figures 2 and 3). The rest of the haplotypes were shared among individuals within the same localities.

All the populations analysed showed a remarkable number of haplotypes (Table 1). The level of gene diversity within populations was $\bar{H}_d$ = 0.987 (0.714 - 1.00). However, the nucleotide diversity exhibited by most of the tested populations ($\pi$ = 0.0015 - 0.0049) was low. Mean pairwise nucleotide diversity (*k*) ranged from 1.71 to 5.69 within populations (average number of nucleotide differences for the whole sample *k* = 19.79; Table 1). The mismatch distribution (MMD) for all the data set was bimodal (not shown), one of the peaks represents the differences between lineages, and the other the differences among individuals within lineages. The two lineages and the different clades obtained in the previous analyses were tested independently. The MMD for Lineage I was unimodal and bimodal for Lineage II (Figure 6), which is expected when populations are geographically subdivided [36]. In both cases, raggedness indices (*r*) were not significant, thus not rejecting the null hypothesis of stationarity. Conversely, Tajima's *D*-statistic and Fu's statistic ($F_s$) were significantly negative and supported the expansion model for the different groups tested (Table 5). Both tests show a significant excess of the number of segregating sites and singletons compared to the average pairwise sequence divergence. These tests indicate that the different groups analyzed are in mutation–migration–drift genetic disequilibrium with respect to mtDNA alleles. The different results obtained by

these three demographic tests might be due to the low power of mismatch distribution based tests (e.g. raggedness), compared with methods based on the mutation frequency (e.g. Tajima's *D*) or haplotype distribution (e.g. Fu's *F*). In a variety of cases and for large samples sizes, Fu's *F* has been proved to be the most powerful test to detect population growth [37]. Values of $\tau$ differed between lineages and among clades. For Lineage I, the mean of the mismatch distribution was $\tau = 5.647$ and the estimated time since population growth was $1.5 \times 10^6$ years before present. Within Lineage I, the estimated time since population expansion for the two clades obtained (Ameca and Chapala-Lower Lerma) were *ca.* $1.4 \times 10^6$ ($\tau = 5.08$) and *ca.* $1.08 \times 10^6$ ($\tau = 5.647$), respectively. In Lineage II, we estimated *ca.* 860,000 years since population expansion according to the value of $\tau = 3.09$. For the clades within Lineage II, the estimates of time since population expansion were *ca.* 790,000 years for Cuitzeo-Zacapu ($\tau = 2.83$) and *ca.* 550,000 years for San Francisco del Rincón ($\tau = 1.95$).

## Discussion

### Phylogeography and evolutionary history

The trees obtained by the different methods (NJ, MP and BI) using both types of markers (cytochrome *b* and microsatellites) distinguished two independent lineages, supporting the conclusions of previous studies [21] in which two divergent groups were identified within *Z. quitzeoensis*. Lineage I inhabits areas west of the Middle Lerma, including the Lower Lerma, Ameca and the Chapala Lake basins. Lineage II occurs at sites east of the Angulo River and the Middle Lerma Basin, including the Cuitzeo and Zacapu lakes. Our NCA also supported this conclusion, indicating the two main lineages were not nested together; moreover, the 21 mutation steps between the two lineages exceeded the 95% parsimony limits (14 mutation steps). This result suggests allopatric fragmentation between the two clades.

The formation of the two lineages of *Z. quitzeoensis* was dated at *ca.* 3.3 Mya based on the molecular clock calibration of 0.9% divergence per million years [21]. These two lineages could be the result of a dispersal process of the ancestral population from the Lower Lerma-Chapala area to the Middle Lerma-Cuitzeo-Zacapu area (Figure 1), followed by an isolation

event. This hypothesis is based on the facts that: Lineage I is the most widely distributed group (distributed in Chapala-Ameca-Lower Lerma drainage), and it shows, overall, a higher genetic diversity than Lineage II (*Hd* = 0.991, *S* = 76 and *Hd* = 0.946, *S* = 54, respectively).

The dispersion event we refer to, from the Chapala-Lower Lerma to the Middle Lerma-Cuitzeo-Zacapu area, could have been promoted by a period of high precipitation and humidity occurred in Central Mexico in the early Pliocene (5.2 - 3.6 Mya). This could have caused an increase of the water bodies level in this area causing them to get in contact, as was previously proposed for other lakes in Central Mexico [38, 39]. The subsequent isolation of the ancestor of the two lineages that induced allopatric fragmentation, could have been the result of the end of the humid period, and/or the formation of a biogeographical barrier promoted by the geologic activity of the Penjamillo Graben [40, 41], the Chapala-Tula fault or the activity and formation of the Corredor Tarasco volcanic field, which commenced during the Late Miocene–Early Pliocene [40] (Figure 1). This climatic change and the high tectonic activity have been proposed as the causes for the isolation of the palaeolakes along the TMVB during the Late Miocene-Pliocene (Figure 1) [38].

Since recent geological times, some of the regions where these two lineages occur have been connected, and at present constitute one single hydrologic system (Lerma River). However, our findings of ancestral isolation between Lower Lerma-Chapala and Middle Lerma-Zacapu-Cuitzeo are supported by at least one pair of sister species with the same cladogenetic pattern, *Skiffia lermae-S. multipunctata,* dated around 3.2 Mya [21]. This indicates that the same biogeographic event could have promoted the isolation of the two divergent groups and consequently they could be considered as two ESUs [42, 43]. Furthermore, considering the morphological differences between groups, and pending of more detailed morphometric studies, they could be considered as two species.

**Within lineage genetic structure and demography**

The results of all our analyses revealed significant genetic structure and differentiation among populations between and within the two lineages, but with certain differences as indicated by the two types of molecular markers.

***Lineage I: Ameca-Lower Lerma-Chapala area***

The two main clades identified within Lineage I correspond to two different hydrologic systems. One clade appears in the Ameca river basin (Clade 3.6) and the other is distributed across the Lower Lerma-Chapala Lake area (Clade 4.2). When Lineage I was tested independently, the statistical associations in the nested contingency analysis were not able to discriminate between range expansion and colonization *versus* restricted dispersal and gene flow. However, the fact that haplotype LUZ-40 emerged as the most probable outgroup in the network, and the significant negative value of the Fu's $F_s$, and Tajima's $D$ statistics seem to better support the range expansion and colonization scenario. The haplotype arrangement found within the three populations sampled on the Ameca river basin also supports this scenario. Haplotypes from Los Veneros are placed in a basal position in the phylogenetic trees and appeared in all the 2-step clades found in the NCA (within 3.6 Clade). Such patterns could indicate that Los Veneros may represent the ancestral population of the Ameca Basin. The Los Veneros population is geographically close to the San Marcos-Atotonilco lakes (~19 Km), which formed part of the Chapala Palaeolake [44], but also it is close to the Zacoalco-Ameca paleolake. This supports the hypothesis that *Z. quitzeoensis* spread from the Chapala Palaeolake region to the Ameca River region via the Zacoalco-Ameca Paleolake (Figure 1). The expansion for the Ameca River populations was calculated in *ca.* $1.4 \times 10^6$ ($\tau = 5.08$), in such case the dispersion event could have taken place at the beginning of the Pleistocene.

A former connection between the Chapala and Ameca basins have been previously proposed via the Atotonilco and San Marcos lakes [45] (Zacoalco-Ameca Paleolake area). Other authors have supported this connection based on the distributions of related species/population pairs of fish, as in *Poeciliopsis* [10], *Notropis* [46], *Chirostoma* [17], *Ictalurus* [19], *Yuriria* [47] and almost three events of Goodeines exchange [6, 21].

Within Lineage I, another two clades showed a significant association in the geographic contingency test: Clade 4.2, in which specimens from the Chapala and Lower Lerma were included; and Clade 3.5, comprised of the geographically close populations of La Luz and Orandino that are ~4 km apart within the same basin. In both cases, long distance colonisation possibly accompanied by subsequent fragmentation or past fragmentation followed by range expansion, was inferred from the NCA. Moreover, the genetic distance between the population of La Platanera (clade 3.7) and the populations of La Luz and Orandino (clade 3.5), within the Lower Lerma region, was larger than the distance between the population of La Alberca (clade 3.4), in the Chapala Lake region, and the populations of Orandino and La Luz (Table 2). These results are in disagreement with a previous biogeographic hypothesis, where the Lower Lerma and Chapala Lake were considered as independent biogeographic entities [6]. The significant outcome of Clade 3.5 (populations within the Lower Lerma region) might also be due to the fact that NCA is likely to give false-positive results, and consequently detect a significant spatial structure, in populations that have suffered processes that affect local haplotype frequencies, such as bottlenecks [48]. This could be the case for La Luz and Orandino where the effects of genetic drift and low population size (e.g. low genetic diversity and significant inbreeding), caused by human activities (e.g. pollution, desiccation and isolation of the water bodies, introduction of exotic species) have been proved [49] and are congruent with the high genetic differentiation and significant inbreeding revealed by the microsatellite data (Table 3, Figure 5, Additional file 5).

The pronounced genetic structure among the contiguous sampling sites of Orandino, La Platanera, and La Luz was also found at the nuclear level (Table 3, Figure 5). Further, most of the differences between the mtDNA and nDNA analyses were found in relation to this area (Figure 5). Thus, all these results suggest that recent demographic events could have shaped, through genetic drift, the genetic structure of the populations in the Lower Lerma basin.

***Lineage II: Middle Lerma-Cuitzeo-Zacapu area***

In Lineage II, two well-differentiated groups were recovered by the mtDNA (i.e. $\Phi_{ST,}$ NCA and phylogenetic trees) and microsatellite analyses (i.e. STRUCTURE, $F_{ST}$ and NJ tree). The first of these groups comprises the San Francisco del Rincón population and the second group includes the populations from Zacapu, La Mintzita, San Cristóbal and Belisario, the last three belonging to the Cuitzeo region. In the NCA, only Clade 4.1, which clustered together all the populations of this lineage, showed a significant geographical association, but insufficient to discriminate between long distance colonization and past fragmentation (Additional file 3).

According to our demographic results, both scenarios provided by the NCA inference key are candidates for the five populations distributed across three different basins (Additional file 3). However, certain features, for instance: the low genetic diversity in the San Francisco del Rincón population suggested by the mtDNA and confirmed by previous microsatellite studies [49] (Additional file 5), and that this population probably expanded more recently (*ca.* 540,000 years), could point to the long distance colonization of organisms from Zacapu-Cuitzeo and past fragmentation as the most plausible scenario. This assumption is supported by the neutrality tests of Fu's $F_s$ and Tajimas's $D$ statistics.

All the populations within this lineage showed evidence of recent gene flow, except the one from San Francisco del Rincón. Our results also indicated non significant genetic differentiation for most of the pairwise comparisons, for mtDNA, among the four populations inhabiting the Zacapu and Cuitzeo basins. Haplotypes were shared by the two basins, a fact that disagrees with the hypothesis that Zacapu and Cuitzeo constitute two well-defined biogeographic entities [6]. Although the two basin populations are close (≈50 km), at present they are geographically separated by a mountain chain. The time since expansion for these populations was estimated at *ca.* 790,000 years ago. Our results support the idea of an ancient connection between Zacapu and Cuitzeo lakes, via a river located in the Chucandiro-Uaniqueo region, and further disrupted by the geologic and volcanic activity during the Plio-Pleistocene (≈1 Mya) [14]. This connection is also stated in previous phylogenetic studies that address population relationships between Zacapu and

Cuitzeo based on species of the genus *Notropis* [20] and other Goodeinae species [21]. However, we found differences in the divergence times, suggesting that more than one event of connection and isolation between the Zacapu and Cuitzeo regions occurred in the last three million years.

**Discrepancies between nuclear and mitochondrial markers**

Mitochondrial and nuclear markers reveal different parts of the evolutionary history of the organisms due to their different inheritance modes and mutation rates. Even though mitochondrial markers coalesce faster than nuclear markers due to the smaller effective size and the lack of recombination, microsatellites have a much faster mutation rate which makes them useful to detect more recent processes [50, 51].

In general, the relationships inferred with the mitochondrial and microsatellite data set were congruent, but some differences were found. Whereas both markers retrieved the existence of two main clades, the relationships within them were not the same (Figure 5). Discrepancies were observed in Lineage I for the comparisons of the Chapala-Lower Lerma populations. In this case, both makers showed significant differences among geographically close populations. High and significant pairwise $F_{ST}$ values were obtained for most of the comparisons of populations from the Lower Lerma basin (Table 3), which also were assigned to different genetic clusters by the Bayesian clustering analysis and microsatellite NJ tree, but formed a single clade according to mtDNA (Figure 5). Some of these localities have recently suffered a severe reduction in the size of their water bodies, and become isolated with a consequent increase of the effects of genetic drift, resulting in genetic differentiation. Moreover, it has been suggested that the Lower Lerma populations suffered a more intense genetic drift than other populations within *Z. quitzeoensis* [49]. Similarly the non-significant correlation between geographic and genetic distances based on the microsatellite data might be explained by the stochastic processes that are recently shaping the genetic structure of *Z. quitzeoensis,* and consequently the high genetic differences between nearby localities.

The differences found in the genetic diversity values were much higher for the microsatellite than the mitochondrial data, which showed a high diversity for all populations (Table 1 and Additional file 5). The populations of Zacapu and San Francisco del Rincón, which showed allelic richness values of only 2.93 and 2.97, still retained haplotypic diversities of 0.917 and 0.857. Thus, eventhough samples sizes are, in some cases, low and unequal we might deduce that the loss of diversity is occurring at a different rate for the two types of markers [52]. Additionally, none of the Tajima's *D* values, either for single populations or lineages, were significantly positive, which would be indicative of a bottleneck [53], whereas seven out of the 10 populations analyzed showed a significant signature for a recent bottleneck, with at least one of the three microsatellite mutational models (Additional file 5).

### Implications for conservation

The importance of fish diversity in Central Mexico has been largely recognized [54] but very few efforts have been made to establish a basis for their conservation. Further, no studies have addressed the evolutionary processes underlying such diversity targeted at maintaining these processes. Our results, derived from both phylogeographic and population analyses, could prompt certain conservation management strategies. First, the two reciprocally monophyletic lineages of *Z. quitzeoensis*, support the idea that future conservation plans should be aimed at managing the populations of both lineages independently and, furthermore, be considered as two ESUs [42, 43].

Moreover, within each lineage, we found genetic structure for the two markers, supporting previously identified Operational Conservation Units (OCU's) for *Zoogoneticus* [49]. When the species under study shows high genetic structure, ideally all the populations should be protected since they contain unique portions of the total variation of the species. This may increase the adaptation and the survival possibilities of the species as a whole.

## Conclusions

The present study is the first attempt to describe, on a fine scale, the evolutionary history of populations of a fish species in Central Mexico. Our results demonstrate the value of the

use of mtDNA combined with nuclear microsatellite loci to detect genetic structure and to elucidate the evolutionary history of fish species. The methodology used integrates independent geological and genetic information to test for interactions between historical and contemporary factors in a highly structured endemic fish in Central Mexico.

Our results indicate that the evolutionary history and genetic structure among populations of this fish species is closely tied to geological and climatic events that promoted changes in the ancient drainages, since the Middle-Pliocene, rather than to the current configuration of those drainages. In addition to this, the results obtained and differences between molecular markers are an evidence of the effects of genetic drift over the genetic structure in some highly polluted aquatic environments.

The information provided by this type of study is essential for the conservation of highly genetically structured species and its phylogeographic hypotheses prompt comparable analyses of other codistributed fish species to test the scenarios proposed.

## Methods

### Specimen collection and DNA extraction

Fin clips were obtained from individuals of *Zoogoneticus quitzeoensis.* Specimens were collected from 12 sites distributed across six regions along the Mesa Central of Mexico (Figure 1 and Additional file 1), representing most of the distribution range of the species. The fishes were sampled using minnow traps, seine nets and by electrofishing. Tissue was preserved in 96% ethanol and most fishes were returned to the water unharmed. A few fish specimens were incorporated into the Goodeid Conservation Program of the Universidad Michoacana de San Nicolás de Hidalgo. Because of the endangered status of *Z. quitzeoensis* [27] and its scarcity at all the collection sites, sample sizes ranged from 7 to 20 individuals per population (Additional file 1). Similar sample sizes have been used successfully in similar phylogeograpic studies of freshwater fauna [55-61]. However, it has long been proved that phylogenetic and population genetic inferences are sensitive to the number of taxa included. The number of specimens and populations needed to resolve their relationships depend on the amount of polymorphism relative to the extent of divergence.

Thus, it would be appropriate to use small samples per groups when most of the variation occurs among groups [62]. However, in such cases other inferences should be taken with caution as some estimates, for example those based on genetic diversity, could be biased. Sampling at each site was conducted during the same field season. Total genomic DNA was extracted according to standard CTAB and phenol-chloroform extraction procedures [63].

In order to compare the genetic structure between mitochondrial and nuclear markers, we used a microsatellite data set from a previous study about the effects of human impacts on the genetic variability of *Z. quitzeoensis* [49]. However, not all the populations included here for the mtDNA study were available at that time, and therefore the number of analysed populations differs between marker types.

**mtDNA sequencing and phylogenetic analysis**

Two overlapping fragments of the cytochrome *b* gene (1140 bp total) were amplified via polymerase chain reaction (PCR) from 80 specimens distributed in 12 sampling sites. The primers used were those used in Machordom & Doadrio [64]. The amplification process was conducted using the conditions described elsewhere [21]. PCR products were sequenced in an ABI PRISM 3700 DNA analyser. Chromatograms and alignments were visually checked and verified. All sequences were deposited in GenBank under accession numbers: EU679420-EU679499.

We used phylogenetic tree-building algorithms to infer the phylogenetic relationships among sequence haplotypes. Maximum Parsimony Analysis (MP) was performed by heuristic searching with the tree-bisection-reconnection (TBR) branch swapping algorithm and random stepwise sequence addition using 10 replicates. Two different weights were given to the characters; first all characters were equally weighted, and second, transversions (Tv) were assigned eight times the weight of transitions (Ti) according to the empirically determined Tv/Ti ratio for *Zoogoneticus* obtained in PAUP 4.0b10[65]. The robustness of the MP topologies was assessed by bootstrapping with 1000 replicates (full heuristic search) of 10 random stepwise addition replicates each. The model of DNA substitution

that best fitted the data set was selected using MODELTEST 3.7 [66] using the Bayesian information criterion (BIC) for each codon position and the Akaike information criterion (AIC). A Neighbour-Joining (NJ) phylogram was obtained using maximum-likelihood distances according to the model selected by AIC. Bootstrap values for this analysis were obtained from 1000 replications. All phylogenetic analyses were performed using PAUP 4.0b10. Bayesian analysis was conducted with MrBayes 3.1.1 [67]. By simulating a Markov Chain Monte Carlo reaction for $2 \times 10^6$ cycles and using the substitution model obtained for each codon position selected by BIC criterion, 20,000 trees were generated, 1000 of which were burned and discarded. Posterior clade probabilities were used to assess node support. To identify ancestral and derived haplotypes, the trees were rooted using *Zoogoneticus tequila,* the sister species of *Z. quitzeoensis,* as outgroup, and a molecular clock of 0.9% per million years was applied to the pairwise uncorrected *p* genetic distances [21].

Evidence of positive selection was sought using a codon-based approach as implemented in Datamonkey [68]. This method does not need to assume equal synonymous substitution rates throughout the sequence and allows to choose the most appropriate model for nucleotide substitution. We used the single likelihood ancestor counting (SLAC) and fixed effects likelihood (FEL) approaches [69] using a *P* value of 0.1. In both cases, ambiguities in the consensus sequence were averaged in the analysis.

**Nested Clade Analysis**

We constructed a 95% statistical parsimonious un-rooted haplotype network using TCS 1.18 [70]. As a complementary method to those performed before, and considering the limitations and drawbacks of Nested Clade Analysis [48, 71], we tested geographical association among haplotypes and clades, based on the most parsimonious haplotype network and followed by a nested cladistic analysis (NCA), as described by Templeton [34, 72]. To test for significant associations between clades and geographical sites, nested contingency analysis [73] was conducted by the program GEODIS 2.2 [74]. The AUTOINFER 1.0 [75] software package was used to infer the most suitable population structure model and historical scenario for the observed geographical associations.

**Genetic structure based on mtDNA**

Pairwise $\Phi_{ST}$ values were calculated among all geographic populations as an estimate of genetic differentiation. To assess the significance of genetic differentiation at different hierarchical levels, an analysis of molecular variance (AMOVA) was performed as described by Excoffier *et al.* [76]. Populations were initially grouped according to previous biogeographical information [6]. In subsequent analyses, we considered the information obtained in both the phylogenetic and the NCA analyses, but other hierarchical arrangements were also tested. Statistical significance was assessed using 20,000 permutations. A Mantel test (100,000 permutations, [77]) served to evaluate correlations between linear geographic distances and genetic distances. All analyses were performed using ARLEQUIN 3.1. [78].

**mtDNA diversity and demographic history**

Population genetic statistics, such as the number of polymorphic sites (S), haplotype diversity ($H_d$, [79]), nucleotide diversity ($\pi$, [79]) and the average number of pairwise nucleotide differences ($k$, [80]) were calculated using DnaSP 4.0 [81].

To investigate the demographic history of the groups identified in the phylogenetic analyses and through the AMOVA results, a mismatch distribution analysis (MMD) of pairwise substitution differences among haplotypes was performed for the whole data set and the lineages obtained. Deviations from the constant population size model were further tested using the Harpending's raggedness index ($r$) [82]. To test for deviations from neutrality we used Tajima's $D$ [53] and Fu's $F$s [83] tests as implemented in DnaSP 4.0 [81]. We used the MMD age expansion parameter ($\tau$) to date the onset of population expansion [84]. This was done by calculating $\tau$ using the equation $\tau = 2\mu t$, were $\mu$ is the sum of per-nucleotide mutation rates in the DNA region under study (0.9% PMY; [21]) and $t$ is time in generations (0.5 for goodeines).

**Microsatellite analysis**

We examined a previous data set, consisting of 135 individuals of *Z. quitzeoensis* from 10 populations, genotyped for five microsatellite loci (Additional file 5) [49] to look for

differences in the allele frequencies of the populations by estimating $F_{ST}$ between all sample pairs, according to Weir & Cockerham [85], using ARLEQUIN 3.1. The significance of these estimates was assessed using 10,000 data permutations corrected by Bonferroni adjustment [86].

Geographical and phylogenetical genetic variation were compared among populations and clades by AMOVA. We assessed genetic isolation by distance[87], testing for independence between $F_{ST}$ estimates and geographical distances using a Mantel test [77]; regression matrices of $F_{ST}/1-F_{ST}$ values *versus* the linear distance between sample pairs. All these analyses are implemented in ARLEQUIN 3.1.

To determine relationships among the sampled populations a neighbour-joining tree was created using the POPTREE program [88] based on $D_A$ modified Cavalli-Sforza distances [89] with 5000 bootstrap replications.

Because of the uncertainty that the geographical assignment of individuals to populations could represent biologically significant entities, a Bayesian clustering method was conducted as implemented in the program STRUCTURE 2.1 [90]. We performed a series of independent runs from $K = 1$ to 8 populations assuming correlated allele frequencies and an admixture model. For each value of $K$, the MCMC scheme was run with a burn-in period of $5 \times 10^5$ steps and chain length of $5 \times 10^6$. Multiple runs were performed for each $K$ to assess convergence of the results. Mean log probabilities were used to calculate $\Delta K$ (i.e. a quantity based on the second-order rate of change of the log probability of data between successive $K$ values), to find the true $K$ following the method of Evanno *et al.* [35]. Global $F_{ST}$ values were calculated for each $K$ to find out which of the structures inferred explained the highest percentage of genetic variation. To assess the level of admixture and gene flow between the central Mexican Basins, we ran a population assignment test using GeneClass 2.0 [91].

## Authors' contributions

ODD conceived the study, collected the samples, participated obtaining the molecular data and analyses and drafted the manuscript. FA contributed in the data analyses and helped to draft the manuscript. GPPDL conceived the study, participated in its design and coordination and helped to draft the manuscript. JLG participated obtaining the molecular data and analyses. ID conceived the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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## Figures

**Figure 1 - Sampling sites in Central Mexico from which the *Zoogoneticus quitzeoensis* specimens were obtained**

1) Magdalena, 2) El Moloya, 3) Los Veneros, 4) La Alberca, 5) La Platanera, 6) La Luz, 7) Orandino, 8) San Francisco del Rincón, 9) Belisario, 10) San Cristóbal, 11) La Mintzita, 12) Zacapu. Light blue outlines represent the areas of the paleolakes that developed in Central Mexico during the Miocene-Pleistocene: A) Sayula, B) Magdalena, C) Zacoalco-Ameca. Arrows represent proposed routes of colonisations. Black dotted lines represent geologic faults and grabens: Am: Ameca Fault, SM: San Marcos Fault, PG: Penjamillo Graben, CT: Chapala-Tula Fault, TC and dotted area: Corredor Tarasco volcanic field. The colours in the water bodies represent clades defined in Figures 2 and 3.

**Figure 2 - NJ tree for the mtDNA haplotypes of the *Zoogoneticus quitzeoensis* populations examined in this study**

Bootstrap support of >70% in NJ (top left) and MP (top right) and posterior probabilities (numbers below) for BI are given for the relevant nodes. The sister species, *Zoogoneticus tequila,* was used as outgroup (not shown).

**Figure 3 - Statistical parsimony (95%) network of 68 mitochondrial DNA haplotypes identified for *Zoogoneticus quitzeoensis*.**

The network was grouped into nesting clades. Each line in the network represents a single nucleotide substitution. Small circles indicate undetected intermediate haplotype states. Ovals represent haplotypes with more than one specimen. The number 21 represents the number of mutational steps between the two upper clades.

**Figure 4 - Correlation between geographic distances and genetic distances among all populations of *Z. quitzeoensis*.**

Plot of geographic distances against the genetic distances for mtDNA ($\Phi_{ST}/1-\Phi_{ST}$) in red, and the genetic distances for microsatellite data ($F_{ST}/1-F_{ST}$) in blue. Mantel test's *r* and *P* values are shown.

**Figure 5 - Phylogenetic relationships of *Zoogoneticus quitzeoensis* populations.**
Left: NJ tree based on distances calculated for the microsatellite loci. Right: Bayesian tree based on mtDNA data for the same populations used in the microsatellite analysis. Centre: Bar plot obtained using STRUCTURE for the most likely number of clusters $K = 5$. LUZ: La Luz, ORA: Orandino, MAG: Magdalena, MOL: Moloya, PLA: La Platanera, SFR: San Francisco del Rincón, BEL: Belisario, MIN: Mintzita, ZAC: Zacapu, SCR: San Cristóbal. Colours represent clades defined in Figures 2 and 3, except Orandino, in yellow, which appears differentiated from La Luz and clustered with El Moloya and Magdalena populations at the nuclear level.

**Figure 6 - Mismatch distribution for the two main lineages obtained in the cladogram and NCA.**
Grey bars indicate the observed values and black lines show the expected distribution under the sudden expansion model.

**Table 1. Measures of mitochondrial DNA diversity observed for the two lineages and other clades identified in this study.**

| Biogeographic region | Population | *N* | *Hn* | *S* | $H_d$ ± SD | π ± SD | *k* |
|---|---|---|---|---|---|---|---|
| Lineage I | | 45 | 40 | 76 | 0.991±0.008 | 0.0067±0.0036 | 7.63±3.73 |
| Ameca River | Magdalena | 6 | 5 | 6 | 0.933±0.122 | 0.0017±0.0013 | 2.00±1.30 |
| | Moloya | 5 | 5 | 6 | 1.0±0.126 | 0.0024±0.0018 | 2.8±1.77 |
| | Veneros | 9 | 9 | 19 | 1.0±0.0524 | 0.0049±0.0029 | 5.64±2.98 |
| | All populations | 20 | 18 | 31 | 0.984±0.018 | 0.0032±0.0028 | 3.64±1.39 |
| Chapala Lake | La Alberca | 7 | 7 | 16 | 1.00±0.076 | 0.0044±0.0028 | 4.97±2.75 |
| Lower Lerma River | La Platanera | 5 | 5 | 8 | 1.00±0.126 | 0.0028±0.0020 | 3.21±1.98 |
| | La Luz | 7 | 4 | 6 | 0.714±0.181 | 0.0015±0.0011 | 1.71±1.13 |
| | Orandino | 6 | 6 | 13 | 1.00±0.0962 | 0.0039±0.0026 | 4.55±2.60 |
| | All populations | 18 | 15 | 29 | 0.961±0.039 | 0.0044±0.0028 | 5.07±1.37 |
| Lineage II | | 35 | 25 | 54 | 0.946±0.025 | 0.0061±0.0033 | 6.97±3.46 |
| Middle Lerma River | San Francisco del Rincón | 7 | 5 | 10 | 0.857±0.137 | 0.0028±0.0019 | 3.25±1.90 |
| Cuitzeo Lake | Belisario | 6 | 6 | 13 | 0.714±0.181 | 0.0049±0.0032 | 5.69±3.18 |
| | San Cristóbal | 7 | 4 | 9 | 1.00±0.0764 | 0.0022±0.0015 | 2.58±1.56 |
| | La Mintzita | 6 | 5 | 9 | 0.936±0.122 | 0.0032±0.0022 | 3.75±2.19 |
| | All populations | 19 | 14 | 27 | 0.936±0.047 | 0.0039±0.0024 | 4.52±1.654 |
| Zacapu Lake | Zacapu | 9 | 7 | 9 | 0.917±0.092 | 0.0017±0.0012 | 2.00±1.24 |
| | Total | 80 | 65 | 139 | 0.987±0.007 | 0.0172±0.0067 | 19.79±1.96 |

N=sample size, *Hn*=number of haplotypes, *S*=number of polymorphic sites, $H_d$=gene diversity, π=nucleotide diversity (Nei 1987), *k*=mean pairwise nucleotide diversity (Tajima 1993)

**Table 2. Maximum likelihood and uncorrected *p* distances between clades and subclades of *Z. quitzeoensis***

| | LUZ-ORA | ALB | PLA | AME | SFR | CUI-ZAC |
|---|---|---|---|---|---|---|
| LUZ-ORA | (0.30/0.31) | 0.54 | 0.61 | 0.93 | 2.59 | 2.94 |
| ALB | 0.53 | (0.43/0.43) | 0.69 | 0.96 | 2.64 | 2.90 |
| PLA | 0.62 | 0.71 | (0.25/0.25) | 0.91 | 2.59 | 3.09 |
| AME | 0.90 | 0.99 | 0.89 | (0.34/0.35) | 2.67 | 2.93 |
| SFR | 2.79 | 2.85 | 2.79 | 2.88 | (0.28/0.29) | 1.21 |
| CUI-ZAC | 2.72 | 3.15 | 2.85 | 3.19 | 1.17 | (0.37/0.38) |

Above diagonal: uncorrected *p* distances; below diagonal: maximum likelihood distances (TIM+G); diagonal: within clade mean (%) distance (*p*/ML). LUZ: La Luz, ORA: Orandino, ALB: La Alberca, PLA: La Platanera, AME: Ameca, SFR: San Francisco del Rincón, CUI: Cuitzeo, ZAC: Zacapu.

**Table 3. Estimate pairwise comparisons of cytochrome b sequences (mtDNA) above the diagonal ($\Phi_{ST}$) and for five microsatellite loci below the diagonal ($F_{ST}$) for the *Zoogoneticus quitzeoensis* populations. MAG, Magdalena; MOL, Moloya; VEN, Veneros; ALB, Alberca; PLA, Platanera, LUZ, La Luz; ORA, Orandino; SFR, San Francisco del Rincón; BEL, Belisario; SCR, San Cristobal; MIN, Mintzita; ZAC, Zacapu.**

| | MAG | MOL | VEN | ALB | PLA | LUZ | ORA | SFR | BEL | SCR | MIN | ZAC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MAG | - | 0.006 | 0.020 | **0.654** | 0.736 | 0.814 | 0.652 | 0.912 | 0.888 | 0.932 | 0.914 | **0.940** |
| MOL | 0.025 | - | 0.012 | 0.624 | 0.702 | 0.790 | 0.618 | 0.901 | 0.874 | 0.923 | 0.902 | **0.933** |
| VEN | - | - | - | **0.545** | **0.574** | **0.656** | **0.519** | **0.855** | **0.839** | **0.877** | **0.857** | **0.889** |
| ALB | - | - | - | - | 0.488 | 0.483 | 0.141 | 0.868 | **0.847** | **0.890** | **0.870** | **0.903** |
| PLA | **0.337** | **0.316** | - | - | - | **0.686** | 0.437 | **0.894** | 0.865 | 0.916 | 0.894 | **0.927** |
| LUZ | **0.363** | **0.354** | - | - | **0.408** | - | **0.325** | **0.347** | 0.889 | 0.932 | 0.915 | **0.939** |
| ORA | **0.132** | **0.111** | - | - | **0.224** | **0.271** | - | **0.091** | 0.843 | **0.893** | 0.870 | **0.906** |
| SFR | **0.315** | **0.330** | - | - | **0.352** | **0.455** | **0.258** | - | **0.682** | **0.785** | **0.738** | **0.808** |
| BEL | **0.321** | **0.300** | - | - | **0.368** | **0.345** | **0.236** | **0.179** | - | 0.076 | 0.137 | 0.115 |
| SCR | **0.283** | **0.268** | - | - | **0.371** | **0.380** | **0.243** | **0.132** | 0.034 | - | 0.477 | 0.005 |
| MIN | **0.278** | **0.258** | - | - | **0.377** | **0.406** | **0.249** | **0.170** | **0.059** | **0.046** | - | **0.518** |
| ZAC | **0.343** | **0.309** | - | - | **0.397** | **0.355** | **0.231** | **0.209** | **0.041** | **0.107** | **0.082** | - |

Significant values after Bonferroni correction are shown in bold.

**Table 4. Hierarchical analysis of molecular variance based on mtDNA haplotypes and microsatellite allele frequencies among *Z. quitzeoensis* populations.**

| Groups | $F_{ST}$ | $F_{CT}$ | $F_{SC}$ | % Among groups | % Within groups | *P* |
|---|---|---|---|---|---|---|
| mtDNA | | | | | | |
| One gene pool (Populations) | 0.836 | - | - | 83.63 | 16.37 | <0.001 |
| Biogeography (Ameca)(Chapala)(Lower Lerma)(Middle Lerma-SFR)(Cuitzeo)(Zacapu) | 0.849 | 0.787 | 0.292 | 78.7 | 15.08 | <0.001 |
| Lineage I Biogeography (Ameca) (Chapala) (Lower Lerma) | 0.613 | 0.462 | 0.280 | 46.23 | 38.69 | <0.05 |
| Lineage II Biogeography (Middle Lerma-SFR)(Cuitzeo)(Zacapu) | 0.643 | 0.487 | 0.305 | 48.71 | 35.65 | ns |
| Two gene pools (Lineage I)(Lineage II) | 0.893 | 0.748 | 0.574 | 74.8 | 10.72 | <0.001 |
| Phylogenetic arrangement (Moloya-Magdalena-Veneros)(Belisario-Zacapu-San Cristóbal-Mintzita)(San Francisco del Rincón)(La Platanera)(Orandino-La Luz-La Alberca) | 0.832 | 0.823 | 0.168 | 84.32 | 14.71 | <0.001 |
| nDNA | | | | | | |
| One gene pool (Populations) | 0.262 | - | - | 26.18 | 73.82 | <0.001 |
| Biogeography (Chapala)(Bajo Lerma)(Middle Lerma-SFR)(Cuitzeo)(Zacapu) | 0.275 | 0.107 | 0.275 | 10.76 | 72.47 | <0.05 |
| Lineage I Biogeography (Ameca)(Chapala)(Bajo Lerma) | 0.323 | 0.082 | 0.263 | 8.27 | 67.63 | ns |
| Lineage II Biogeography (SFR)(Cuitzeo)(Zacapu) | 0.135 | 0.094 | 0.046 | 9.37 | 86.49 | ns |
| Two gene pools (Lineage I)(Lineage II) | 0.298 | 0.112 | 0.210 | 11.18 | 70.14 | <0.01 |

**Table 5. Estimates of demographic parameters and neutrality tests within the two species and main clades obtained.**

| | Clade | τ | $F_s$ | $D$ | $H_{ri}$ |
|---|---|---|---|---|---|
| Lineage I | 5.1 | 5.647 | -38.98** | -2.03* | 0.0049ns |
| | 3.6 Ameca | 5.08 | -18.40** | -2.27* | 0.0249ns |
| | 4.2 Chapala-Lower Lerma | 5.647 | -15.71** | -1.97* | 0.0161ns |
| Lineage II | 4.1 | 3.09 | -13.26** | -1.76* | 0.0124ns |
| | 3.1-3.2 Cuitzeo-Zacapu | 2.83 | -15.22** | -2.13* | 0.0218ns |

τ = age expansion parameter, $F_s$ = Fu's statistic, $D$ = Tajima's $D$ test, $H_{ri}$ = Harpending's raggedness index.
* $p < 0.05$, ** $p < 0.01$, ns=not significant

Figure 1

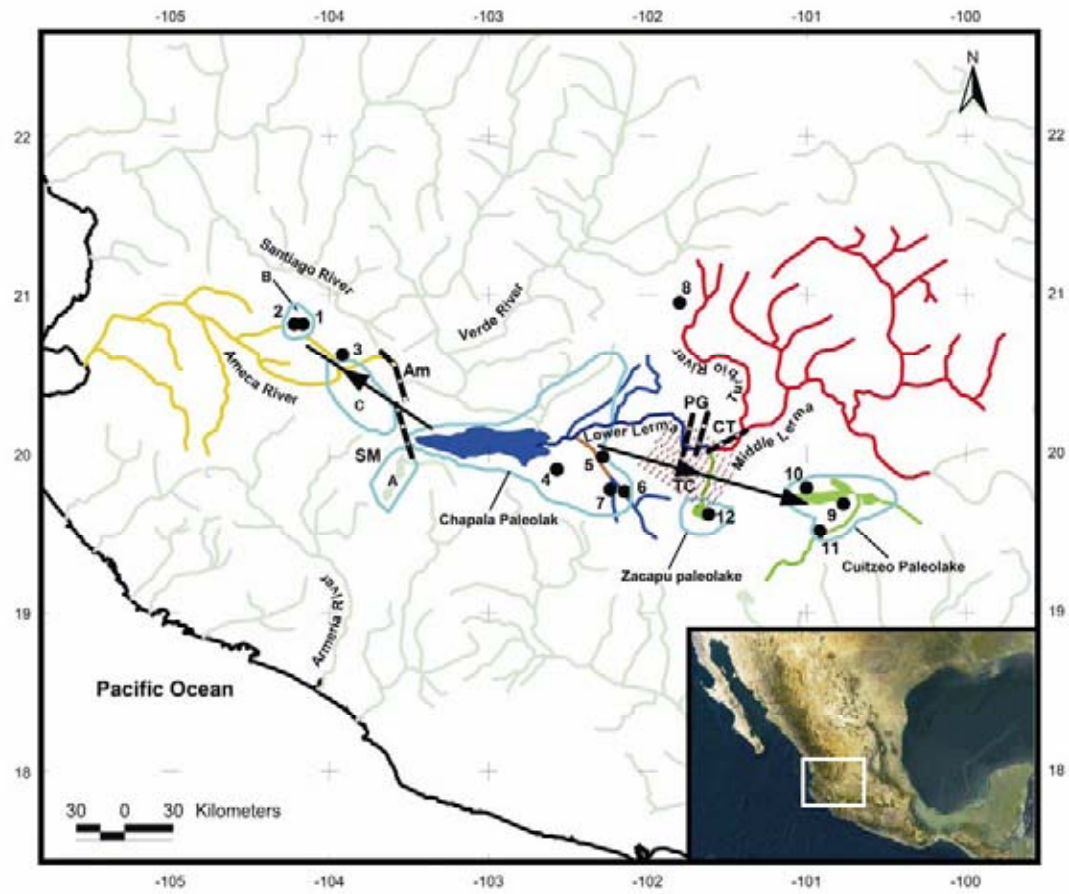

-105
-104
-103
-102
-101
-100
22
21
20
19
18
N
Santiago River
Verde River
Ameca River
Lerma River
Lower Lerma
Middle Lerma
Chapala Paleolak
Zacapu paleolake
Cuitzeo Paleolake
Armería River
Pacific Ocean
Am
SM
PG
CT
TC
A
B
C
1
2
3
4
5
6
7
8
9
10
11
12
30 0 30 Kilometers

Figure 2

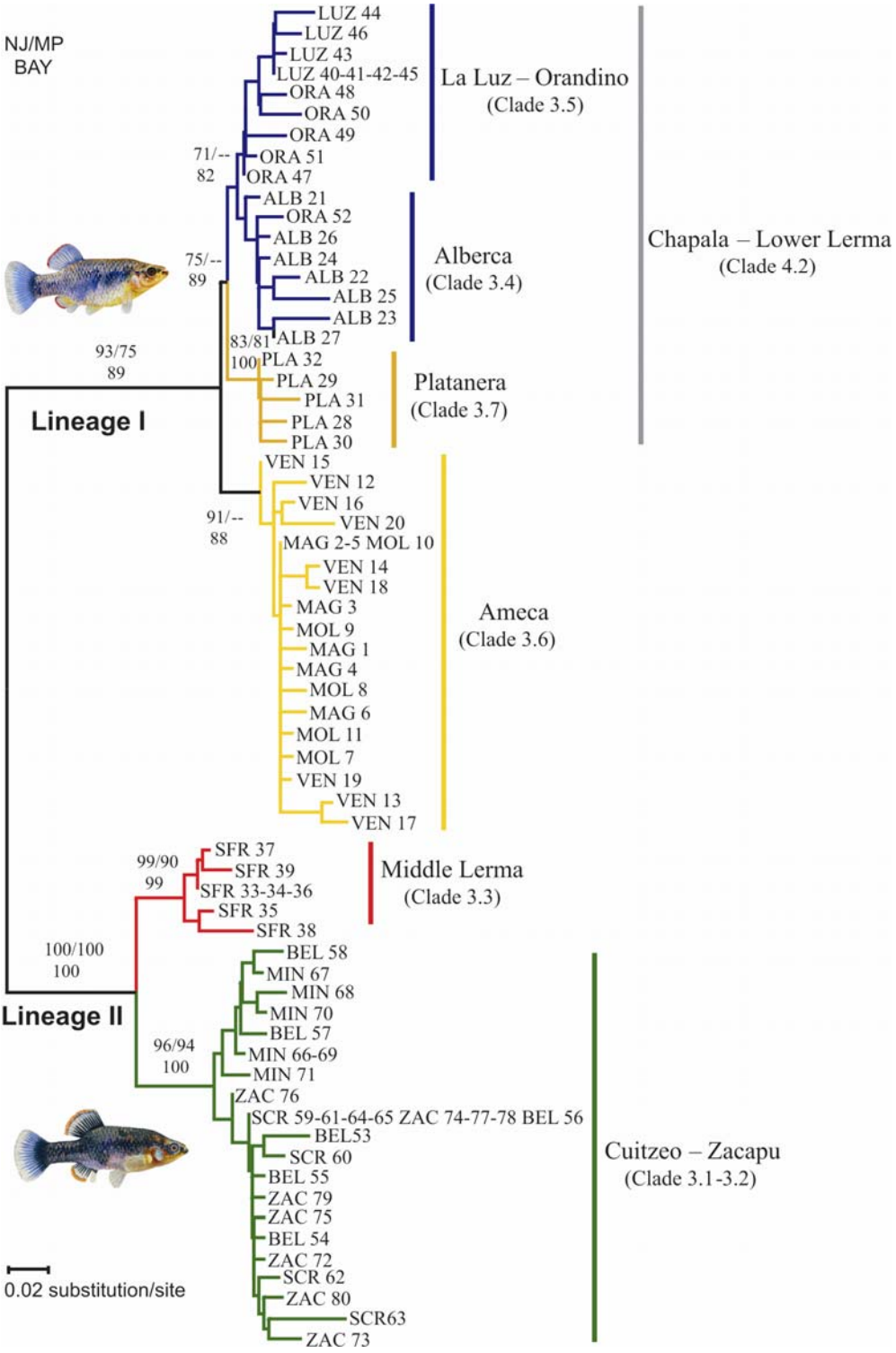

NJ/MP
BAY
LUZ 44
LUZ 46
LUZ 43
LUZ 40-41-42-45
ORA 48
ORA 50
ORA 49
ORA 51
ORA 47
La Luz – Orandino
(Clade 3.5)
71/--
82
ALB 21
ORA 52
ALB 26
ALB 24
ALB 22
ALB 25
ALB 23
Alberca
(Clade 3.4)
75/--
89
Chapala – Lower Lerma
(Clade 4.2)
83/81
100
ALB 27
PLA 32
PLA 29
PLA 31
PLA 28
PLA 30
Platanera
(Clade 3.7)
93/75
89
Lineage I
VEN 15
VEN 12
VEN 16
VEN 20
91/--
88
MAG 2-5 MOL 10
VEN 14
VEN 18
MAG 3
MOL 9
MAG 1
MAG 4
MOL 8
MAG 6
MOL 11
MOL 7
VEN 19
VEN 13
VEN 17
Ameca
(Clade 3.6)
99/90
99
SFR 37
SFR 39
SFR 33-34-36
SFR 35
SFR 38
Middle Lerma
(Clade 3.3)
100/100
100
Lineage II
BEL 58
MIN 67
MIN 68
MIN 70
BEL 57
96/94
100
MIN 66-69
MIN 71
ZAC 76
SCR 59-61-64-65 ZAC 74-77-78 BEL 56
BEL53
SCR 60
BEL 55
ZAC 79
ZAC 75
BEL 54
ZAC 72
SCR 62
ZAC 80
SCR63
ZAC 73
Cuitzeo – Zacapu
(Clade 3.1-3.2)
0.02 substitution/site

Figure 3

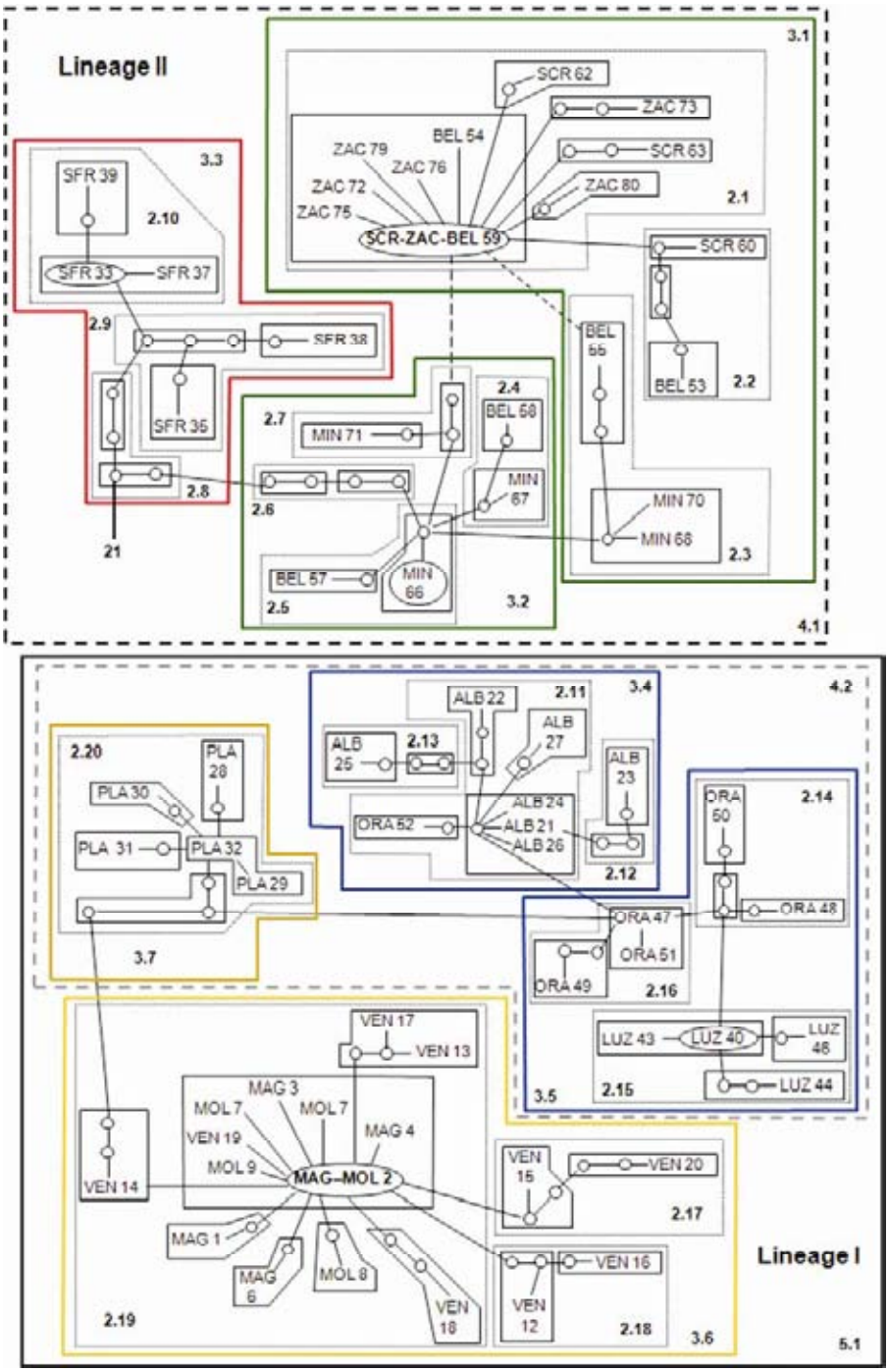

Lineage II
3.1
SCR 62
ZAC 73
BEL 54
ZAC 79
ZAC 76
SCR 63
ZAC 72
ZAC 80
ZAC 75
2.1
SCR-ZAC-BEL 59
SCR 60
3.3
SFR 39
2.10
SFR 33
SFR 37
BEL 55
BEL 53
2.2
2.9
SFR 38
SFR 35
2.4
2.7
MIN 71
BEL 58
MIN 67
2.8
2.6
MIN 70
MIN 68
2.3
21
BEL 57
MIN 66
2.5
3.2
4.1
4.2
3.4
2.11
ALB 22
ALB 27
2.20
PLA 28
ALB 25
2.13
ALB 23
PLA 30
ALB 24
ORA 52
ALB 21
ALB 26
2.14
ORA 60
2.12
PLA 31
PLA 32
PLA 29
ORA 48
ORA 47
ORA 51
3.7
ORA 49
2.16
LUZ 43
LUZ 40
LUZ 46
LUZ 44
3.5
2.15
VEN 17
VEN 13
MAG 3
MOL 7
MOL 7
VEN 19
MAG 4
MOL 9
MAG-MOL 2
VEN 14
VEN 15
VEN 20
2.17
MAG 1
MAG 6
MOL 8
VEN 16
VEN 18
VEN 12
2.18
2.19
3.6
Lineage I
5.1

Figure 4

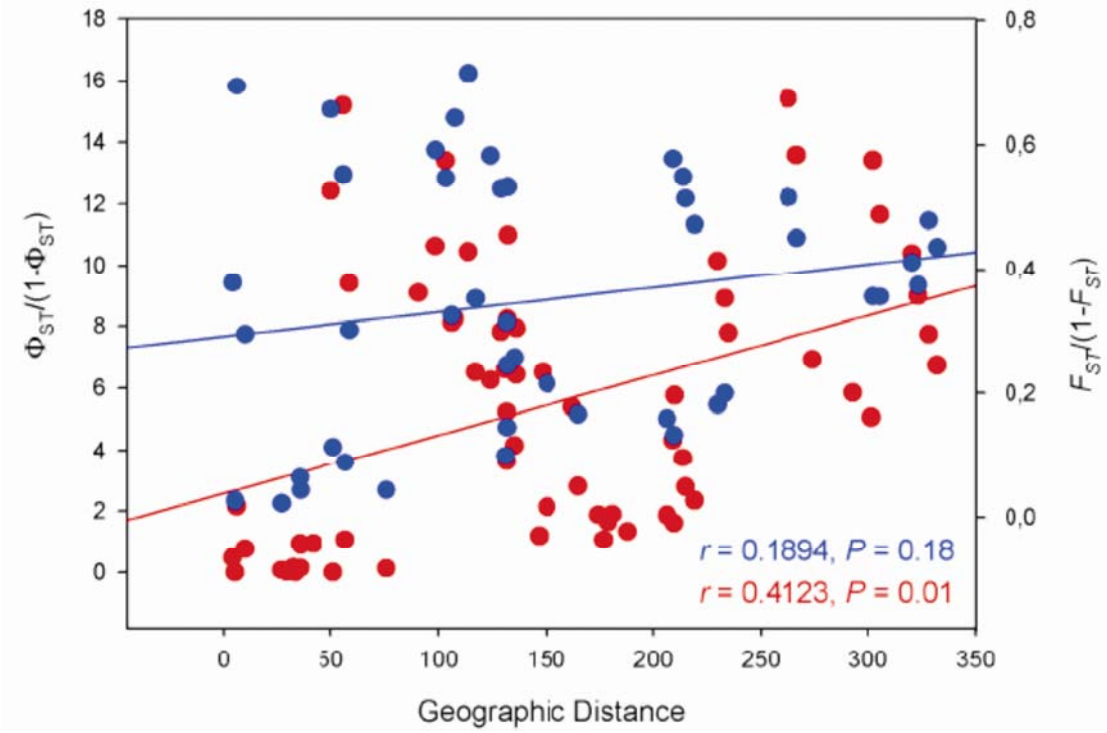

18
16
14
12
10
8
6
4
2
0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
200
250
300
350
Geographic Distance
$\Phi_{ST}/(1-\Phi_{ST})$
$F_{ST}/(1-F_{ST})$
r = 0.1894, P = 0.18
r = 0.4123, P = 0.01

Figure 5

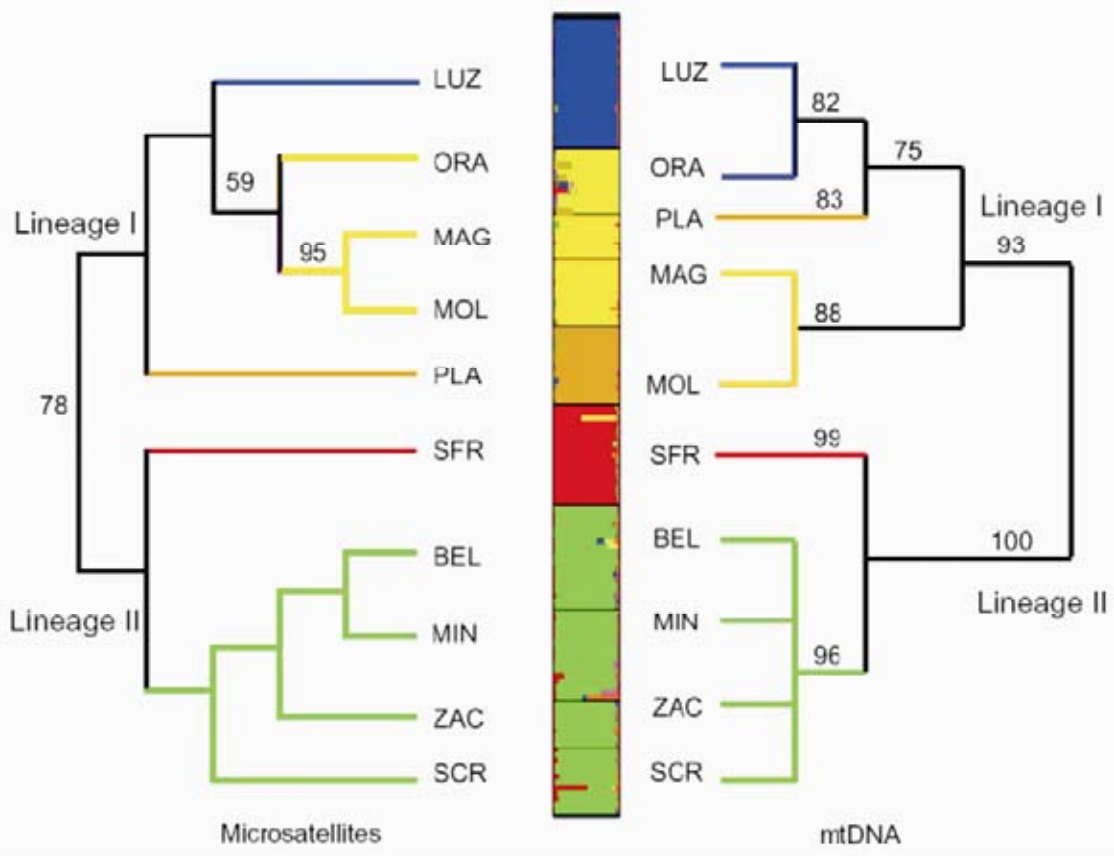
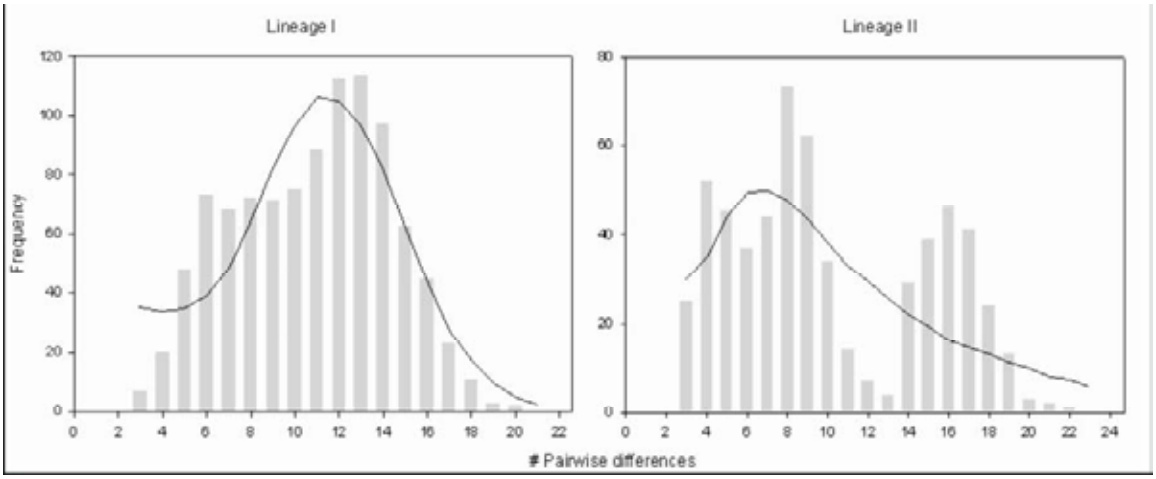

LUZ
ORA
MAG
MOL
PLA
SFR
BEL
MIN
ZAC
SCR
59
95
78
Lineage I
Lineage II
Microsatellites
LUZ
ORA
PLA
MAG
MOL
SFR
BEL
MIN
ZAC
SCR
82
83
75
88
99
96
93
100
Lineage I
Lineage II
mtDNA

Figure 6



Additional file 1

**Title:** Sampling sites and individuals analysed of *Zoogoneticus quitzeoensis*

**Description:** The table provided summarizes the collection sites of *Zoogoneticus quitzeoensis*, their geographical coordinates, the number of individuals analysed for cytochrome *b* and microsatellites and Genebank accession numbers.

| Basin | Site | *n* | Coordinates | GenBank Acc |
|---|---|---|---|---|
| Ameca River | Magdalena | 6 / 7 | 20º 53' 29.4" N | |
| | | | 104º 01' 55.2" W | |
| | Moloya | 6 / 10 | 20° 54´ 4.4" N | |
| | | | 104° 4´ 46.7´´ W | |
| | Veneros | 9 / 0 | 20º71' 22.9" N | |
| | | | 103º30' 29.9" W | |
| Chapala Lake | La Alberca | 7 / 0 | 20º03'32.93" N | |
| | | | 102º36' 33.1" W | |
| Lower Lerma River | La Platanera | 5 / 12 | 19º55'15.72" N | |
| | | | 102º15'04.91" W | |
| | La Luz | 7 / 20 | 19º56'08" N | |
| | | | 102º18'02.1 W | |
| | Orandino | 6 / 10 | 19° 57' 21.8" N | |
| | | | 102° 19' 29.7" W | |
| Middle Lerma River | San Francisco del Rincon | 7 / 19 | 21º02' 47.3" N | |
| | | | 101º50'9.3" W | |
| Cuitzeo Lake | Belisario | 6 / 19 | 19º53'42.41" N | |
| | | | 101º04'16.84" W | |
| | San Cristóbal | 7 / 12 | 19º57'42" N | |
| | | | 101º18'57" W | |
| | La Mintzita | 6 / 17 | 19º38'40" N | |
| | | | 101º16'28" W | |
| Zacapu Lake | Zacapu | 9 / 9 | 19º49'35" N | |
| | | | 101º47'10" W | |

*n*=Number of specimens for mtDNA / microsatellites

Additional file 2

**Title:** Substitution parameters and evolutionary models obtained for the different criterion and partitions of the cytochrome *b* data

**Description:** The data provided summarizes the parameters of the evolutionary models obtained for the cytochrome b data after the AIC and BIC. Ti/Tv ratio, Empirical base frequencies, Gamma shape parameter and Proportion of invariant sites are provided.

| Criterion | Codon position | Model of evolution | Substitution model Ti/Tv ratio | Empirical base frequencies | Gama distribution shape parameter | Proportion of Invariables sites |
|---|---|---|---|---|---|---|
| **AIC** | All | TIM+G | --- | A=0.2473<br>C=0.2717<br>G=0.1544<br>T=0.3266 | 0.5996 | --- |
| **BIC** | First | K80 | 10.31 | Equal | --- | --- |
| | Second | HKY | 7.73 | A=0.2069<br>C=0.2695<br>G=0.1366<br>T=0.3870 | --- | --- |
| | Third | TrN+I | --- | A=0.3047<br>C=0.3063<br>G=0.0620<br>T=0.3270 | --- | 0.5085 |

Additional file 3

**Title:** Inferences for all clades showing a significant association in the nested clade analysis results provided in Figure 3.

**Description:** The table describes the chain of inference obtained for all the statitistically significant clades in the Nested Clade Analysis.

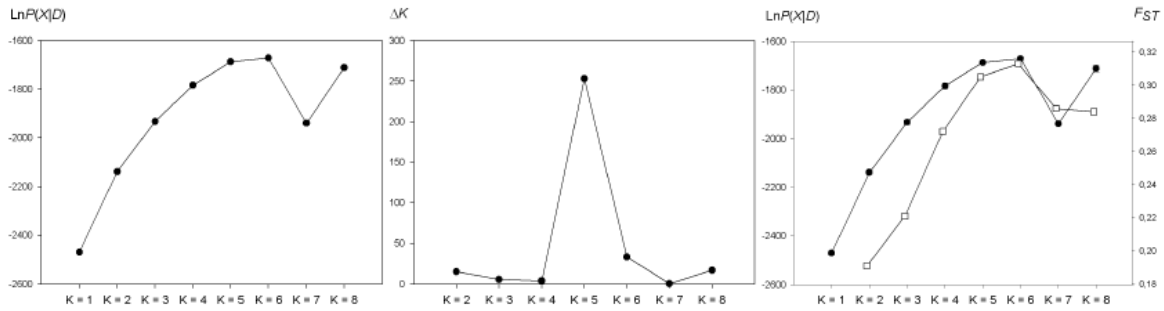
| Clade number | Populations studied | Statistics | Chain of inference | Population inference |
|---|---|---|---|---|
| 5.1 | Lineage I | $\chi 2$=45.00 *P*=0.000 | 1-2-3-5-NO | Insufficient genetic resolution to discriminate between range expansion/colonization and restricted dispersal/gene flow. |
| 3.5 | La Luz – Orandino | $\chi 2$=12.00 *P*=0.001 | 1-2-3-5-6-13-YES | Long distance colonization possibly coupled to subsequent fragmentation followed by range expansion. |
| 3.6 | Ameca | $\chi 2$=18.41 *P*=0.011 | 1-2-3-5-6-13-14-NO-21 | Long distance colonization and/or past fragmentation. Insufficient evidence to discriminate between long-distance movements of the organisms and the combined effects of gradual movements during past range expansion and fragmentation. |
| 4.2 | Chapala – Lower Lerma | $\chi 2$=45.65 *P*=0.000 | 1-2-3-5-6-13-YES | Long distance colonization possibly coupled to subsequent fragmentation followed by range expansion. |
| 4.1 | Lineage II | $\chi 2$=50.20 *P*=0.000 | 1-2-3-5-6-13-14-NO-21 | Long distance colonization and/or past fragmentation. Insufficient evidence to |

| | | | |
|---|---|---|---|
| | | | discriminate between long-distance movements of the organisms and the combined effects of gradual movements during past range expansion and fragmentation. |
| Whole cladogram | $\chi 2$=80.00<br>*P*=0.000 | 1-19-NO | Allopatric fragmentation. |

Additional file 4

**Title:** (Left) Mean Ln*P*(*X*|*D*) for each of the *K* populations inferred by STRUCTURE. (Middle) Number of *Zoogoneticus* populations with the highest posterior probability expressed as the *ΔK* (Evanno et al. 2005). (Right) Comparisons between Ln*P(D)* (black circles) and $F_{ST}$ values (white squares) obtained for the different *K* values inferred by STRUCTURE.

**Description:** This figure represents graphically the values of *LnP(X|D), ΔK* and $F_{ST}$ for each of the different genetic arrangements inferred by STRUCTURE



Additional file 5

**Title:** Summary table of diversity and BOTTLENECK statistics for the microsatellite data of *Zoogoneticus quitzeoensis*. Modified from Domínguez-Domínguez et al. (2007).

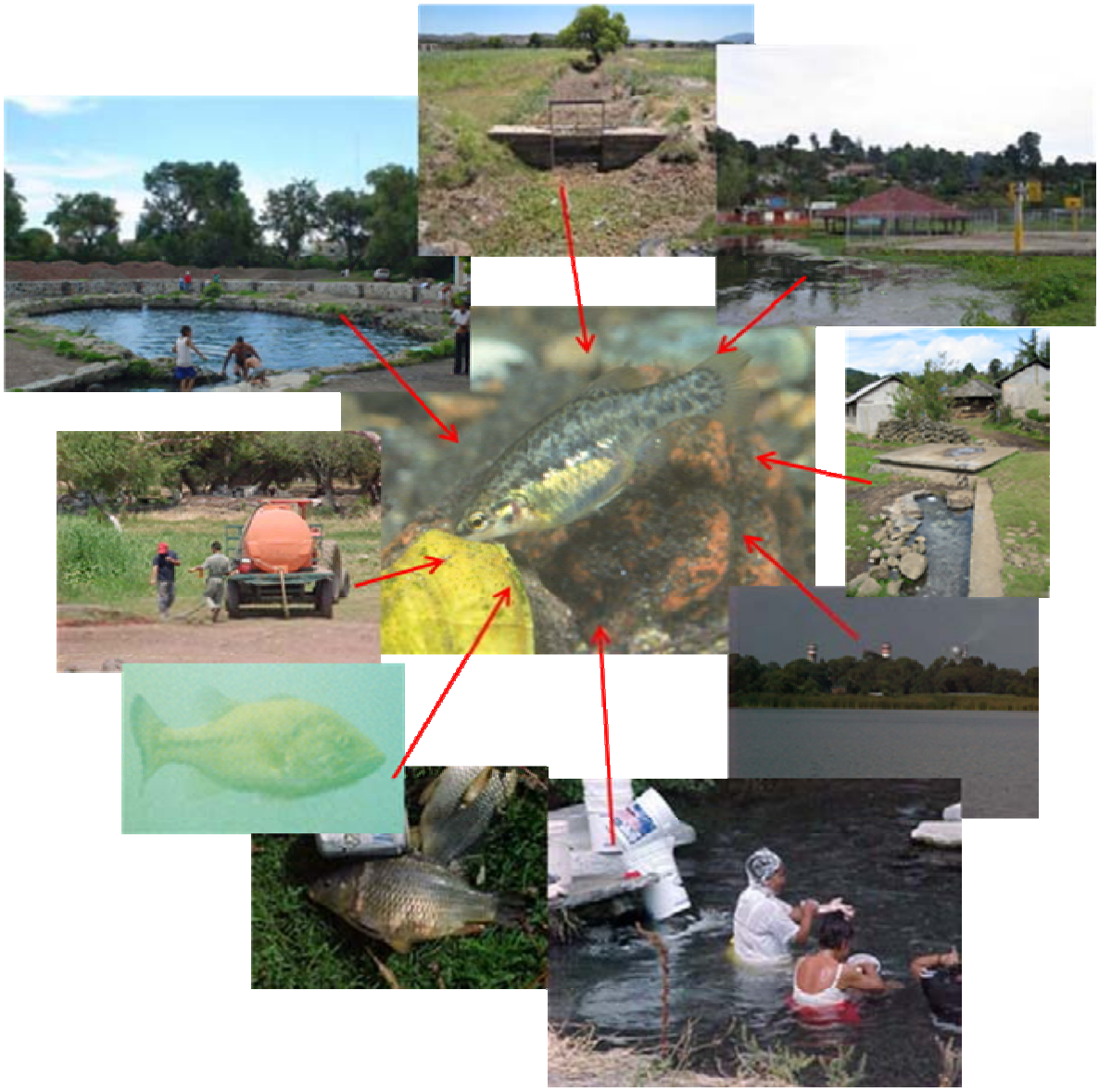
**Description:** The table describes values of genetic diversity based on microsatellite data.

| Population | Biogeographic region | N | A | Ne | NPA | HO | HE | FIS | BOTTLENECK (IAM/SMM/TPM) |
|---|---|---|---|---|---|---|---|---|---|
| 1.- El Moloya | Ameca River | 10 | 5 | 3.69 | 4 | 0.61 | 0.6 | -0.081 | +/-/- |
| 2.- Magdalena | Ameca River | 7 | 4.2 | 3.09 | 2 | 0.6 | 0.65 | -0.018 | +/+/+ |
| 3.- Platanera | Lower Lerma | 12 | 3.6 | 2.27 | 3 | 0.45 | 0.46 | -0.185 | +/-/+ |
| 4.- Orandino | Lower Lerma | 10 | 6.2 | 4.17 | 9 | 0.58 | 0.63 | 0.085 | -/-/- |
| 5.- La Luz | Lower Lerma | 9 | 4.6 | 2.29 | 8 | 0.45 | 0.49 | 0.163* | -/-/- |
| 6.- San Francisco | Middle Lerma | 19 | 3.8 | 2.4 | 3 | 0.38 | 0.42 | 0.093 | -/-/- |
| 7.- Zacapu | Zacapu Drainage | 20 | 3 | 2.09 | 0 | 0.51 | 0.4 | -0.235 | +/+/+ |
| 8.- San Cristobala | Cuitzeo Drainage | 12 | 6.2 | 4.17 | 1 | 0.5 | 0.46 | 0.145 | +/+/+ |
| 9.- La Mintzita | Cuitzeo Drainage | 17 | 4.8 | 3.05 | 1 | 0.48 | 0.47 | -0.025 | +/+/+ |
| 10.- Belisario | Cuitzeo Drainage | 19 | 5.6 | 3 | 3 | 0.45 | 0.48 | 0.144 | +/-/+ |

N: sample size; A: average number of alleles per locus; $N_e$: effective number of alleles; $N_{PA}$: number of private alleles; $H_O$: observed and $H_E$: expected heterozygosity (Nei 1973); $F_{IS}$: inbreeding index; *: significant departure from the Hardy-Weinberg Equilibrium ($p<0.001$); BOTTLENECK: results from the heterozygosity excess estimated by Wilcoxon sing-rank test ($P<0.05$), based on infinite allele model (IAM), stepwise mutation model (SMM) and two phase model (TPM).

# CONSERVACIÓN

## ARTICULO II



# Human Impacts on Drainages of the Mesa Central, Mexico, and Its Genetic Effects on an Endangered Fish, *Zoogoneticus quitzeoensis*

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**Abstract:** *The Mesa Central of Mexico is of special conservation interest due to its high richness of freshwater fish species, of which the goodeines are one of the most representative groups. Through an integrated approach, we determined conservation priorities for goodeine populations. We based our recommendations on the genetic diversity (variation in five microsatellite DNA loci) in 10 populations of* Zoogoneticus quitzeoensis *and on an analysis of ecological (e.g., presence of exotic species), social (e.g., political situation), and environmental (e.g., pollution) information for 52 historical occurrence points for species in the genus* Zoogoneticus. *Patterns of genetic erosion and genetic diversity indices were closely associated with human impact. Recent bottleneck events were most evident in the populations from remnants of the lakes drained at the beginning of the twentieth century. We identified seven operational conservation units (OCUs), all of which should be conserved because they contain unique portions of the total variation of the species. Special attention needs to be given to increase genetic variability, recover population sizes, and reestablish contact among populations within OCUs. It is imperative to create an integrative and effective approach for the recovery and conservation of the freshwater fish diversity of Central Mexico that is based on social and natural sciences.*

**Keywords:** conservation units, genetic diversity, Goodeidae, human impacts, Mexican fishes

Impactos Humanos en Cuencas Hidrológicas de la Meseta Central. México, y Sus Efectos Genéticos sobre una Especie de Pez en Peligro de Extinción, *Zoogoneticus quitzeoensis*

**Resumen:** *La Meseta Central de México es de especial interés para la conservación debido a su alta riqueza de especies de peces dulceacuícolas, de los que los goodeinos son uno de los grupos más representativos. Por medio de un método integral, determinamos prioridades de conservación para poblaciones de goodeinos. Basamos nuestras recomendaciones en la diversidad genética (variación en el loci de cinco microsatélites de ADN) en 10 poblaciones de* Zoogoneticus quitzeoensis *y en un análisis de información ecológica (e.g., presencia de especies exóticas), social (e.g., situación política) y ambiental (e.g., contaminación) de 52 puntos de ocurrencia histórica de especies en el género* Zoogoneticus. *Los patrones de erosión genética y los índices de diversidad genética estuvieron estrechamente asociados con el impacto humano. Eventos de cuello de botella recientes fueron más evidentes en las poblaciones de los remanentes de lagos drenados a inicios del siglo veinte. Identificamos siete unidades operacionales de conservación (UOC), que deben ser conservadas porque contienen porciones únicas de la variación total de las especies. Se necesita dar atención especial para incrementar la variabilidad*

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*genética, recuperar los tamaños poblacionales y reestablecer contacto entre las poblaciones dentro de las UOC. Es imperativa la creación de un método integral y efectivo para la recuperación y conservación de la diversidad de peces dulceacuícolas en el centro de México que se base en información derivada de las ciencias sociales y naturales.*

**Palabras Clave:** diversidad genética, Goodeidae, impactos humanos, peces mexicanos, unidades de conservación

## Introduction

About 520 freshwater fish species are known from Mexico, of which 163 are endemic (CONABIO 1998; Miller 2005). The Mesa Central de Mexico has especially high species richness and endemicity (70%) of fishes and is considered by the World Conservation Monitoring Centre as a region of special interest for the conservation of freshwater fishes (Guzmán-Arroyo 1994; Groombridge & Jenkins 1998). Freshwater environments in this region have been severely altered by anthropogenic activities. Introduction of exotic species and their parasites, pollution, water extraction, deforestation, and overfishing are just some of the causes for the decline of native species (e.g., Lyons et al. 1998; Soto-Galera et al. 1998; Domínguez-Domínguez et al. 2005*a*).

One of the most representative fish groups in the Mesa Central de Mexico are the goodeines, a subfamily composed of 41 species from 19 genera, most of which are endemic to the region (Doadrio & Domínguez-Domínguez 2004; Webb et al. 2004). This group presents unique reproductive characteristics, such as internal fertilization, matrotrophy, and viviparity (Parenti 1981), and is considered one of the most at-risk groups in the world (Duncan & Lockwood 2001).

The goodeine genus *Zoogoneticus* is of special concern with respect to conservation. One of the two species in the genus, *Z. quitzeoensis*, is federally listed in Mexico as endangered and the other species, *Z. tequila*, is extinct in the wild (Mexican Official Norm of Ecology-NOM-ECOL-059-2001). The populations of *Z. quitzeoensis* show high levels of genetic (mtDNA) and morphological divergence (Doadrio & Domínguez-Domínguez 2004). In addition, the species has declined greatly in distribution, and local extinctions have occurred (de la Vega-Salazar et al. 2003; Domínguez-Domínguez et al. 2005*b*).

There has been considerable discussion regarding how to use genetic information to identify "operational groups" for conservation. Genetic diversity is generally recognized as the base level of biodiversity (as recommended by the Convention on Biological Diversity, held in Brazil in 1992), and the exclusive use of molecular data to identify conservation units for development of conservation strategies is tempting. Nevertheless, such data should be evaluated together with historical, ecological, and social information and species distributions to obtain a more accurate and comprehensive perspective (Crandall et al. 2000). Recently, other sources of information have been incorporated in conservation strategies, including the use of spatial distribution of genetic diversity and ecology and ecosystem functions to delimit conservation units, at the species and/or the geographic level (Moritz 2002; Luck et al. 2003; Manel et al. 2003). For the conservation of freshwater fish diversity, Doadrio et al. (1996) introduced the OCU concept, which is defined as "a continuous area limited by geographical boundaries and inhabited by one or more populations sharing the same genetic pattern." Other factors such as conservation status and socioeconomic issues have been used to delimit OCUs (Dodson et al. 1998). We used geographic variation at microsatellite DNA loci and analyses of land use, anthropogenic disturbance, and the historical and present areas of occupancy to identify OCUs and make recommendations for the conservation of *Z. quitzeoensis*.

## Methods

### Samples and Data Collection

We obtained the historical locations of *Z. quitzeoensis* from the University of Michigan, Museum of Zoology; the Icthyological Collection, Universidad Michoacana de San Nicolás de Hidalgo; and the National Collection of Fishes, Instituto de Biología, Universidad Nacional Autónoma de México. To evaluate the present distribution of *Z. quitzeoensis* and to assess environmental threats, we sampled 52 localities of historical occurrence from 2000 through 2005. The fieldwork included inspection of habitat, collection of information on the presence of exotic species, evidence of pollution, and other habitat alterations.

We used minnow traps and seine nets for sampling. All fish captured were identified and released unharmed. All field and historical records were compiled in a georeferenced database. Only localities visited at least four times were included in the study.

We preserved (in ethanol) caudal fin clips from 135 fish representing 10 populations from five biogeographic regions: Cuitzeo, Zacapu, Ameca, Middle Lerma, and the Lower Lerma (sensu Domínguez-Domínguez et al., 2006). Because of the endangered status of *Z. quitzeoensis* and

**Table 1. Microsatellite diversity indices for *Zoogoneticus quitzeoensis* populations sampled in central Mexico.***

| *Population* | *Biogeographic region* | n | *Average no. of alleles/locus* | $A_R$ | $H_o$ | $H_e$ | $F_{IS}$ | $H_eW_{t(IAM/SMM/TPM)}$ |
|---|---|---|---|---|---|---|---|---|
| El Moloya | Ameca River | 10 | 5 | 4.65 | 0.61 | 0.6 | −0.081 | +/−/− |
| Magdalena | Ameca River | 7 | 4.2 | 4.2 | 0.6 | 0.65 | −0.018 | +/+/+ |
| Platanera | Lower Lerma | 12 | 3.6 | 3.16 | 0.45 | 0.46 | −0.185 | +/−/+ |
| Orandino | Lower Lerma | 10 | 6.2 | 5.33 | 0.58 | 0.63 | 0.085 | −/−/− |
| La Luz | Lower Lerma | 9 | 4.6 | 3.18 | 0.45 | 0.49 | 0.163* | −/−/− |
| San Francisco | Middle Lerma | 19 | 3.8 | 2.97 | 0.38 | 0.42 | 0.093 | −/−/− |
| Zacapu | Zacapu drainage | 20 | 3 | 2.93 | 0.51 | 0.4 | −0.235 | +/+/+ |
| San Cristóbal | Cuitzeo drainage | 12 | 6.2 | 3.67 | 0.5 | 0.46 | 0.145 | +/+/+ |
| La Mintzita | Cuitzeo drainage | 17 | 4.8 | 3.91 | 0.48 | 0.47 | −0.025 | +/+/+ |
| Belisario | Cuitzeo drainage | 19 | 5.6 | 3.93 | 0.45 | 0.48 | 0.144 | +/−/+ |

**Key:* n, *sample size;* $A_R$, *mean allelic richness per locus based on the minimum sample size;* $H_o$ *and* $H_e$, *observed and unbiased expected heterozygosity, respectively (Nei 1973);* $F_{IS}$, *inbreeding index (*)significant departure from the Hardy-Weinberg equilibrium,* $p < 0.001$; $H_eW_t$, *heterozygosity excess estimated by Wilcoxon sign-rank test, based on infinite allele model (IAM), stepwise mutation model (SMM), and two-phase model (TPM); +, significant values for bottleneck detection* ($p < 0.05$).

the scarcity of the species at all collection sites, sample sizes ranged from 7 to 20 individuals per population (Table 1). Individuals used for molecular analysis were from a single collection at each locality.

### DNA Extraction and Microsatellite Loci Amplification

We isolated total genomic DNA following a standard cetyl-trimethylammonium bromide (CTAB) and phenol-chloroform extraction protocol (Doyle & Doyle 1987; Sambrook et al. 1989). Five microsatellites were amplified in all samples (ZT1.2, ZT1.6, ZT1.7, ZT1.9, and ZT1.43) following Boto and Doadrio (2003). The polymerase chain reaction (PCR) products were analyzed on an ABI 3700 automatic sequencer and alleles determined using GeneScan (version 3.5.1) and Genotyper (version 3.6) (Applied Biosystems, Weiterstadt, Germany).

### Microsatellite Analysis

We assessed tests of Hardy-Weinberg equilibrium and linkage disequilibrium with GENEPOP (Raymond & Rousset 2000). The alpha level was corrected with the sequential Bonferroni test (Rice, 1989). Using Popgene 1.32 (Yeh & Boyle 1997), we computed for each population the number of alleles per locus ($A$), observed and unbiased expected heterozygosities ($H_o$ and $H_e$), and the inbreeding coefficient ($F_{IS}$, Weir & Cockerham 1984). The observed number of alleles is highly dependent on sample size (El Mousadik & Petit 1996). To avoid this problem, we used FSTAT 2.9.3.2 (Goudet 1995) to obtain estimates of allele richness ($A_R$) from 1000 resamplings of the data adjusted to the minimum sample size.

We used SPAGEDI (Hardy & Vekemans 2002) to determine the relative impact of drift ($F_{ST}$) versus mutation ($R_{ST}$) on genetic divergence. Different allele sizes at each locus were randomly permuted among allelic states (20,000 permutations) to provide a simulated distribution of $R_{ST}$ values ($pR_{ST}$) with 95% confidence intervals. Under the null hypothesis $R_{ST} = F_{ST}$, divergence is explained by genetic drift, whereas if $R_{ST} > F_{ST}$, stepwise mutation is more important (Hardy et al. 2003).

We used Bottleneck (Cornuet & Luikart 1996) to test for recent bottleneck events. We calculated expected average heterozygosity through simulation in the infinite allele model, the stepwise mutation model, and the two-phase mutation model. Population allele frequency distributions were also examined for mode shifts. Given the number of loci and sample sizes in our data set, we used the Wilcoxon sign-rank test to test the significance of excess heterozygosity in the three mutation models (Luikart et al. 1998).

### Genetic and Field Information Plot

Indexes of environmental quality and biotic integrity were obtained from the literature for most sites (Soto-Galera et al. 1998; Lyons et al. 2000; Mercado et al. 2002). Based on the most recent national land-use/land-cover assessment (IFN 2000), we identified areas potentially damaged by human activities by using information on land use (i.e., the transformation of native vegetation to urban areas, croplands, and cultivated or induced grasslands). Associations between habitat impact and land transformation with respect to genetic diversity and bottlenecks (i.e., the number of private alleles, mean allelic richness, observed heterozygosity, and inbreeding coefficient) were visualized with ArcView (version 3.2, Environmental Systems Research Institute, Redlands, CA).

Finally, we performed a Mantel test to determine whether the percentages of land transformation and water body reduction were significantly correlated with bottleneck detection and genetic diversity, expressed as $H_o$ and $A_R$. We used NTSYS version 2.1 (Rohlf 2000) and 10,000 replications. To calculate the percentage of land that was transformed, we "trimmed" the land use/land cover maps with a map representing the sub-basin system

**Table 2. Ecological changes in 52 historical localities within the eight biogeographic regions in central Mexico.***

| *Biogeographic region* | n | *Disappeared localities* | *PL* | *LW-LWo* | *LE* | *LZP* |
|---|---|---|---|---|---|---|
| Ameca drainage | 6 | 1, desiccation | 2 | 5-0 | 5 | 4 |
| Armeria drainage | 2 | 0 | 1 | 2-0 | 2 | 0 |
| Santiago drainage | 8 | 3, construction<br>2, diversion | 3 | 2-1 | 3 | 0 |
| Chapala drainage | 11 | 2, desiccation<br>2, diversion | 4 | 6-1 | 5 | 2 |
| Lower Lerma drainage | 7 | 1, diversion | 4 | 5-1 | 5 | 4 |
| Middle Lerma drainage | 4 | 1, desiccation | 2 | 2-1 | 2 | 1 |
| Zacapu drainage | 2 | 0 | 1 | 2-0 | 2 | 1 |
| Cuitzeo drainage | 12 | 4, desiccation<br>2, construction | 4 | 5-1 | 4 | 5 |
| Total | 52 | 18 | 21 | 29-5 | 28 | 17 |

**Abbreviations:* n, *number of localities sampled with historical records of* Z. quitzeoensis; *PL, polluted localities; LW, localities with fishes; LWo, localities without fishes; LE, localities with exotic fishes; LZP, localities with* Z. quitzeoensis *populations.*

of Central Mexico (sensu Domínguez-Domínguez et al., 2006) in ArcView version 3.2. In addition, we used aerial photographs 1:20000 taken by the Instituto Nacional de Estadística Geografía e Informática (INEGI) during the 2003 campaign to calculate the percentage of water surface that had been drained.

## Results

### Population Status

Fifty-two localities with historical information about the presence of the species were sampled between 2000 and 2005. Eighteen of these localities disappeared completely (Table 2). In the 34 remaining localities *Z. quitzeoensis* was present in only 17, indicating a local extinction of 67% (Fig. 1).

Twenty-one localities showed evidence of pollution (e.g., fetid smell due to organic decomposition, foam, or chemicals), and five of these were devoid of fish (Table 2 & Fig. 2). Fish were present at 29 of the localities sampled, and 28 of those had at least one exotic species (Table 2). The most common introduced species were carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), tilapia (*Oreochromis* spp.), and several poeciliids (Table 3).

At the basin level, Santiago and Armeria basins had the greatest percentage (100%) of locally extinct populations, followed by the Chapala (80%) and Middle Lerma (75%) basins (Table 2 & Fig. 1). The Ameca basin had the fewest local population extinctions (17%; Table 2). Fewer than 10 specimens of *Z. quitzeoensis* were found in 7 of the 17 localities where the species was collected, indicating low abundances in most remnant populations. A few habitat localities, mainly springs and the upper reaches of rivers, still had large populations (i.e., localities 1, 4, 6, 8, and 9 in Fig. 1). Most of these sites were isolated and geographically distant from the closest populations.

For the 10 localities included in the microsatellite analyses, La Mintzita had the highest number of native species (15), followed by Zacapu (10) and Orandino (8), and Magdalena had the lowest number of native species (5). Three localities had 1 endemic species and Zacapu had 2. La Mintzita had the largest number of locally extinct species (3), followed by San Cristóbal (2) and Orandino, Magdalena, San Francisco, and Belisario (1). With the data obtained from the literature we determined that the only site with a low index of biotic integrity was San Cristóbal (Table 2). The historical sites where *Z. quitzeoensis* had been extirpated were generally associated with areas showing relatively severe changes in land use (Fig. 2).

### Intrapopulation Diversity

Exact tests of linkage disequilibrium for each pair of microsatellite loci over all populations yielded no significant values ($p > 0.05$). Only the La Luz population showed a significant departure from Hardy-Weinberg equilibrium ($p < 0.001$, over all loci).

All loci showed high polymorphism with number of alleles per locus ranging from 11 at locus ZT1.6 to 25 at locus ZT1.7. Locus ZT1.2 showed the highest number of alleles in a single population (10 at Orandino), and ZT1.43 was monomorphic in six populations (Platanera, Orandino, San Cristóbal, La Mintzita, Zacapu, and San Francisco). Allele variation expressed as the average number of alleles per locus ($A$) and allele richness ($A_R$) was generally low (Table 1). The Orandino and San Cristóbal populations had the highest average number of alleles ($A = 6.2$), whereas Orandino and El Moloya showed the highest allelic richness ($A_R = 5.33$ and $A_R = 4.65$, respectively). The lowest allelic variation was in Zacapu Lake ($A = 3$ and $A_R = 2.93$). The observed heterozygosity values ranged from 0.38 in San Francisco to 0.61 in El Moloya (Table 1, Fig. 3). The Mantel tests showed that land transformation was significantly correlated with observed heterozygosity ($r = 0.235$; $p = 0.04$) but not with allele richness ($r = 0.089$; $p > 0.05$).

### Interpopulation Diversity

All analyzed loci and 9 of the 10 sampled populations of *Z. quitzeoensis* contained unique alleles. Although Orandino and La Luz are geographically close and within the same biogeographic region, they had the highest number of private alleles (nine and eight, respectively).

Global estimates of $F_{ST}$ and $R_{ST}$ were high and significant ($F_{ST} = 0.2584$ and $R_{ST} = 0.4124$; $p < 0.001$). The only nonsignificant pairwise $F_{ST}$ values ($p > 0.05$) were those for two pairs of populations that were recently isolated from each other: San Cristóbal-Belisario and El Moloya-Magdalena. All other pairwise comparisons were highly significant ($p < 0.001$), with $F_{ST}$ values ranging

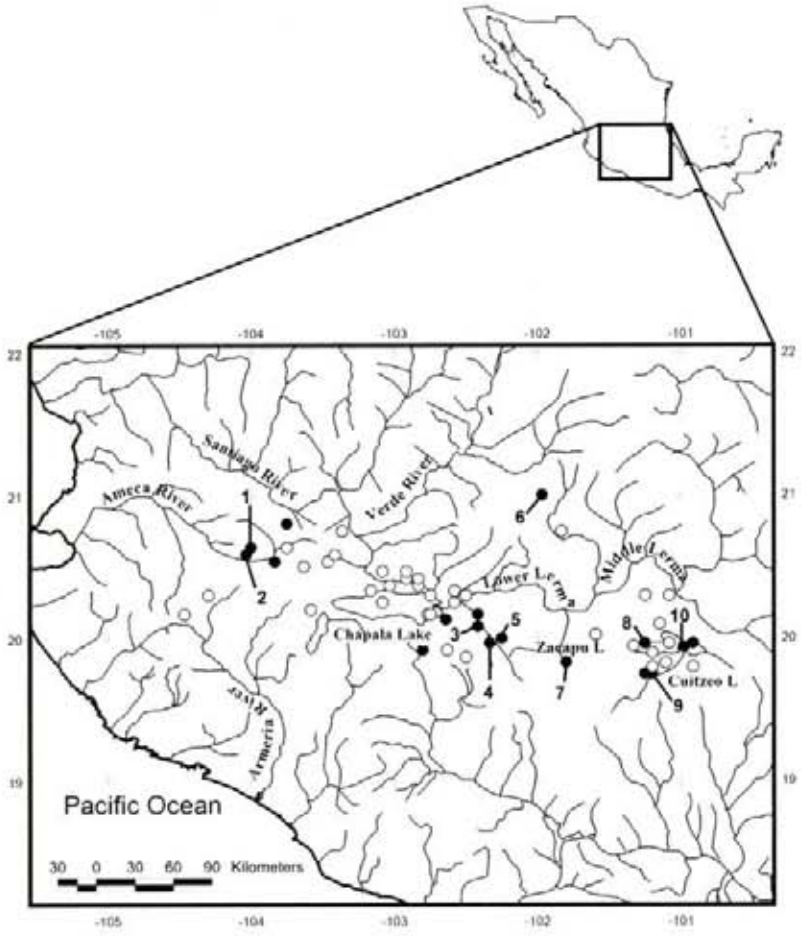


*Figure 1. Local extinction in 52 populations of* Zoogoneticus quitzeoensis *in the Mesa Central, Mexico: open circles, localities where the species has not been found in the last 5 years; filled circles, localities where the species was collected in the last 5 years; numbers, localities included in microsatellite analysis: 1, El Moloya Spring; 2, Magdalena Lake; 3, La Platanera Spring; 4, Orandino Spring; 5, La Luz Spring; 6, San Francisco del Rincón Spring; 7, Zacapu Lake; 8, San Cristóbal Spring; 9, La Mintzita Spring; 10, Belisario.*

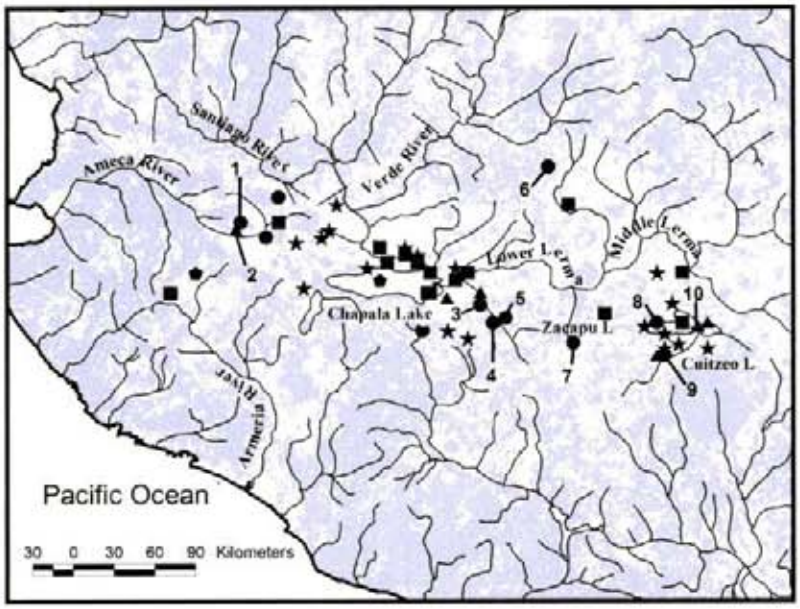


*Figure 2. Land-use and land-cover assessment map and the historical localities sampled in the Mesa Central, Mexico, during field trips (2000–2005): gray, original vegetation present; white, natural vegetation totally transformed to agriculture, industry, cities, or grasslands; circles, localities with Z.* quitzeoensis*; squares, polluted localities where no Z.* quitzeoensis *were collected; triangles, polluted localities with Z.* quitzeoensis*; pentagons, no apparent perturbance, but no Z.* quitzeoensis *collected; stars, drained localities; numbers, localities included in the microsatellite analysis.*

**Table 3. Environmental and conservation information for the 10 populations used in the microsatellite analyses.[a]**

| *Population* | *Native species* | *Introduced species* | *Endemic species* | *EQ*[b] | *Extinctions* | *Drainage alterations* | *Immediate threats* |
|---|---|---|---|---|---|---|---|
| El Moloya (WP) | *Z. quitzeoensis*[c,d] | *Oreochromis* sp. | 0 | NI | 0 | after the Magdalena Lake was drained, spring remained isolated | modification of spring for recreational swimming pool; introduction of exotic species |
| | *Xenotoca eiseni*[d] | | | | | | |
| | *X. melanosoma*[d] | | | | | | |
| | *Ameca splendens*[c,d] | | | | | | |
| | *Goodea atripinnis* | | | | | | |
| | *Poeciliopsis infans* | | | | | | |
| Magdalena (WP) | *Allotoca maculata*[e,d] | *Cyprinus carpio* | 1 | NI | 1 | drained in 1926 for agricultural purposes; since then lake was reduced to a few irrigation channels (Camacho 1998) | drainage for agricultural purposes; introduction of exotic species; organic pollution |
| | *Z. quitzeoensis*[c,d] | *Ctenopharingodon idella* | | | | | |
| | *G. atripinnis* | *Oreochromis* sp. | | | | | |
| | *X. melanosoma*[d] | | | | | | |
| | *X. eiseni*[d,f] | | | | | | |
| Platanera (WP) | *Alloophorus robustus*[d] | *C. carpio* | 0 | NI | 0 | reduction of migration rates due to isolation of the spring induced by highly polluted waters and diversion of natural water flow for agricultural purposes (Medina-Nava 2003); 17% of spring drained for agricultural purposes and water extraction | increase of the pollution in the Lerma River (Soto-Galera et al. 1998); introduction of exotic species |
| | *Z. quitzeoensis*[c,d] | *C. idella* | | | | | |
| | *Xenotoca variata* | *Oreochromis* sp. | | | | | |
| | *G. atripinnis* | *Micropterus salmoides* | | | | | |
| | | *Poecilia reticulata* | | | | | |
| | *Skiffia multipunctata*[d] | | | | | | |
| | *P. infans* | | | | | | |
| | *Chapalichthys encaustus*[d] | | | | | | |
| | *Chirostoma jordani* | | | | | | |
| Orandino (WP) | *S. multipunctata*[d] | *C. carpio* | 1 | medium*** | 1 | reduction of migration rates due to isolation of the spring induced by highly polluted waters and diversion of natural water flow for agricultural purposes (Medina-Nava 2003) | polluted environments persist in area; introduction of exotic species |
| | *C. encaustus*[d] | *C. idella* | | | | | |
| | *Z. quitzeoensis*[c,d] | *Xiphophorus helleri* | | | | | |

*continued*

**Table 3. (continued)**

| *Population* | *Native species* | *Introduced species* | *Endemic species* | *EQ*[b] | *Extinctions* | *Drainage alterations* | *Immediate threats* |
|---|---|---|---|---|---|---|---|
| | *G. atripinnis* | *Poecilia sphenops* | | | | | |
| | *P. infans* | | | | | | |
| | *Algansea tincella* | | | | | | |
| | *Notropis joseltvarezi*[e,f] | | | | | | |
| | *A. robustus*[d] | | | | | | |
| La Luz (WP) | *S. multipunctata*[d] | *C. carpio* | 0 | medium*** | 0 | reduction of migration rates due to isolation of spring induced by highly polluted waters and diversion of natural water flow for agricultural purposes (Medina-Nava 2003) | polluted environments persist in area; introduction of exotic species; extraction of water for human and irrigation supply |
| | *C. encaustus*[d] | *Oreochromis* sp. | | | | | |
| | *Z. quitzeoensis*[c,d] | *X. hellerii* | | | | | |
| | *G. atripinnis* | | | | | | |
| | *P. infans* | | | | | | |
| | *Lampetra geminis*[c] | | | | | | |
| | *A. robustus*[d] | | | | | | |
| San Francisco (EP) | *Z. quitzeoensis*[c,d] | *Oreochromis* sp. | 0 | medium*** | 1 | highly polluted environments around spring (Soto-Galera 1998) reduced migration rates between contiguous water bodies | pollution persists in surrounding area; introduction of exotic species; water use for recreational and human supply |
| | *X. variata* | *Lepomis macrochirus* | | | | | |
| | *G. atripinnis* | | | | | | |
| | *A. tincella* | | | | | | |
| | *Notropis calientis*[f] | | | | | | |
| | *P. infans* | | | | | | |
| | *A. robustus*[d] | | | | | | |
| Zacapu (EP) | *Z. quitzeoensis*[c,d] | *Algansea lacustris* | 2 | medium*** | 0 | drainage of original marshy area (15,000 ha) in 1907 for agricultural purposes; lake now only 32 ha (Guzmán 1985) | introduction of exotic species; occasionally polluted by wastewaters |
| | *Allotoca zacapuensis*[d,e] | *C. carpio* | | | | | |
| | *G. atripinnis* | *C. idella* | | | | | |
| | *X. variata* | | | | | | |
| | *Hubbsina turneri*[c,d,e] | | | | | | |
| | *A. robustus*[d] | | | | | | |
| | *Skiffia lermae*[c,d] | | | | | | |
| | *Chirostoma humboldtianum* | | | | | | |
| | *A. tincella* | | | | | | |
| | *N. calientis* | | | | | | |
| San Cristóbal (EP) | *Z. quitzeoensis*[c,d] | *Oreochromis* sp. | 0 | medium*** | 2 | isolation due to total drainage of west part of Cuitzeo Lake (Tamayo & West 1964); situation persists today | use of the spring water for human supply; introduction of exotic species |

*continued*

**Table 3. (continued)**

| Population | Native species | Introduced species | Endemic species | EQ[b] | Extinctions | Drainage alterations | Immediate threats |
|---|---|---|---|---|---|---|---|
| | *G. atripinnis* | *Xiphophorus maculatus* | | | | | |
| | *S. lermae*[c,d,f] | *X. helleri* | | | | | |
| | *A. robustus*[d] | | | | | | |
| | *Xenotoca variata*[d] | | | | | | |
| | *P. infans* | | | | | | |
| | *C. jordani*[f] | | | | | | |
| La Mintzita (EP) | *Z. quitzeoensis*[c,d] | *C. carpio* | 0 | medium** | 3 | 70% loss due to agricultural expansion and continuous extraction of ground water, which reduced 25% of water flow of spring (Guzmán 1985) | reduction of spring flow due to ground water extraction and its use for human supply; introduction of exotic species |
| | *A. robustus*[d] | *C. idella* | | | | | |
| | *Allotoca catarinae*[d] | *M. salmoides* | | | | | |
| | *Allotoca dugesii*[c,d,f] | *Oreochromis* sp. | | | | | |
| | *G. atripinnis* | *P. reticulata* | | | | | |
| | *X. variata*[d] | | | | | | |
| | *S. lermae*[c,d] | | | | | | |
| | *H. turneri*[c,d,f] | | | | | | |
| | *N. calientis* | | | | | | |
| | *Notropis sallei*[f] | | | | | | |
| | *Scartomyzon austrinus* | | | | | | |
| | *C. bumboldtianum* | | | | | | |
| | *Chirostoma chavarri*[c] | | | | | | |
| | *A. tincella* | | | | | | |
| | *P. infans* | | | | | | |
| Belisario (EP) | *Z. quitzeoensis*[c,d] | *Oreochromis* sp. | 1 | poor* | 1 | complete drainage of the Cuitzeo Lake in winter 1941 when construction of Cointzio Dam was finished (De Buen 1943) | introduction of exotic species; water pollution; drainage |
| | *A. dugesii*[c,d] | *M. salmoides* | | | | | |
| | *Neotoca bilineata*[c,d] | *C. carpio* | | | | | |
| | *C. peraticus*[d] | | | | | | |
| | *H. turneri*[c,d,f] | | | | | | |
| | *C. jordani* | | | | | | |

[a] *Abbreviations: EQ, index of environmental quality; NI, no information; WP, populations that correspond to the west group; EP, populations that correspond to the east group.*
[b] *Citations: *Mercado et al. 2002; **Lyons et al. 2000; ***Soto-Galera et al. 1998.*
[c] *Species with status (NOM-ECOL-059-2001).*
[d] *Includes the information from Domínguez-Domínguez et al. (2005b).*
[e] *Endemic species.*
[f] *Species locally extinct.*

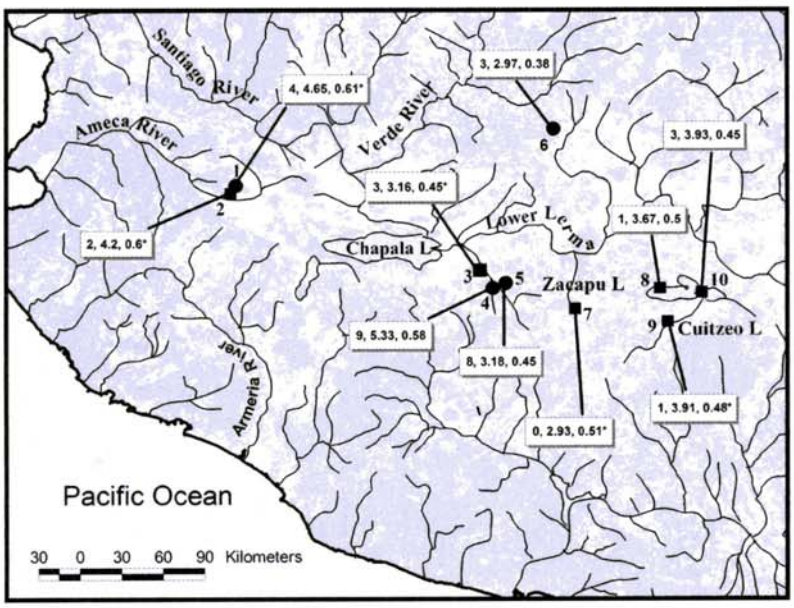


*Figure 3. Land-use and land-cover assessment map of the Mesa Central Mexico: gray, original vegetation present; white, natural vegetation totally transformed for agriculture, industry, cities, or grasslands; squares, populations with bottlenecks; circles, no significant bottlenecks; numbers in boxes, numbers of private alleles, mean allele richness, and observed heterozygosity, respectively; *, populations with negative* $F_{IS}$ *values; numbers, localities included in microsatellite analysis.*

from 0.046 for La Mintzita-San Cristóbal to values as high as 0.455 for La Luz-San Francisco.

Based on the allele size permutation test, $R_{ST}$ values were significantly greater than $F_{ST}$ values in most of the cases where comparisons were made between populations west of the Lower Lerma River (WP, western populations) and those east of the Zacapu and the Middle Lerma River (EP, eastern populations). In these instances stepwise mutation had a greater effect on genetic differentiation than drift (i.e., $F_{ST}$ underestimated the actual levels of genetic structure; thus, $R_{ST}$ would be a better estimator). The highest pairwise $R_{ST}$ values were close to the upper theoretical boundary for comparisons of La Platanera with San Cristóbal, La Mintzita, and Zacapu ($R_{ST}$ = 0.855–0.882).

### Population Bottleneck

The tests for population bottleneck supported previously documented declines in most of the populations of *Z. quitzeoensis*. Seven of the studied populations showed significant values of heterozygosity excess ($p < 0.05$) for at least two of the three mutation models tested (Table 1). Four of the populations (Magdalena, Zacapu, San Cristóbal, and La Mintzita) had significant values for all three models. Although the qualitative mode-shift model cannot be used with a high confidence for samples with fewer than 30 individuals (Cornuet & Luikart 1996), in our analyses samples from Magdalena, Platanera, Zacapu, San Cristóbal, and La Mintzita showed allele frequency shifts indicative of recent population declines. The results are in most cases comparable with those obtained for the Wilcoxon test. Only Belisario, which showed significant heterozygosity excess values for infinite allele model and two-phase mutation model, showed a normal L-shaped distribution as would be expected for a stable population in mutation-drift equilibrium (Luikart et al. 1998). The Mantel test showed a significant correlation between percentage of water body drainage and significant bottleneck detection ($r = 0.372$; $p = 0.016$).

## Discussion

This study is one of the first to apply extensive fieldwork, information on habitat degradation, geographic information systems (GIS), and genetics to the conservation of fishes, particularly to the endangered Mexican ichthyofauna. The OCUs (Doadrio et al. 1996) were determined based on information from previously described biogeographic regions (Domínguez-Domínguez et al. 2006), genetic, environmental, and ecological data. We considered the OCUs the most appropriate criterion for the identification of management units for freshwater fish species (Doadrio et al. 1996): those that have limited dispersal capability and are circumscribed to territories with well-defined boundaries such as rivers or lakes.

### Environmental and Genetic Information

Across the distribution of *Zoogoneticus quitzeoensis* we found a high percentage of local population extinction and a dramatic reduction in population sizes, as well as evidence of fragmentation. One of the most common human alterations in the area is the drainage of water bodies. Evidence for this is provided by the absence of water in 18 localities we studied. The devastation of water bodies

of Central Mexico was induced for agricultural purposes at the beginning of the twentieth century (Table 3) and caused the direct drainage of lakes, lower water inflow due to the diversion of rivers, and reduced water tables. The most affected areas are the La Mintzita, Zacapu, Chapala, Magdalena, and Cuitzeo lakes, which in some cases have lost up to 90% of their surface area (e.g., Zacapu Lake). Other important alterations to the water system of the Mesa Central include the government plan for the introduction of exotic fish species as a food supply or as biological control (Tables 2 & 3). This has had a negative impact on the native species, leading in some cases to their extinction (Zambrano et al. 1999; Dominguez-Dominguez et al. 2005*b*).

All these situations are reflected in the estimates of genetic diversity, the values of which were similar or lower than those found for other endangered freshwater fishes (Salgueiro et al. 2003; Mesquita et al. 2005; Lage & Kornfield 2006), and the evidence of significant bottlenecks in all populations studied from these areas, with the exception of El Moloya (Table 1). Additional evidence of the impact of humans on the genetic structure of the species includes the significant $F_{ST}$ values but nonsignificant $F_{ST}$ versus $R_{ST}$ values among populations from the Cuitzeo basin (La Mintzita and Belisario), which are only 34 km apart. The same situation was observed between La Luz and Orandino, which are 4 km apart. This indicates that genetic drift due to relatively recent fragmentation is more important than ancient isolation and mutation in explaining genetic divergence between these two pairs of populations.

The pollution caused by industry, wastewater from urban areas, and changes in land use (Fig. 2) is also reducing and fragmenting habitat in the water bodies of Central Mexico. Pollution is most evident in the Santiago, Lower and Middle Lerma river basins, and the Lake Chapala Basin, where there was a large reduction in occurrence points (Table 2 & Fig. 1). This damage has been documented in other studies of fish diversity in the region (Soto-Galera 1998; Soto-Galera et al. 1999; Lyons et al. 2000; Mercado-Silva 2002). The populations with the lowest values of genetic diversity were located in these highly polluted basins (Table 1). Populations at La Platanera (which had the highest number of introduced species) and San Francisco showed the lowest overall genetic diversity ($A_R = 3.16$, $H_o = 0.45$, and $A_R = 2.97$, $H_o = 0.38$, respectively).

Most of the surviving wild populations are restricted to springs or water bodies fed by springs. Pollution levels are lower in these areas than in rivers because the spring flows maintain a good water quality. The populations in some of these springs, such as those at El Moloya, Orandino, and La Mintzita, are the most genetically diverse of those surveyed. Water bodies that have been historically stable in terms of water quality and size (e.g., Orandino and San Francisco) showed no evidence of reduced population size (Table 1 & Fig. 3). It appears that the size of the water body (which affects population size) is important in maintaining genetic diversity. For example, in the Lower Lerma Basin, Orandino (approximately 5.2 ha) had the highest diversity ($A = 6.2$, $A_R = 5.7$, and $H_o = 0.58$), whereas La Luz and Platanera, which are almost three times smaller (approximately 1.7 ha and 2.1 ha), had diversity estimates around 30–40% lower ($A = 4.6$, $A_R = 3.18$, and $H_o = 0.45$ and $A = 3.6$, $A_R = 3.16$, and $H_o = 0.45$, respectively). The importance of water bodies fed by springs is corroborated by the high richness of native species found within them (i.e., La Mintzita, Orandino, and Zacapu) (Table 3).

Although springs are important refuges for many species, conservation strategies cannot focus only in these areas. Their geographical isolation and the lack of gene flow among some of them combine to increase the possibility of genetic degradation (e.g., La Luz and San Cristobal, Table 1).

### Genetic Structure

The results of the allele-size permutation test demonstrated that stepwise mutation had a greater effect than drift on genetic divergence ($R_{ST} > F_{ST}$) between western (WP) and eastern (EP) populations of *Z. quitzeoensis*. This indicates that these two groups of populations have been isolated long enough for mutations to accumulate (Hardy et al. 2003). This corroborates the finding that sequence divergence in the mitochondrial cytochrome *b* gene was higher between EP and WP populations of *Z. quitzeoensis* (2.5–3%) than in any other intraspecific comparison of goodeines (Doadrio & Domínguez-Dominguez 2004).

For comparisons between populations belonging to EP and WP, values of $R_{ST}$ ranged from 0.535 for Magdalena-San Francisco to 0.882 for La Platanera-Zacapu. The main exception was a western population (La Luz) for which $R_{ST}$ was not significantly greater than $F_{ST}$ in any comparisons with populations from EP, and its lowest $F_{ST}$ value (0.271) was obtained when it was compared with Orandino; they are in the same basin. All other comparisons involving La Luz, either with EP or WP, showed similar values of genetic differentiation ($F_{ST} = 0.345$–$0.455$). La Luz was the only population with a significant departure from Hardy-Weinberg equilibrium and no evidence of a recent bottleneck event, but it did have a high inbreeding index (Table 1). This, together with the high number of private alleles in this population (the highest after the Orandino population) and the significant $F_{ST}$ values but nonsignificant $F_{ST}$ versus $R_{ST}$ when compared with the populations from Platanera, Orandino, and La Luz, indicates that genetic drift in the Lower Lerma Basin has been more intense than in other areas occupied by *Z. quitzeoensis*.

The current population structure within each of the two groups (WP and EP) of *Z. quitzeoensis* could be explained by two alternative hypotheses: (1) the migration rate among populations has been larger than the mutation rate, so new mutational variants spread over all populations (e.g., Moloya-Magdalena, Belisario-San Cristóbal) or (2) human activities have caused relatively recent fragmentation and reduction of populations. The first explanation is counterindicated by the high $F_{ST}$ values among populations and by the high rate of population loss in the area. The second hypothesis is supported by our environmental data and genetic results. The results of the allele size permutation test indicated that genetic drift explains the divergence among populations within both WP and EP.

### OCU Identification

Because the conservation of freshwater fishes in Central Mexico needs to be an integrative task, we identified which populations are in greater need of protection taking into consideration biogeographic, genetic, environmental, social, and political issues. From the 10 populations studied, we identified seven OCUs: (1) Cuitzeo, including the Belisario, La Mintzita, and San Cristóbal populations; (2) Magdalena, including the Magdalena and El Moloya populations; (3) Zacapu; (4) San Francisco del Rincón; (5) Orandino; (6) La Luz; and (7) La Platanera, with OCUs 3–7 corresponding to single populations. All OCUs represent separate biogeographical regions, except La Luz, Orandino, and La Platanera, which belong to the same biogeographic region.

El Moloya and Magdalena within the Ameca drainage were not divergent based on $F_{ST}$ and shared most of their alleles. In such cases environmental and social aspects should be taken into consideration when conservation strategies are proposed. El Moloya and Magdalena show high values of $A_R$ and $H_o$ (Table 1), but the former has more private alleles (4) than the latter (2). The environmental quality of El Moloya is higher because it is a spring with a high water flow. The endangered restricted species *Ameca splendens* occurs at this locality. In contrast, the Magdalena population suffers from intense human pressure because of changes in land use and the diversion of water for irrigation, but it still contains the endemic species *Allotoca maculata*.

In Cuitzeo basin the La Mintzita population showed low but significant $F_{ST}$ values compared to Belisario and San Cristóbal. La Mintzita and Belisario are small lakes fed by large spring flows, but La Mintzita had a greater number of native species and is classified as a regional natural park. In contrast, Belisario suffers from high human pressure, high fluctuations in hydrologic regime, and pollution.

The three populations from Lower Lerma (Orandino, Platanera, and La Luz) differed in levels of genetic diversity and inbreeding and had high numbers of private alleles. This, together with the high environmental quality of these water bodies suggests that they should be considered different OCUs in the Lower Lerma Basin.

For OCUs corresponding to single populations, most showed high values of $F_{ST}$ when compared with other populations, and most of these OCUs had private alleles. Zacapu Lake was the only population with low $F_{ST}$ values when compared with populations from the Cuitzeo basin. In addition, no private alleles were detected, and it had the lowest average of alleles per locus and $A_R$. The classification of Zacapu Lake as a separate OCU was based on its high environmental quality, high number of native and endemic species, and its designation as a regional natural park.

### Conservation and Management Implications

The devastation of the basins of Central Mexico apparently has affected the genetic variability and structure of *Z. quitzeoensis*. This is exemplified by the bottlenecks detected in the populations inhabiting the lakes drained at the beginning of the twentieth century, such as Zacapu, La Mintzita, Magdalena, and the two Cuitzeo Lake populations (San Cristóbal and Belisario). The probability of population extinction is heightened by increased demographic stochasticity, which causes inbreeding, loss of genetic variation, and fixation of deleterious alleles (Saccheri et al. 1998). Habitat reclamation and stability should be the most important goal of conservation strategies if genetic degradation in depressed populations (following a bottleneck, such as in Zacapu and La Mintzita) is to be reversed. In populations such as San Cristóbal and Magdalena, where apparently the bottleneck was not as dramatic as in other populations, reestablishment of natural conditions, recovery of population numbers, and maintenance of genetic diversity needs to be taken into consideration.

Pollution and introduction of exotic species have forced some populations of native species to live in refuges (i.e., La Luz and San Francisco del Rincón), which has led to reductions in population size and genetic variability. Although high genetic diversity indices are found in small populations such as Orandino, special attention still needs to be given to genetic and demographic characteristics if conservation and management strategies are to be successful. The degradation of habitat should be stopped and habitat conservation is the most important conservation tool.

The fact that each OCU identified here contains unique portions of the total variation of the species suggests that they all should be conserved. In addition, we conclude that gene flow among OCUs is restricted or does not exist. This indicates there is little opportunity for reestablishment of populations and their particular genetic characteristics after local extinction. The reestablishment of

contact between populations, where historical contact can be demonstrated (e.g., El Moloya and Magdalena), is a key factor for the long-term conservation of *Z. quitzeoensis*, even more so when one considers the increasing effects of human activities on its natural range.

None of the natural protected areas of Central Mexico include populations of *Z. quitzeoensis*, and, except for Cuitzeo and Chapala, none of the hydrologic priority regions from Central Mexico include the species. Therefore, other sources of information should be considered in recognizing important areas for the conservation of the freshwater diversity of Central Mexico.

The success, or lack thereof, of translocation programs and their effect on the reversal of genetic degradation in wild populations is an ongoing debate (Tallmon et al. 2004; Geist & Kuehn 2005). Considering the high levels of genetic divergence we found, translocations between different OCUs should be avoided except as a last resort. Other approaches to management and recovery need to be implemented in response to the high degree of degradation of the aquatic ecosystems in Central Mexico and its impact on the population dynamics and genetic diversity. We recommend the development of captive breeding programs and semiartificial breeding and perhaps translocations of populations with previous contact as a last resort.

Integration of data on the distribution of genetic diversity with historical or current environmental and ecological data should play a role in any future conservation plans for *Z. quitzeoensis*. It is imperative to create an integrative approach involving social and natural sciences for the recovery and conservation of such highly fragmented populations. The survival of this species depends critically on minimizing or reversing the habitat degradation that has occurred.

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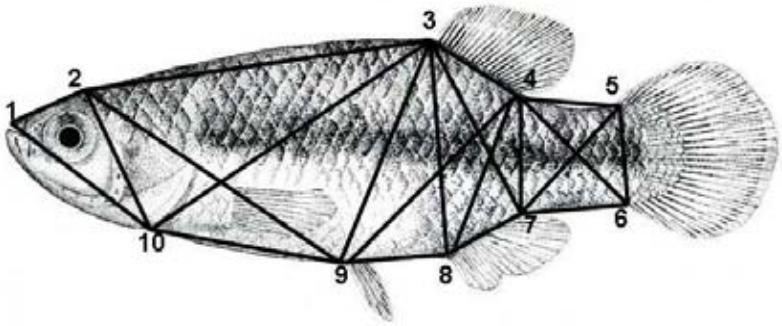
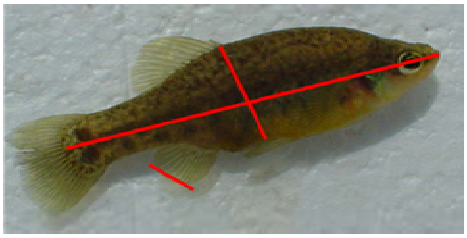
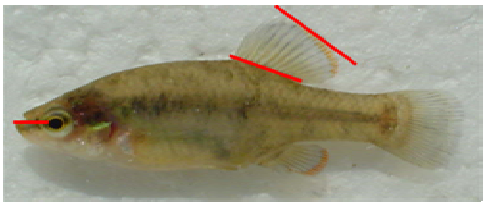
# TAXONOMÍA

## ARTICULO III





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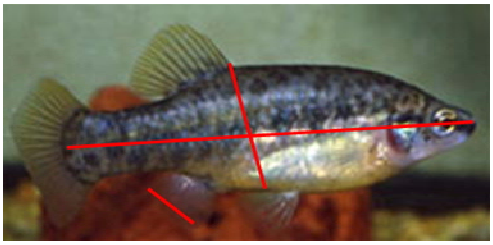

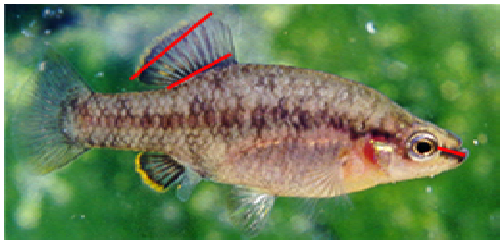

*Aceptado en Revista Mexicana de Biodiversidad*

**Domínguez-Domínguez et al.- Taxonomía de *Zoogoneticus*.**

# Morphologic and genetic comparative analyses of populations of *Zoogoneticus quitzeoensis* (Bean, 1898) (Cyprinodontiformes:Goodeidae) from Central Mexico, with description of a new species.

# Análisis comparativo, morfológico y genético de diferentes poblaciones de *Zoogoneticus quitzeoensis* (Bean, 1898) (Cyprinodontiformes:Goodeidae) del Centro de México, con la descripción de una nueva especie.

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**Resumen.**

Se realizó un estudio genético y morfométrico en poblaciones de *Zoogoneticus quitzeoensis* pertenecientes a las cuencas de los ríos Lerma y Ameca y los lagos de Cuitzeo, Zacapu y Chapala en el Centro de México. Para el análisis genético se analizaron siete poblaciones, identificándose dos grupos monofiléticos bien diferenciados, con distancias genéticas entre ellos de $D_{HKY}$ = 3.4% (3-3.8%), uno de los grupos se distribuye por las cuencas de los ríos Ameca y bajo Lerma y en el lago de Chapala, mientras que el otro incluye las poblaciones de los lagos de Zacapu y Cuitzeo. Cuatro poblaciones fueron empleadas para los análisis morfométricos identificándose dos morfotipos, uno de la localidad del manantial La Luz en la cuenca del bajo Lerma y el otro a los lagos de Zacapu y Cuitzeo. Con estas dos fuentes de evidencia, la población de La Luz es considerada como una nueva especie *Zoogoneticus purhepechus* sp. nov. La nueva especie difiere de su especie hermana, *Z. quitzeoensis* por tener una distancia preorbital más corta (PrOL/SL $\bar{x}$ = 0.05 -0.06), la base de la aleta dorsal más larga (DFL/SL $\bar{x}$ = 0.17-0.20) y presentar entre 13 y 14 radios en la aleta dorsal. La nueva especie difiere de las dos especies descritas en el género (*Zoogoneticus tequila* y *Z. quitzeoensis*) en 10 posiciones nucleotídicas fijadas para el gen citocromo *b*. *Zoogoneticus purhepechus* se distribuye por las cuencas de los ríos Ameca, Armería, Santiago y bajo Lerma, así como en el lago de Chapala. *Z. puhrepechus* debe ser considerada en peligro de extinción de acuerdo a los criterios del MER (Aii,Bi,Ci,Di) y de la UICN (A-1,b,c,e).

Palabras Clave: *Zoogoneticus* – cytochrome b – Mesa Central – México – morfometría.

**Abstract**

A genetic and morphometric study of populations of *Zoogoneticus quitzeoensis* from the Lerma and Ameca basins and Cuitzeo, Zacapu and Chapala Lakes in Central Mexico was conducted. For the genetic analysis, seven populations were sampled and two monophyletic groups were identified with a genetic difference of $D_{HKY}$= 3.4% (3-3.8%), one corresponding to populations from the lower Lerma basin, Ameca and Chapala Lake, and the other corresponding to populations from Zacapu and Cuitzeo Lakes. For the morphometric analysis, four populations were sampled and two morphotypes identified, one corresponding to La Luz Spring in the lower Lerma basin and the other from Zacapu and Cuitzeo Lakes drainages. Using these two sources of evidence, the population from La Luz is regarded as a new species *Zoogoneticus purhepechus* sp. nov. The new species differs from its sister species *Zoogoneticus quitzeoensis* in having a shorter preorbital distance ( $\bar{x}$Prol/SL = 0.056, SD = 0.01), longer dorsal fin base length (DFL/SL $\bar{x}$ = 0.18, SD = 0.03) and 13-14 rays in dorsal fin. The new species differs from both of its sister taxa (*Zoogoneticus tequila* and *Z. quitzeoensis*) at 10 fixed nucleotide positions in the cytochrome *b* gene. We have determined that *Zoogoneticus purhepechus* is distributed in the lower Lerma, upper Ameca, Armeria and Santiago river basins, and Chapala Lake. This new species should be considered endangered of extinction according to the criteria of the MER (Aii,Bi,Ci,Di) and for the IUCN (A-1,b,c,e).

*KEY WORDS: – Zoogoneticus* – cytochrome *b* – new species – Mesa Central – México – morphometry.

## Introduction

The Mesa Central of Mexico is characterized by its high diversity of freshwater fishes (Barbour, 1973; Echelle & Echelle, 1984; Domínguez-Domínguez et al., 2005). A total of 100 native species having been reported, of which 70% are endemic (Guzmán-Arroyo, 1994). This important biological diversity has been attributed to the complex geological and zoogeographic history of central Mexico (Miller & Smith, 1986; Domínguez-Domínguez et al., 2006a). Of the endemic fish fauna of the Mesa Central, the cyprinodontiform fish subfamily Goodeinae (family Goodeidae) is one of the most diverse and interesting. The subfamily exhibits internal fertilization, matrotrophy and viviparity (Parenti, 1981; Grudzien et al., 1992). When the genus *Zoogoneticus* Meek, 1902 was described, the 14 species of goodeines were included in the Poeciliidae, which also comprised the presently recognized families Profundulidae, Fundulidae, Rivulidae, Cyprinodontidae, and Anablepidae (sensu Parenti, 1981). Meek (1904) placed *Fundulus robustus* Bean 1892, *Platypoecilus quitzeoensis* Bean, 1898 and *Fundulus dugesii* Bean, 1887 in *Zoogoneticus*, and simultaneously described two new species (*Z. diazi* Bean, 1887 and *Z. miniatus* Bean, 1887). Regan (1908) proposed the synonymy of *Z. miniatus* with *Z. diazi* and *Z. maculatus* Regan 1904 with *Z. robustus* (Bean, 1892). The revision by Hubbs & Turner (1939), based on the anatomy of the ovary and the trophotaeniae, restricted the genus to include only *Z. quitzeoensis* (Bean, 1898), removing other taxa to what are presently three different genera of goodeines (*Allotoca* Hubbs and Turner, 1937, *Alloophorus* Hubbs and Turner, 1937 and *Allodontichthys* Hubbs and Turner, 1937). Based on molecular characters the genus *Zoogoneticus* is currently placed in the tribe Chapalichthyini (sensu Doadrio & Domínguez, 2004). Actually the genus is comprised of the species *Z. quitzeoenesis* and *Z. tequila* Webb and Miller, 1988. *Zoogoneticus quitzeoensis* is widely distributed in Central Mexico whereas *Z. tequila* has a restricted area of distribution, the former is considered endangered and the second has been considered extinct in the wild (Espinosa-Pérez et al., 1993; Webb & Miller, 1998; DOF 2001), although recently was reported one small and restricted population (De la Vega-Salazar et al., 2003). Genetic studies have shown that populations of *Z. quitzeoensis* have a geographical structure with a consistently high degree of genetic divergence among populations (Doadrio & Domínguez, 2004; Domínguez-Domínguez et al., 2007). The

causes of the ancient population structure may be explained by several volcanic and tectonic events during the Plio-Pleistocene; the population has been subject to different events of dispersion and vicariance that differ in spatial and temporal scale (Domínguez-Domínguez et al., 2006a). Thus, genetic and morphologic differences within and between populations have been observed in other non-goodeid fishes from Central Mexico including: *Poeciliopsis infans* (Woolman, 1894) (Mateos et al., 2002) and the genus *Notropis* Rafinesque 1818 (Schönhuth & Doadrio, 2003).

According to a phylogenetic hypothesis proposed by Doadrio & Domínguez (2004), the westernmost populations of *Z. quitzeoensis* are genetically different from populations from the Lake Zacapu and La Mintzita, the latter in the Lake Cuitzeo systems. These results suggest that a morphologic and more extensive molecular revision of *Zoogoneticus* needs to be conducted to better establish the taxonomic identity of the different populations of the genus that still exist in nature. These patterns of variation between population in the lower and middle Lerma drainages, with evidence of separated closely related clades, are paralleled by the sister species *Skiffia lermae* Meek 1902, and *S. multipunctata* (Pellegrin, 1901). It is hypothesized that both groups of species owe their origins to the same vicariant event between 1 and 3.5 Mya (Domínguez-Domínguez et al., 2006a).

The purpose of this study, therefore, is to analyze the morphologic and genetic differences among populations of the genus *Zoogoneticus* and to provide the description of a newly recognized species.

**Materials and Methods**

The study was based on specimens collected using hand and seine nets and electrofishing. All of the sampled specimens were preserved in 70% ethanol, voucher specimens are housed at the Universidad Michoacana de San Nicolás de Hidalgo (CPUM), the Museo Nacional de Ciencias Naturales de Madrid (MNCN) and the Instituto de Biología Universidad Nacional Autonoma de Mexico (CNP). The tissues used in genetic analysis are housed at the Museo Nacional de Ciencias Naturales de Madrid (voucher numbers MEX 38, 4271, 4272, 506 and 508)

*Morphological analysis*

Specimens from the type locality (San Cristobal, Cuitzeo Lake, Michoacan) and three others populations of *Z. quitzeoensis*, (La Luz-Spring, Zamora, Michoacan; La Mintzita

Spring, Morelia, Michoacan and Zacapu Lake, Zacapu, Michoacan) were analyzed (Table 1 and Fig. 1). Twenty morphometric characters were measured with digital calipers (0.01 mm) and four meristic variables were recorded using a stereoscopic microscope. The abbreviations used for morphometric variables are: SL, standard length; HL, head length; PrOL, preorbital length; ED, eye diameter; InOW, interorbital width; BD, body depth; BLD, body least depth; PAD, pelvic-anal fin distance; PDD, pelvic-dorsal fin distance; PODE, pelvic-fin origin to dorsal-fin posterior extent distance; DAD, dorsal-anal fin distance; DOAE, dorsal-fin origin to anal fin posterior extent distance; DFL, dorsal-fin length; DEAO, dorsal-fin posterior extent to anal-fin origin distance; AFL, anal-fin length; AEDE, anal-fin posterior extent to dorsal-fin posterior extent distance; EDUP, end of dorsal fin-upper extreme of caudal peduncle distance; EDLP, end of dorsal fin-lower extreme of caudal peduncle distance; EAUP, end of the anal fin-upper extreme of caudal peduncle distance; EALP, end of the anal fin- lower extreme of caudal peduncle distance. The abbreviations for meristic characters are: D, dorsal-fin rays; A, anal-fin rays; P, pectoral-fin rays; GR, gill rakers. All the measurements are in millimetres.

A two-way analysis of variance (ANOVA), comparing both morphometric and meristic characters, was conducted to test sexual dimorphism and variation between populations. Burnaby's method was used to correct size effect (Burnaby, 1966; Rohlf & Bookstein, 1987; Doadrio et al., 2002). All analyses were conducted with the corrected matrix. For the purpose of finding the variation pattern and identifying the measurements that contributed more to the variability between populations, a Principal Components Analysis (PCA) was conducted with covariance matrix for morphometric characters and with correlations matrix for meristic characters. The classificatory hypothesis obtained by PCA was tested by a Discriminate Function Analysis (DFA). All analyses were conducted with the statistics packages NTSYS v.2.1 (Rohlf, 2000) and SPSS v.13.0. Both sets of characters, morphometric and meristic, were analyzed independently. Because of sexual dimorphism involved in the morphometric measurements, these were analysed separately for males and females.

*Genetic analysis*

Six sequences of the gene Cytochrome *b* of *Zoogoneticus* genus from different localities (Table 1 and Fig. 1) were obtained from GenBank (AF510751-AF510755 and AF510757)

and one was obtained for the outgroup (*Xenoophorus captivus* (Hubbs, 1924), AF510758). The other sequences (including specimens from the type locality of the new species and specimens from the type locality for *Z. quitzeoensis*) were obtained using the following protocol. Total cellular DNA was isolated from tissues by a standard proteinase K and phenol/chloroform extraction method (Sambrook et al., 1989). Two overlapping fragments of the cytochrome *b* gene (total of 1140 bp) were amplified via polymerase chain reaction (PCR) for each individual DNA sample. The primers used for cytochrome *b* in all species were those discussed in Machordom & Doadrio (2001). The amplification process was conducted as follows: 94 ºC (2 min), 35 cycles at 94 ºC (45 s), 48 ºC (1 min), 72 ºC (90 s), and 72 ºC (5 min). PCR mixtures were prepared in 25 µl reactions with a final concentration of 0.4 µM of each primer, 0.2mM of each dNTP, 1.5mM MgCl2, and 1U of Taq DNA polymerase (Biotools). PCR products were checked on 1.5% agarose gels, and cloned using the pGEM-T vector (Promega) into *Escherichia coli* JM109. Positive clones were sequenced using the Big Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers. All samples were sequenced on an Applied Biosystems 3700 DNA sequencer following manufacturer's instructions. Chromatograms and alignments were visually checked. The model of DNA substitution that best fitted the data set was selected using MODELTEST 3.7 (Posada & Crandall 1998) using the Bayesian information criterion (BIC). The aligned data were analysed with the Bayesian inference method with the program Mr. Bayes 3.1.1 (Hueselsenbeck & Ronquist, 2001) by simulating a Markov chain for 1,000,000 cycles. Based on the HKY+G model obtained by Modeltest, a genetic distance between the two groups was obtained using the program Sequencer 6.1.0 (written by B. Kessing and available at http://nmg.si.edu/).

**Results**

*Morphometrics*

Analysis of variance showed significant differences ($\leq 0.05$) between species for most of the morphometric variables mainly due to differences in standard length, except for the dorsal-fin origin to end of the anal fin. We inferred from this result that the longer dorsal-fin base in *Z. purhepechus* sp. nov influences the DOAE measurement 0.32-0.25 ($\bar{x} = 0.29$) (Table 2). ANOVA for sexual dimorphism showed significant differences in all variables

except in the end of the anal-fin to end of the dorsal-fin distance, thus showing that females have a narrower caudal peduncle than males. The interaction between populations and sexes in most of the morphometric characters do not show significant differences, except for preorbital length. On the contrary, in the meristic characters, the significant differences are only between populations (Table 2). These results justify the separation of morphometric but not meristic analyses by sex.

In an exploratory PCA with the morphometric measurements, PCI explains 90.29% of the variation in males and 90.03% in females; for both sexes, the eigenvectors show closed values with the same symbol, suggesting an influence of the standard length in the results (Doadrio et al., 2002). A second PCA with a Burnaby corrected matrix, accumulates 45.53% of the variance in the PCII in males and 52.42% in females. For males, the high values in eigenvectors were preorbital length, dorsal fin length and anal fin length in the PCI, and interorbital width and FAD-ESPC in the PCII. For females, the high values in eigenvectors were preorbital length, dorsal fin length and FAD-ESPC in the PCI, and anal fin length in the PCII (Table 3). In both sexes, the variation patterns were determined by the preorbital length and dorsal fin length in the PCI. This analysis shows a clear tendency to form two groups (ellipses in Fig. 2 A and B), which correspond to the lower Lerma basin (La Luz Spring) and middle Lerma (San Cristobal and La Mintzita Springs in the Cuitzeo drainage and Zacapu Lake). With respect to the PCA with meristic characters, PCII accumulates 76.05% of the explained variance, and the high values in the eigenvectors were dorsal fin rays and gill rakers in the PCI, and pectoral fin rays in the PCII (Table 4). Similar to morphometric characters, the meristic characters show a variation pattern with the formation of two groups (ellipses in Fig. 3).

Starting with the classificatory hypothesis (formation of two groups), the DFA shows a significant difference ($\alpha \leq 0.05$) with the intermediate distances of morphometric characters in males ($P= 0.003$) and females ($P= 0.010$), and with the meristic characters ($P= 0.000$). These results agree with the proposed classificatory hypothesis and corroborate the variation pattern found in the PCA.

*Genetics*

In the data set, 106 characters were variable, and 35 were parsimony informative. Third codon positions were the most informative characters (24 informative characters), followed

by the first codon position (10 characters). Saturation of transition and transversion changes was checked by plotting the absolute number of changes of each codon position against patristic distances. There was no ingroup evidence of saturation at any of the three positions (not shown). The HKY-G model was selected as the best fit to the data set. Rate matrix parameters were: -lnL = 2205.0105; K = 5; BIC = 4456.7324. The base frequencies were: freqA = 0.2522; freqC = 0.2685; freqG = 0.1390; freqT = 0.3402. Among-site rate variation was approximated with gamma distribution shape parameter $\alpha = 0.1976$. The phylogenetic tree obtained by the Bayesian analysis after burning 500 chains and discarded (Fig. 4) shows the formation of two well differentiated groups, with a posterior probability of the branches of 100 for the *Z. quitzeoensis* clade (including the type locality) and 96 for the clade which contains the new species *Z. purhepechus* sp nov. The genetic distance obtained by the model HKY-G within groups was 3.4% (3-3.8%).

**Description.**

*Zoogoneticus purhepechus*, sp. nov. Domínguez-Domínguez O. R. Pérez-Rodríguez e I. Doadrio (Figures 5 A-B, Table 5)

D = (13) 14; A = 13-14; P = (11-12) 13-15; GR = (7) 9-12. Morphometric measurements are shown in Table 5. Body relatively deep, laterally compressed and elongated, maximum height $\bar{x}$ = 3.1 (range = 2.8-3.6) times the standard length in males and $\bar{x}$ = 3.3 (range = 2.9-3.7) times in females. Minimum body height $\bar{x}$= 6.5 (range = 6.2-7) times the standard length for males and $\bar{x}$ = 7 (range = 6.2-7.7) in females. Head short, cephalic length $\bar{x}$ = 3.5 (range = 3.3-3.7) times standard length in males and $\bar{x}$ = 3.7 (range = 3.3-4.1) in females. Preorbital distance short, preorbital distance $\bar{x}$ = 16.6 (range = 14.6-21.8) times standard length in males and $\bar{x}$ = 20 (range = 12.8-27.4) in females. Anal fin inserted before origin dorsal fin at same axis. Dorsal fin length long $\bar{x}$ = 5.2 (range = 3.4-7) standard length in males and $\bar{x}$ = 5.8 (range = 5.3-6.6) in females.

***Pigmentation pattern***. When alive, both sexes exhibit a light brown coloration, with dark brown and moderately large spots on the posterior part of the body, starting at the base of the caudal fin. In the anterior part of the body, a mottling pattern of small spots can be

distinguished at the top of the ventral region. They show a pair of dark brown spots laterally aligned at the base of the caudal peduncle, in the region of the hypural plate. In males, during the breeding season, these spots could not be distinguished. The ventral region lacks spots. Adult males are slightly darker than females and may show a slightly bluish or greenish hue on the lateral side of the body and some scales can produce iridescence. The males from the type locality show an intense red band at the end of the pelvic and dorsal fins. However, in specimens from other locations where the species is now reported, this band may be an intense orange. Alcohol preserved specimens show a light brown coloration on the body with the abdominal region yellowish, and from the caudal peduncle approximately to the insertion of the anal fin showing moderately large, dark brown spots. From the anal fin anteriorly, such spots with undefined form and smaller in size. Red band in the dorsal and anal fins and the slightly bluish or greenish hue faint or are less intense.

***Sexual dimorphism and reproduction***. As in all members of the subfamily Goodeinae, males show a 60 to 70% reduction in the length of the first anal rays (2-7), which form a short lobe that is inferred to function in sperm transfer (Parenti, 1981) (Fig. 5A). Differences in size were found between sexes, with females being larger than males. As in *Z. quitzeoensis*, the caudal peduncle is narrower (SL/BLD $\bar{x}$ = 7.0 , range= 6.2-7.7) and longer (SL/EDUP $\bar{x}$ = 4.1, range= 3.4-4.7) in females than in males (SL/BLD $\bar{x}$ = 6.5 , range= 6.1-7.0 and SL/EDUP $\bar{x}$ = 3.8 , range= 3.5-4.4). The males have a stripe with intense red coloration at the end of the dorsal and anal fin. The sex of males can be distinguished at a few weeks after birth. In captive conditions (temperature 21ºC ± 2ºC), the males and females can become sexually mature between 8 and 12 weeks. Gestation requires 7 to 9 weeks. Reproduction occurs throughout the year, but peaks when the temperature is between 20 and 21ºC. The number of offspring in captivity oscillates between 15 and 45, and are usually 9 to 12 mm in standard length at birth. They feed on the second day after birth and first parturition females usually have a low number of offspring.

**Taxonomic summary.**

***Material examined***. *Holotype*. (Table 5, Fig. 5A) CPUM 1509, adult male 34.12 mm SL, La Luz Spring (lower Lerma), Zamora, Michoacán, México, Ludo Couvreur, Jan de Moree, Kees de Jong, Juan C. Merino & Luis Escalera-Vázquez, November 2002.

*Paratypes*. CPUM 1055, (10 individuals); MNCN 246184 (15 individuals); CNP-IBUNAM 14425-14427 (3 individuals). Collected with the holotype.

***Distribution***. According with the molecular and morphometric diagnostic characters, we hypothesized that the populations from Ameca, Santiago and Armería basins and Chapala Lake, identified as *Z. quitzeoensis*, should henceforth be considered as *Z. purhepechus.* Thus, the distribution of *Z. purhepechus* occupies the lower Lerma basin, the upper part of the Armeria, Santiago and Ameca basins and Chapala Lake (Fig. 1).

***Habitat***. The type locality of *Z. purhepechus* is La Luz Spring. La Luz is a permanent spring of lentic and clear waters and forms a small pond of approximately 1500 $m^2$. Once, the water of this spring flowed to the Duero River, which forms part of the lower Lerma River basin. Currently, the water of this spring is used for irrigation and as a water supply to the population. It has an average depth of 1.5 m with a maximum depth of 3.5 m. The bottom is rocky in its periphery and muddy in most of the pond. Aquatic vegetation is *Iris* sp. and *Ceratophylum* sp, emergent vegetation *Tipha* sp. and *Cirpus* sp., and the introduced species *Eichornia* sp, and terrestrial vegetation is of the subtropical forest type.

The associated fish fauna are the native species, *Alloophorus robustus* (Bean, 1892); *Chapalichthys encaustus* (Jordan & Snyder, 1899); *Goodea atripinnis* Jordan 1880; *Skiffia multipunctata* (Goodeidae); *Poeciliopsis infans* (Poeciliidae); and *Lampetra geminis* (Alvarez, 1964) (Petromyzontidae); and the introduced species *Poecilia mexicana* Steindachner 1863; *Xiphophorus hellerii* Heckel 1848, (Poeciliidae); *Oreochromis* spp (Cichlidae); and *Cyprinus carpio* Linnaeus 1758, *Ctenopharingodon idella* (Valenciennes 1844) (Cyprinidae).

***Conservation***. Although this species has been taken from a number of localities, and is widely distributed in different drainages along the occidental part of Central Mexico, in the last five years, a reduction in its distribution of almost 75% of the historical occurrence points has been observed (Fig. 1). The most common alterations reported in the localities where the species has disappeared are the introduction of exotic species, water pollution and desiccation (De la Vega-Salazar et al., 2003; Domínguez-Domínguez et al., 2005; Domínguez-Domínguez et al., 2006b). Genetic erosion related with human perturbations of the population of this new species was demonstrated recently (Domínguez-Domínguez et al., 2007). This species should be considered as endangered of extinction, following the

criteria and categories of the MER-Aii,Bi,Ci,Di (DOF-2001) and the International Union for the Conservation of Nature and Natural Resources IUCN-A,1a,c,e. (IUCN, 2001- http://app.iucn.org/webfiles/doc/SSC/RedList/ RedListGuidelines.pdf).

***Etymology***. The name "*purhepechus*" comes from purhepecha, the name of the indigenous ethnic group which inhabited part of the distribution range of this species, including the type locality.

**Remarks.**

*Zoogoneticus purhepechus* sp. nov. differs from its sister species, *Z. quitzeoensis* by the following combination of characters: 13-14 branched rays in the dorsal fin (*vs*. 12, rarely 11 or 13 branched dorsal rays in *Z. quitzeoensis*), long dorsal fin base length (DFL/SL $\bar{x}$ = 0.18, SD = 0.03 *vs.* $\bar{x}$ = 0.16, SD = 0.01 in *Z. quitzeoensis*) and short pre-orbital length (Prol/SL $\bar{x}$ = 0.056, SD = 0.01 *vs* $\bar{x}$ = 0.066, SD = 0.008 in *Z. quitzeoensis*); two conspicuous dark brown spots in the hypural plate region, except in males within the reproductive season; 10 molecular autapomorphies in the cytochrome *b* gene also differentiate *Zoogoneticus purhepechus* sp. nov. from *Z. tequila* and *Z. quitzeoensis* (Table 6). Divergences in the cytochrome *b* gene is $D_{HKY}$ = 3.4% (3-3.8%) compared to *Z. quitzeoensis* and $D_{HKY}$ = 11% (9-13%) compared to *Z. tequila*.

**Discussion.**

The genus *Zoogoneticus* is characterized by the presence of some distinctive characteristics, exhibiting two to six melanic patches in the post-ventral region of the body (Webb & Miller, 1998). Two dark brown spots in the hypural plate region are characteristic, more evident in *Z. quitzeoensis* and *Z. purhepechus* than in *Z. tequila,* in which the spots are less evident or are fused. The trophotaenia is a ribbon-type with 9-14 termini. The genetic and morphometric variation pattern within the populations of the two species analysed here show the separation of two well-defined groups, supporting previous findings made with molecular characters (Doadrio & Domínguez, 2004). The ANOVA analysis between populations shows that DOAE distance differs between them. We inferred that this is a result of the largest DFL distance in *Z. purhepechus* and this inference is supported by the PCA. In the same manner, differences in PrOL were obtained and supported by the minus preorbital length obtained as a diagnostic character in PCA. The morphometrically diagnostic characters are preorbital length and dorsal fin length in the PCI. Accordingly,

with this classification, which was corroborated by the DFA, the *Z. quitzeoensis* populations (San Cristobal Spring and La Mintzita Spring in the Cuitzeo drainage and Zacapu Lake) have a larger superior mandible and a shorter base of the dorsal fin. On the other hand *Z. purhepechus* (La Luz Spring) exhibits a shorter upper mandible, a larger dorsal fin base and a higher number of dorsal rays.

This grouping model is congruent with the results of the Bayesian analysis, and the high genetic divergences between *Z. quitzeoensis* and *Z. purhepechus* ($D_{HKY}$= 3.4%, range= 3-3.8%). These values are higher than those described for the family Goodeidae, were an intraspecific pairwise uncorrected "p" distance of 0.001 to 1.7% was found, and are similar to those found in interspecific distances of 1.7 to 11% (Doadrio & Domínguez, 2004). In the same manner, the middle Lerma and lower Lerma basins are considered to have undergone a pattern of isolation and union, which correlates with vicariant events inferred to promote cladogenetic processes in at least 2 pairs of sister species within the Goodeinae (e.g. *Skiffia lermae-Skiffia multipunctata* and *Zoogonetius quitzeoensis-Z. purhepechus*) (Domínguez-Domínguez et al., 2006a).

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FIGURE LEGENDS

**Figure 1.** Distribution range of *Zoogoneticus quitzeoensis*; open circles correspond to historical occurrence points where specimens have not been collected in the last 5 years, solid circles correspond to sites where specimens were collected in the last 5 years, and for *Z. purhepechus*; open boxes correspond to historical occurrence points where specimens have not been collected in the last 5 years, solid boxes correspond to sites where specimens were collected in the last 5 years. Numbers correspond to localities shown in table 1.

**Figure 2.** Plots of first two principal components for 20 morphometric variables corrected by Burnaby's methods for males (A) and for females (B). L) La Luz Spring, C) Cuitzeo Lake (San Cristobal Spring), M) La Mintzita Spring, and Z) Zacapu Lake.

**Figure 3.** Plots of the first two principal components for 4 meristic variables. L) La Luz Spring, C) Cuitzeo Lake (San Cristobal Spring), M) La Mintzita Spring, and Z) Zacapu Lake.

**Figure 4.** Phylogenetic tree of 11 analysed specimens of the genus *Zoogoneticus* recovered from cytochrome *b* sequences (1140 bp) according to the Bayesian analysis based on the best model of evolution that fit our data using the program Modeltest 3.7 (Posada & Crandall 1998). The numbers on the branches represent the Bayesian posterior probability.

**Figure 5.** (A) Holotype, Male (CPUM-1509) and (B) Paratype, female (CPUM-1510) of *Zoogoneticus purhepechus* from La Luz Spring.

Table 1. Localities and sample sizes for *Zoogoneticus* spp populations used for morphometric, meristic and genetic analysis. *Correspond to the type locality for *Z. quitzeoensis.* ** Correspond to the type locality for *Z. tequila*. ***Correspond to the type locality for *Z. purhepechus* sp. nov. +Correspond to sequences obtained from Genbank.

| *Zoogoneticus* spp | | Morphometrics | | Meristics | | Genetics |
|---|---|---|---|---|---|---|
| Locality | Drainage | Males | Females | Males | Females | |
| 1. *San Cristobal Spring | Cuitzeo Lake | 5 | 11 | 5 | 11 | 2 |
| 2. La Mintzita Spring | Cuitzeo Lake | 16 | 16 | 16 | 16 | 1+ |
| 3. Zacapu Lake | Middle Lerma River | 11 | 7 | 11 | 7 | 2+ |
| 4. ***La Luz Spring | Lower Lerma River | 15 | 14 | 15 | 14 | 1 |
| 5. Orandino Spring | Lower Lerma River | ---- | ---- | ---- | ---- | 2 |
| 6. Jaripo Stream | Chapala Lake | ---- | ---- | ---- | ---- | 1+ |
| 7. ** El Rincon Spring | Ameca River | ---- | ---- | ---- | ---- | 2+ |

Table 2. Two-way analysis of variance testing for sexual dimorphism, population variation and their interaction (Pop*Sex). n.s. not significant differences. Mean squares from SigmaStat v. 3.0.1.

| Variable | Population | Sex | Pop*Sex |
|---|---|---|---|
| Meristics | | | |
| D | 0.0210 | 0.000112n.s. | 0.000617n.s. |
| A | 0.000719n.s. | 0.000141n.s. | 0.000800 |
| P | 0.00282 | 0.0103 | 0.00259 |
| GR | 0.0131 | 0.000697 n.s. | 0.000822 n.s. |
| Morphometrics | | | |
| SL | 0.0948 | 0.139 | 0.00403n.s. |
| HL | 0.0935 | 0.0734 | 0.00715n.s. |
| PrOL | 0.166 | 0.0761 | 0.0279 |
| ED | 0.0926 | 0.0408 | 0.00884n.s. |
| InOW | 0.112 | 0.0791 | 0.00903n.s. |
| BD | 0.128 | 0.103 | 0.0102 n.s. |
| BLD | 0.0557 | 0.0376 | 0.00917 n.s. |
| PAD | 0.0951 | 0.181 | 0.0147n.s. |
| PDD | 0.114 | 0.113 | 0.00770n.s. |
| PODE | 0.103 | 0.107 | 0.00845n.s. |
| DAD | 0.114 | 0.0547 | 0.0128n.s. |
| DOAE | 0.121n.s. | 0.102 | 0.0122n.s. |

| | | | |
|---|---|---|---|
| DFL | 0.133 | 0.0502 | 0.0110 n.s. |
| DEAO | 0.111 | 0.0855 | 0.00851n.s. |
| AFL | 0.124 | 0.000000381n.s. | 0.00390 n.s. |
| AEDE | 0.0925 | 0.139 | 0.00629n.s. |
| EDUP | 0.0706 | 0.139 | 0.00833n.s. |
| EDLP | 0.0839 | 0.145 | 0.00514n.s. |
| EAUP | 0.0933 | 0.148 | 0.00327n.s. |
| EALP | 0.0838 | 0.145 | 0.00314 n.s. |

Table 3. Eigenvectors and eigenvalues for the first three principal components for 20 morphometric variables in males and females. Variable codes are given in the method section.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Eigenvectors | I | II | III | I | II | III |
| SL | 0.000 | 0.004 | 0.006 | 0.009 | 0.001 | 0.003 |
| HL | 0.006 | -0.005 | 0.001 | -0.006 | 0.000 | -0.005 |
| PrOL | 0.031 | -0.028 | 0.015 | **-0.073** | 0.015 | -0.003 |
| ED | 0.024 | -0.016 | 0.004 | -0.013 | 0.003 | -0.017 |
| InOW | 0.007 | -0.036 | -0.007 | 0.000 | 0.004 | -0.012 |
| BD | 0.002 | 0.004 | -0.009 | -0.002 | -0.008 | -0.001 |
| BLD | 0.001 | 0.018 | -0.021 | 0.002 | -0.007 | 0.003 |
| PAD | 0.002 | -0.009 | -0.024 | 0.017 | -0.004 | -0.031 |
| PDD | -0.001 | 0.003 | -0.009 | 0.001 | -0.016 | -0.006 |
| PODE | -0.007 | 0.001 | -0.004 | 0.010 | -0.016 | -0.006 |
| DAD | -0.006 | 0.001 | -0.004 | 0.006 | -0.012 | 0.006 |
| DOAE | -0.010 | 0.001 | -0.005 | 0.008 | -0.003 | 0.001 |
| DFL | -0.046 | -0.015 | 0.012 | 0.025 | 0.008 | 0.014 |
| DEAO | -0.011 | 0.006 | 0.001 | 0.005 | -0.001 | 0.000 |
| AFL | -0.024 | -0.003 | 0.015 | 0.021 | 0.044 | 0.004 |

| | | | | | | |
|---|---|---|---|---|---|---|
| AEDE | 0.008 | 0.014 | -0.009 | -0.001 | -0.006 | 0.002 |
| EDUP | 0.014 | 0.022 | 0.010 | -0.026 | -0.008 | 0.015 |
| EDLP | 0.009 | 0.016 | 0.006 | -0.002 | -0.007 | 0.013 |
| EAUP | 0.005 | 0.014 | 0.016 | 0.005 | -0.004 | 0.009 |
| EALP | 0.011 | 0.016 | 0.015 | -0.001 | 0.000 | 0.017 |
| Eigenvalues | 0.005 | 0.004 | 0.002 | 0.008 | 0.003 | 0.002 |
| Percentage | 24.327 | 21.206 | 12.578 | 37.988 | 14.435 | 12.337 |
| Accumulated% | 24.327 | 45.532 | 58.111 | 37.988 | 52.423 | 64.761 |

Table 4. Eigenvectors and eigenvalues for the first three principal components for 4 meristic variables. Variable codes are given in the method section.

| Eigenvectors | I | II | III |
|---|---|---|---|
| D | 0.653 | 0.458 | 0.405 |
| A | -0.093 | 0.345 | 0.176 |
| P | -0.475 | 0.647 | -0.349 |
| GR | -0.905 | -0.045 | 0.457 |
| Eigenvalues | 1.479 | 0.749 | 0.526 |
| Percentage | 50.474 | 25.578 | 17.954 |
| Accumulated% | 50.474 | 76.052 | 94.006 |

Table 5. Statistical parameters for morphometric characters in *Z. purhepechus* sp. nov. Each variable is divided by standard length. Variable codes are given in the method section (SD = standard deviation).

| | | **14 Males** | | | **14 Females** | | |
|---|---|---|---|---|---|---|---|
| Variables | Holotype | Range | Mean | SD | Range | Mean | SD |
| SL | 34.12 | 36.95-19.31 | 28.41 | 5.45 | 52.40 - 24.30 | 33.26 | 7.08 |
| HL | 0.28 | 0.30 - 0.27 | 0.29 | 0.01 | 0.30 - 0.24 | 0.27 | 0.02 |
| PrOL | 0.08 | 0.07 - 0.04 | 0.06 | 0.01 | 0.08 - 0.04 | 0.05 | 0.01 |
| ED | 0.09 | 0.10 - 0.08 | 0.09 | 0.01 | 0.10 - 0.07 | 0.08 | 0.01 |
| InOW | 0.11 | 0.12 - 0.09 | 0.10 | 0.01 | 0.11 - 0.10 | 0.09 | 0.01 |
| BD | 0.34 | 0.35 - 0.28 | 0.32 | 0.02 | 0.35 - 0.27 | 0.30 | 0.02 |
| BLD | 0.15 | 0.16 - 0.14 | 0.15 | 0.01 | 0.16 - 0.13 | 0.14 | 0.01 |
| PAD | 0.18 | 0.19 - 0.13 | 0.17 | 0.02 | 0.22 - 0.15 | 0.18 | 0.02 |
| PDD | 0.35 | 0.37 - 0.29 | 0.34 | 0.02 | 0.38 - 0.27 | 0.32 | 0.03 |
| PODE | 0.40 | 0.41 - 0.34 | 0.38 | 0.02 | 0.40 - 0.34 | 0.37 | 0.02 |
| DAD | 0.32 | 0.35 - 0.27 | 0.32 | 0.02 | 0.33 - 0.27 | 0.30 | 0.02 |
| DOAE | 0.29 | 0.32 - 0.25 | 0.29 | 0.02 | 0.30 - 0.25 | 0.28 | 0.01 |
| DFL | 0.20 | 0.29 - 0.14 | 0.20 | 0.03 | 0.20 - 0.15 | 0.17 | 0.01 |
| DEAO | 0.27 | 0.29 - 0.24 | 0.26 | 0.01 | 0.26 - 0.23 | 0.24 | 0.01 |
| AFL | 0.12 | 0.12 - 0.09 | 0.11 | 0.01 | 0.11 - 0.07 | 0.09 | 0.01 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AEDE | 0.17 | 0.19 - 0.16 | 0.17 | 0.01 | 0.20 - 0.16 | 0.17 | 0.01 |
| EDUP | 0.23 | 0.28 - 0.23 | 0.26 | 0.02 | 0.29 - 0.21 | 0.24 | 0.03 |
| EDLP | 0.28 | 0.33 - 0.28 | 0.30 | 0.01 | 0.33 - 0.26 | 0.30 | 0.02 |
| EAUP | 0.29 | 0.32 - 0.28 | 0.30 | 0.01 | 0.31 - 0.26 | 0.29 | 0.01 |
| EALP | 0.22 | 0.28 - 0.24 | 0.26 | 0.01 | 0.30 - 0.23 | 0.26 | 0.02 |

Table 6. Molecular diagnostic characters for the cytochrome *b* gene in *Zoogoneticus* spp. (BPP = base pair position).

| Species \ BPP | 136 | 237 | 327 | 481 | 585 | 798 | 816 | 870 | 883 | 895 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Z. tequila* | C | T | T | T | T | C | A | A | C | C |
| *Z. quitzeoensis* | T | T | T | G | A | A | A | C | C | C |
| *Z. purhepechus* | A | C | C | A | C | T | G | T | T | T |

Figure 1

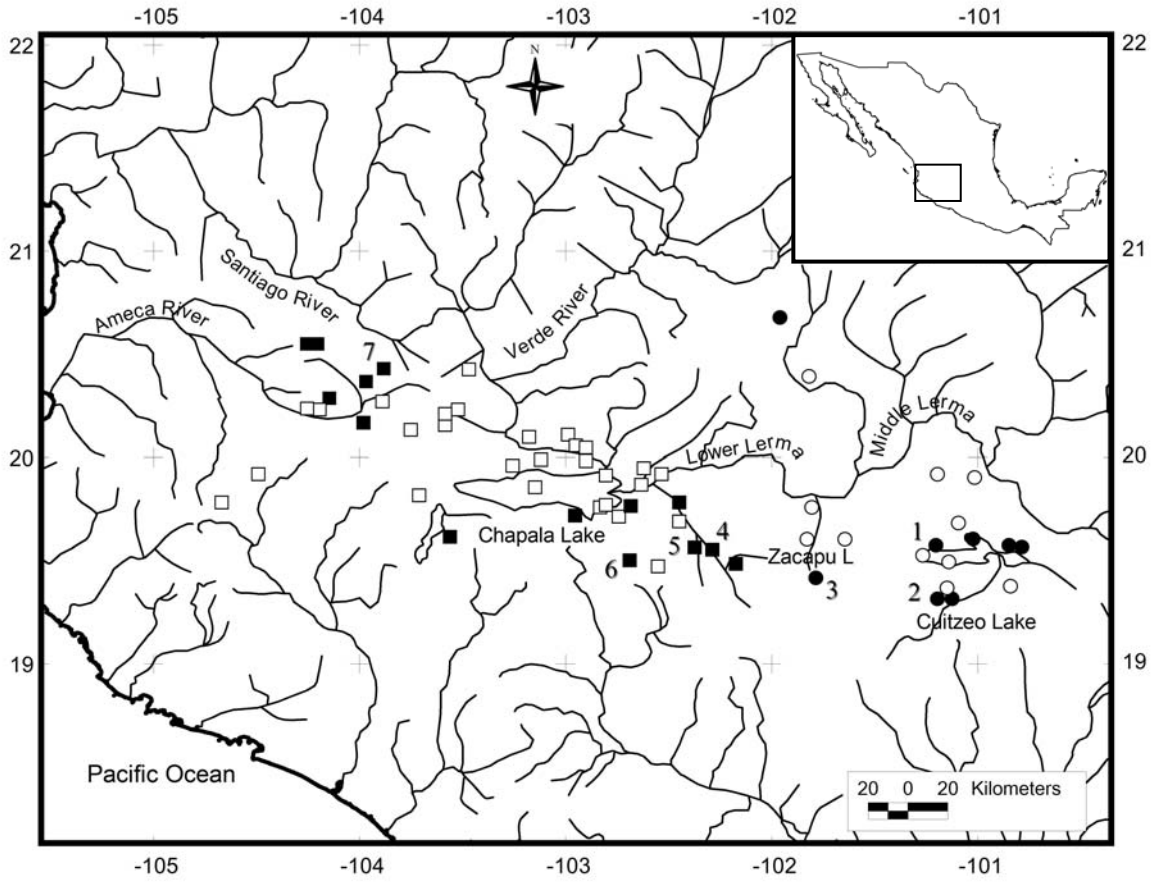

-105
-104
-103
-102
-101
22
21
20
19
N
Santiago River
Ameca River
Verde River
Lower Lerma
Middle Lerma
Chapala Lake
Zacapu L
Cuitzeo Lake
Pacific Ocean
1
2
3
4
5
6
7
20 0 20 Kilometers

Figure 2 A

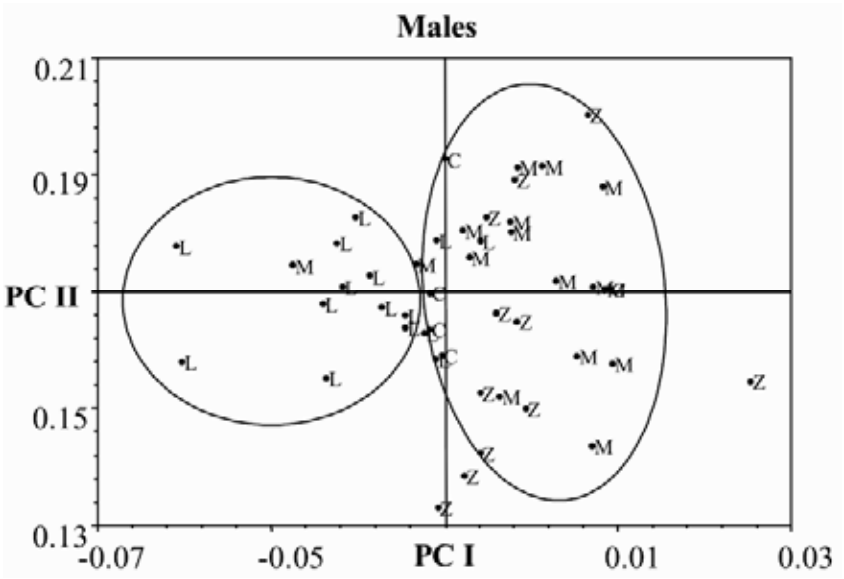



Figure 2 B

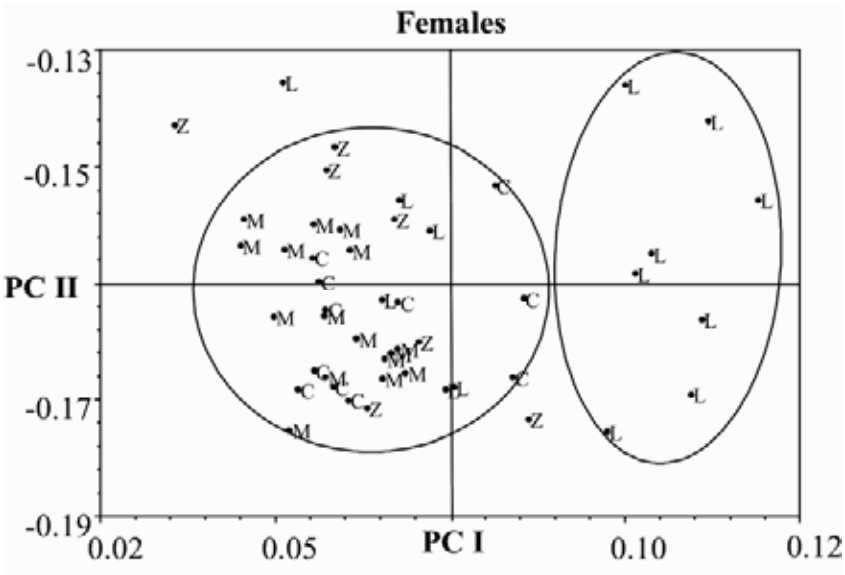


Figure 3

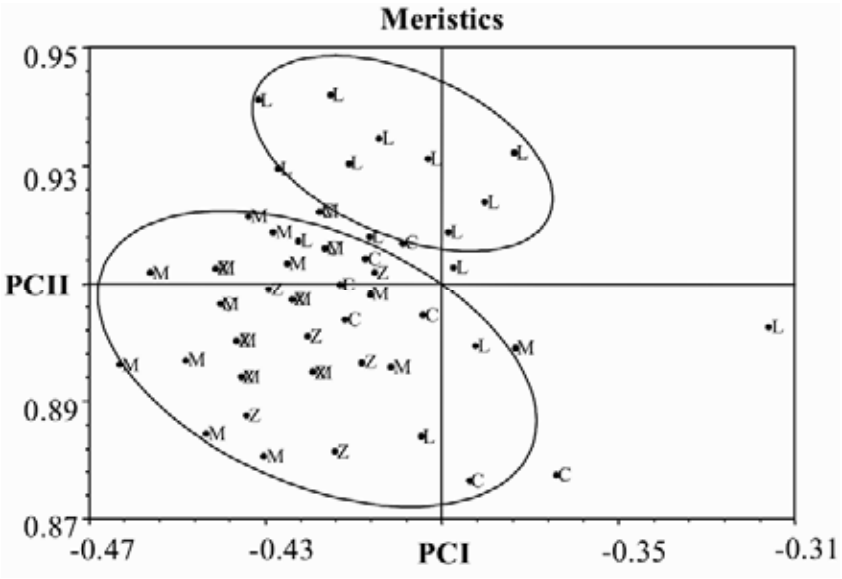


Figure 4

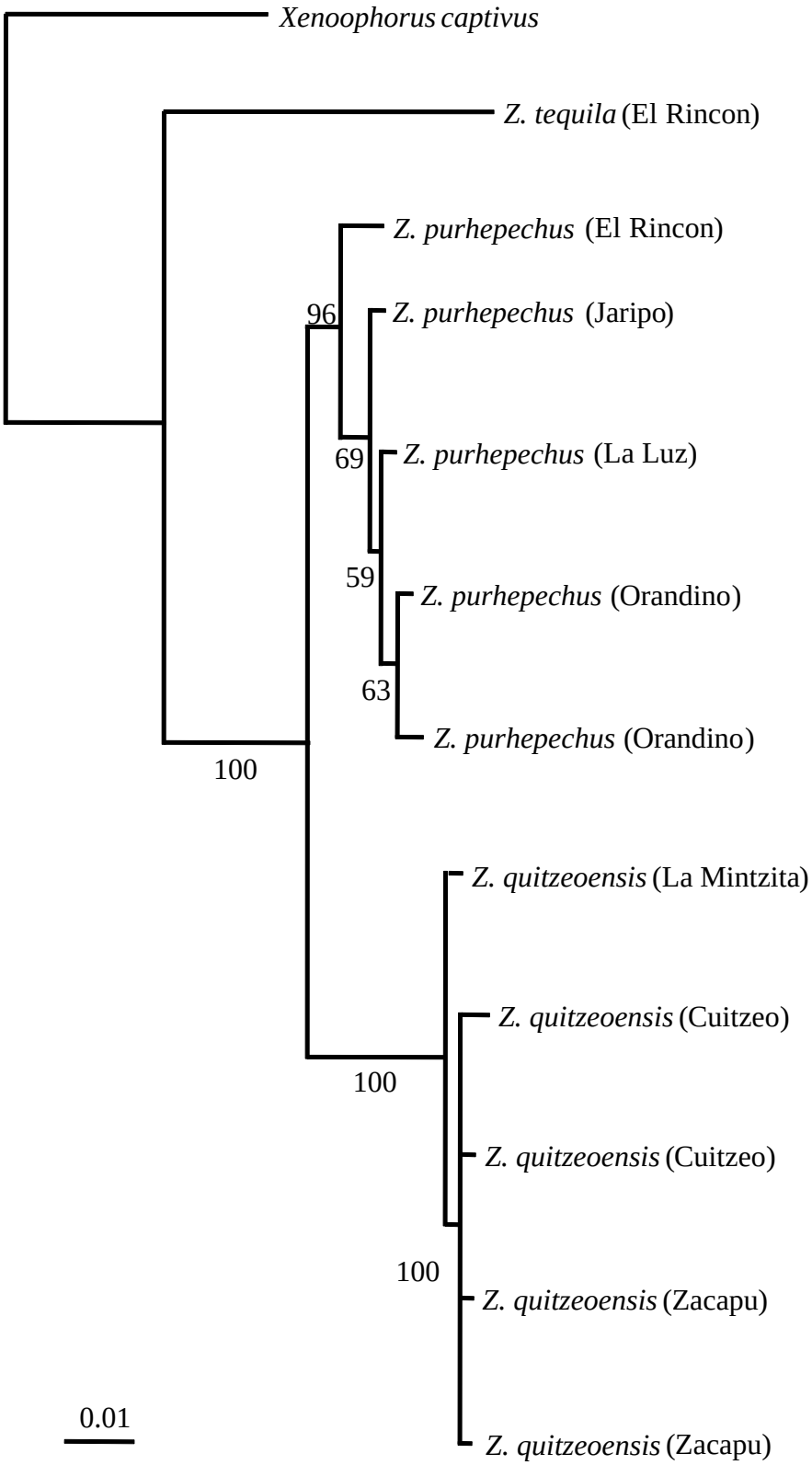
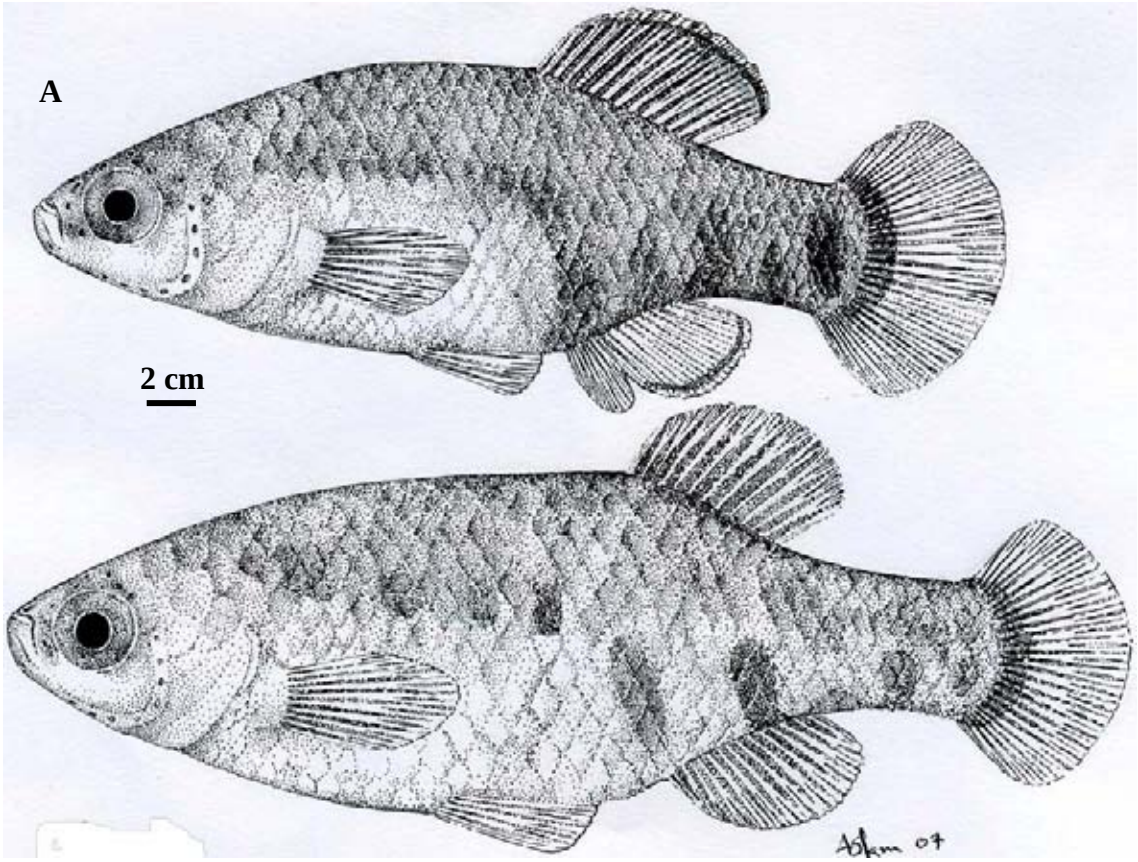

*Xenoophorus captivus*
*Z. tequila* (El Rincon)
*Z. purhepechus* (El Rincon)
96
*Z. purhepechus* (Jaripo)
69
*Z. purhepechus* (La Luz)
59
*Z. purhepechus* (Orandino)
63
*Z. purhepechus* (Orandino)
100
*Z. quitzeoensis* (La Mintzita)
*Z. quitzeoensis* (Cuitzeo)
100
*Z. quitzeoensis* (Cuitzeo)
100
*Z. quitzeoensis* (Zacapu)
0.01
*Z. quitzeoensis* (Zacapu)

Figure 5

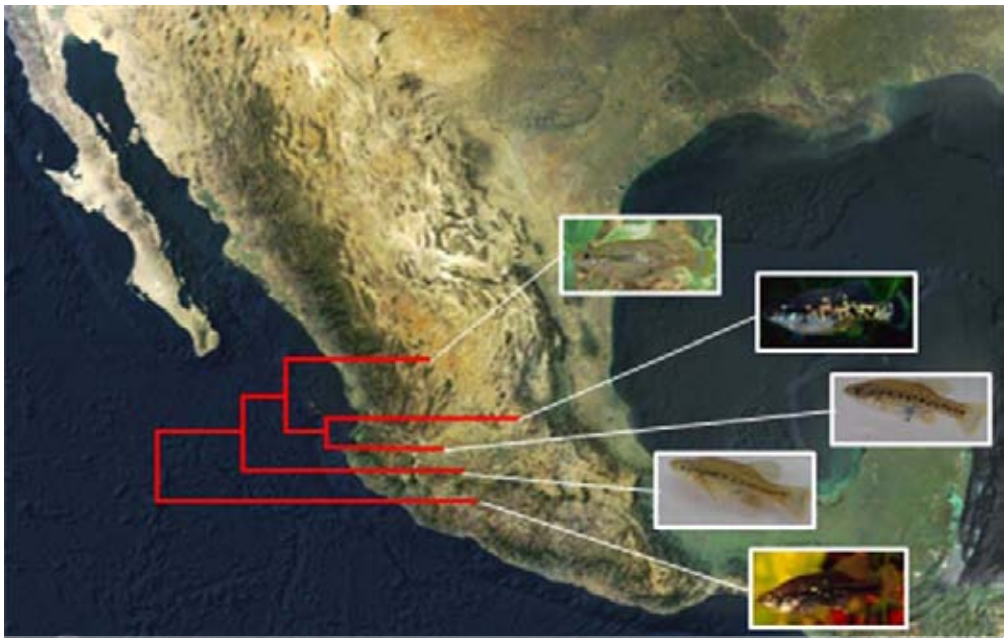


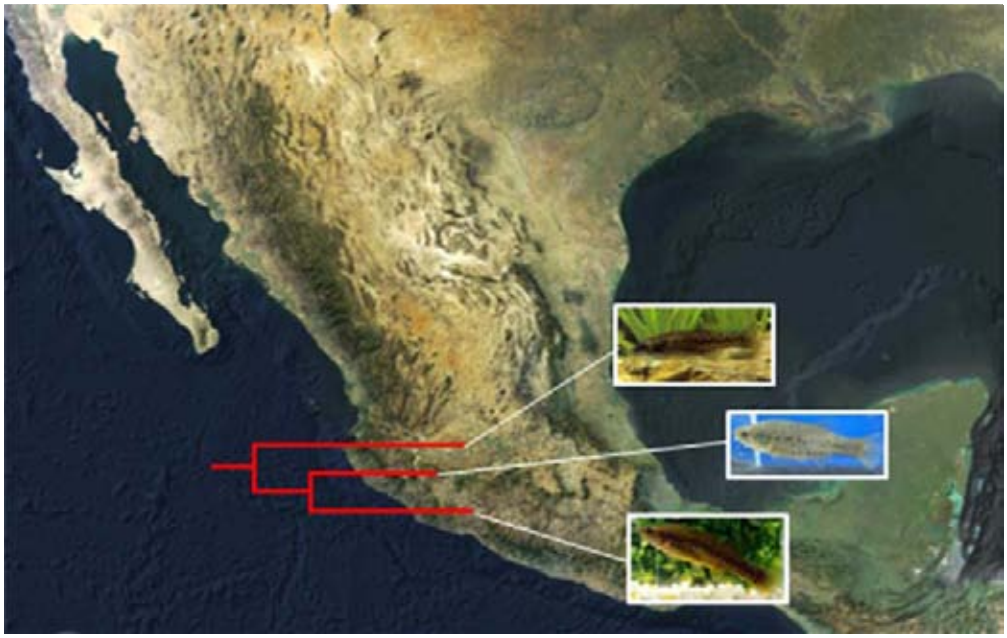
# CAPITULO 2

## *Xenotoca variata* y *Alloophorus robustus*

# FILOGEOGRAFÍA Y CONSERVACIÓN

## ARTICULO IV





Domínguez-Domínguez Omar

*A ser enviado a Moelcular Ecology*

# Comparative mtDNA phylogeography of *Xenotoca variata* and *Alloophorus robustus* (Cyprinodontiformes: Goodeidae) in drainages of Central Mexico.

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**ABSTRACT**

From a biogeographical perspective, common geological and climatic events that have influenced the evolutionary history of co-distributed species represent the first explanation to describe processes among concordant taxa. In cases of no concordance, other processes have to be invoked such as particular ecological aspects sexual selection, etc. Freshwater fishes of Central Mexico represent a model study to understand the evolution of complex areas and biotas. The ichthyofauna of Central Mexico is a key component in the evolutionary history of the area. The species *Xenotoca variata* and *Alloophorus robustus* are widely distributed along Central Mexico, sharing most of its distributional range. Although they belong to the same tribe within the Goodeinae, exhibit important intrinsic differences. Based on extensive sampling effort for *X. variata* and *A. robustus*, we investigate the biogeographic and demographic history of the two species, and the possible role of geologic and climatic events on the evolutionary history of each species, and also the role of the intrinsic characteristics of the species. Finally, we discuss the implications of our results within a conservation perspective. We found that the evolutionary history of *X. variata* and *A. robustus* not shared the same biogeographic scenario. *Xenotoca variata* is a species genetically well structured, with five independent evolutionary groups showing high genetic divergences among them, significant $\Phi_{ST}$ values when comparisons between basins are made, relative low migration rate, high effective population size and high genetic diversity within populations. Instead, *A. robustus* resulted to be a species with low genetic structure, with at least two independent evolutionary groups exhibiting low divergences between them, significant $\Phi_{ST}$ values found in the comparisons between clades, but not when basins within clades where compared, high migration rates, low population size and low genetic diversity within populations. The geologic and climatic activity of the area is closely related with the early diversification of the different groups within each species, but the differences in phylogeographic patterns shed some light into the possible influence of the intrinsic characteristics of the species in the evolutionary history of the fresh water fish fauna of a complex geological and climatic area, as the Mexican Plateau. Five main OCU's should be considered for *X. variata*: Zacapu, Lerma-Panuco, Chapala, Aguanaval and Cuitzeo, and three for A. *robustus*: San Francisco del Rincon, Cuitzeo-Patzcuaro-Zacapu-Zirahuen, and Chapala-Cotija-Lower Lerma-Middle Lerma-Uruapan.

**Key words:** Phylogeography, Central Mexico, Conservation, Goodeinae, discordant paterns.

## INTRODUCTION

The diversification of the biological diversity has been widely linked to global climatic changes as one of the main factors that enforce evolutionary changes, however, it has been argued that these climatic changes affect organisms in different ways in the same region (Knowles, 2000; Barraclough and Vogler, 2000; Doadrio and Domínguez, 2004; Hewitt, 2004; Kadereit *et al.,* 2004; Weir, 2006). In taxa restricted to the freshwaters, effects related to climatic changes (mainly the glaciar cycles) that promote changes in baseline see level and in the water balance of inland water bodies also promote changes in fish populations and dispersal routes (Williams *et al.,* 1998). Geological events are other source of evolutionary forces that shape the evolution and dispersal of freshwater fish since these events are linked with the evolution of hydrographic systems (e.g. river capture, connection and isolation of water bodies), which directly influence the historical biogeography of fishes due to events such as vicariance, dispersal, and range expansion (Waters *et al.,* 2001; Domínguez-Domínguez, 2006a; Kozak *et al.,* 2006; Burridge *et al.,* 2007; Waters *et al.,* 2007). Even though the important role played by geological events and climatic change causing contact and isolation of freshwater environments and promoting speciation events and divergence, there are other factors such as sexual selection, life histories or ecological factors (e.g. competitive exclusion) responsible of population divergence, however, the way these factor contribute is still a matter of discussion (Van Alphen and Seehausen, 2001; Masta and Maddison, 2002; Carson, 2003; Boul *et al.,* 2007).

From a biogeographical perspective, common geological and climatic events that have influenced the evolutionary history of co-distributed species represents the first explanation to describe processes among concordant taxa (Bermingham and Martín, 1998; Domínguez-Domínguez *et al.,* 2006a). Moreover, when a phylogenetic perspective is incorporated in biogeographic hypothesis, and when expectations are derived from models incorporating random changes in a geographic range, phylogenetic data suggest allopatric speciation as the predominant mode in several groups, with an overlapping distribution occurring due to range expansion after speciation (Barraclough and Vogler, 2000; Edwards, 2005; Domínguez-Domínguez *et al.,* 2006a). In some cases, when area cladograms are obtained through different biogeographical hypotheses, some geographic regions are not resolved even though it is assumed that taxa occupying these regions respond to the same

historical events. In this case, other processes have to be invoked such as dispersal, extinction, particular ecological aspects or sexual selection (Bermingham and Martín, 1998; Panhuis *et al.*, 2001; Domínguez-Domínguez *et al.*, 2008b).

A number of studies have been conducted to elucidate the historical biogeography of the freshwater fauna of Central Mexico. Some studies were based on descriptive methods (De Buen, 1943; Barbour, 1973; Miller and Smith, 1986; Moncayo-Estrada *et al.*, 2001; Pérez Ponce de León, 2003; Pérez-Ponce de León and Choudhury, 2005). Other studies used the Parsimony Analysis of Endemicity method (Morrone and Escalante, 2002; Aguilar-Aguilar *et al.*, 2003; Huidobro *et al.*, 2006), while others used phylogenetic information in the context of a cladistic biogeographical analysis (Mateos *et al.*, 2002; Doadrio and Domínguez, 2004; Domínguez-Domínguez *et al.*, 2006a, 2008b). More recently, historical biogeography and diversification of freshwater fishes from this region has been elucidated by incorporating either a phylogeographic approach (see Mateos, 2005; Mejía-Madrid *et al.*, 2007; Domínguez-Domínguez *et al.*, 2008a) or by describing population divergence and speciation due to ecological factors such sexual selection (see Ritchie *et al.*, 2005; Ritchie *et al.*, 2007). All these studies show that freshwater fishes in a complex geographical region such as central Mexico represent a model study to understand the evolution of complex areas and biotas. In this sense, the ichthyofauna of Central Mexico is a key component in the evolutionary history of the area.

Particularly, goodeids have been used recently as a model to uncover evolutionary and historical biogeography patterns in the so-called Mesa Central de Mexico. This freshwater fish group originated in the Middle-Miocene and experienced a diversification process in river basins of central Mexico in the last *ca.* 15-11 millions years (Doadrio and Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008b). Goodeines include 41 species with unique reproductive traits such as viviparity and matothrophy (Fitzsimons, 1972; Parenti, 1981), with particular ecological requirements (Moncayo-Estrada, 1996; Mercado-Silva *et al.*, 2002; Salazar-Tinoco, 2007), and with sexual dimorphism and sexual selection (Ritchie *et al.*, 2005; Ritchie *et al.*, 2007).

Phylogeographic studies of freshwater fishes have proven to be useful to understand the biotic and geologic evolution of particular regions at both spatial and temporal scales (Lundberg 1993; Durand *et al.* 1999). This discipline has been commonly used to describe

evolutionary patterns at intraspecific scales (Avise, 2000), and also to infer demographic processes such as gene flow, effective population size, colonization sequence, bottlenecks, species limits and also to identify conservation units (Avise *et al.*, 1987). In this way, the use of geographical distribution of phylogenetic data may help to uncover historical events such as habitat fragmentation or expansion of distributional range, lineage extinction and some other processes affecting haplotype frequencies in temporal and spatial scales (Hardy *et al.*, 2002; Johnson *et al.*, 2007). Additionally, a comparative analysis of phylogeographical patterns of co-distributed populations belonging to various taxa is useful to postulate common events of dispersal and vicariance, as well as to identify the geological, ecological and biological causes that influence these events (Lanteri and Confalonieri, 2003). Comparative phylogeography has allowed to uncover biogeographical patterns (Arbogast and Kenagy, 2001), to reveal cryptic and deeply divergent evolutionary lineages not shown by conventional taxonomical techniques, and to discover nominal species usually known as no natural groups (Riddle *et al.*, 2000). Resides, the combined use of molecular data with biological and ecological information is useful to show patterns of genetic structure influences by events such as life-history, behavior and genetic drift than to common historical, climatic and geological events (Zink, 1996; Weir, 2006; Domínguez-Domínguez *et al.*, 2008a).

Within the Goodeinae, the species *Xenotoca variata* and *Alloophorus robustus* are widely distributed along basins of Central Mexico, sharing most of its distributional range. Although they belong to the same tribe within the Goodeinae (Doadrio and Domínguez, 2004), they exhibit important differences. *Xenotoca variata* is tolerant to the environmental conditions, is omnivore, possess a small size (mean SL for adult males ≈ 60 mm), and shows a deep sexual dimorphism and high phenotypic plasticity. Instead, *Alloophorus robustus* is sensitive to environmental conditions, is carnivore, with a relative large size (mean for adult male SL ≈ 150 mm), and shows a slight sexual dimorphism (Fitzsimons, 1972; Mercado-Silva *et al.*, 2002, 2006; Moyaho *et al.*, 2004, 2005; Domínguez-Domínguez *et al.*, 2005; Macías and Ramirez, 2005; Ritchie *et al.*, 2005; Ritchie *et al.*, 2007).

Based on a extensive sampling effort in practically all the distribution range of both species (*X. variata* and *A. robustus*) and using data of 1000 bp of the mitochondrial gene

cytochrome b, in this study we investigate: i) The biogeographic history of the two species in basins of Central Mexico, and the possible role of geologic and climatic events on the evolutionary history of each species, ii) The demographic history of each species and its possible link with the intrinsic characteristics of the species, and iii) The implications of our results within a conservation perspective.

## MATERIALS AND METHODS

### *Specimens collection*

Fish were sampled in 11 biogeographic discrete regions and 19 sampling sites in Central Mexico (Domínguez-Domínguez *et al.*, 2006a) between 2004 and 2007 using seine nets and electofishing. A total of 223 fin clips were cut and stored in 95% ethanol, including 128 individual samples of *X. variata* (1 to 19 per population), and 95 individuals of *A. robustus* (1 to 15 per population) (Table 1, Fig. 1). Sample size varied between population and species due the variation in abundance in the collection sites for each species (see Domínguez-Domínguez *et al.*, 2005; 2007; 2008c). Locations with low number of specimens were not considered in some size dependent analyses (e.g. San Francisco del Rincon for *Alloophorus robustus*).

### *DNA extraction, sequencing and phylogenetic analysis*

Total cellular DNA was extracted from ethanol preserved tissue by a standard proteinase K and phenol/chloroform extraction method (Sambrook *et al.*, 1989). Two overlapping fragments of mitochondrial cytochrome *b* (1000 bp) were amplified via polymerase chain reaction (PCR) from each individual DNA sample. Primers used for PCR amplification were those mentioned in Machordom and Doadrio (2001). The amplification process for cytochrome b was conducted as follows: 94ºC (5 min), 35 cycles at 94ºC (1 min), 54ºC (1 min), 72ºC (1.30 min). PCR mixtures were prepared in a final volume of 25 µl containing 1-2 µl DNA, 0.5 µM each primer, 0.2 mM each dNTP, 1.5 mM MgCl2, and 1 unit of Taq DNA polymerase (Invitrogen). After checking PCR products on 1 % agarose gels, the genetic fragments were purified with the kit ExoSAP-ITTM (USB) and directly sequenced. All samples were sequenced on an Applied Biosystems 3700 DNA sequencer following the manufacturer's instructions.

that mentioned in Machordom and Doadrio (2001). All sequences were deposited in GenBank under accession Numbers XXXX-XXXX.

Phylogenetic relationships between haplotypes were inferred via three methods of phylogenetic reconstruction: a distance based method (Neighbour-Joinin or NJ), maximum likelihood (ML) and Bayesian inference (BY). The best-fit model of DNA substitution and the parameter estimates used for tree reconstruction were chosen under the Akaike Information Criterion (AIC; Akaike, 1974) in Modeltest (v.3.7, Posada and Crandall, 1998). We determined that the best-fit model for *X. variata* was the HKY + I and K81uf+I for *A. robustus*.

Neighbour-joining (NJ) phylogram was prepared using maximum-likelihood distances according to the model selected using PAUP version 4.0b10 (Swofford, 2002). Maximum-likelihood (ML) tree with Modeltest-derived parameters was constructed with the PHYML program using the method of Guindon and Gascuel (2003), because of the simultaneous adjustment of the topology and branch lengths this algorithm rapidly reaches an optimum and avoids getting trapped in local optima. The robustness of inferences was assessed by bootstrap resampling (BP) (Felsenstein, 1985) using 1000 random repetitions for the NJ and 10 000 random repetitions for the ML analyses. Bayesian inference was conducted by simulating a Markov Chain Monte Carlo reaction for $5 \times 10^6$ cycles and using the best-fit model using MrBayes 3.0 (Huelsenbeck *et al.*, 2001), posterior probabilities were obtained from the 50% majority rules consensus of trees sampled every 100 generations, after removing trees obtained before chains reached apparent stationary ('burn in' determined by empirical checking of likelihood values at 500 000 generations). To identify ancestral and derived haplotypes, and according with previously phylogenetic information (Doadrio and Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008b), the trees were rooted using *Chapalichthys encaustus* for *Xenotoca variata* and *Z. quitzeoensis* for *Alloophorus robustus* as outgroups. The average ratio of non-synonymous/synonymous substitutions (dN/dS) was calculated using DnaSP 4.0 (Rozas *et al.*, 2003).

***Nested Clade Analysis***

To infer a population history, a 95% plausible parsimony network connecting the haplotypes was inferred using statistical parsimony (Templeton, 1998; Templeton *et al.*, 1992) as implemented in the program TCS version 1.13 (Clement *et al.*, 2000). The program was set to estimate the upper limit of the number of mutational steps between haplotypes. As a complementary method to those performed before, and considering the

limitations and drawbacks of Nested Clade Analysis (Panchal and Beaumont, 2007; Petit, 2007), the resulting gene network was then grouped into one, two, three and four-step clades by hand, according to the methods described by Templeton *et al.* (1987) and employing special modifications of these rules described in Templeton and Sing (1993) and Templeton (1998) to handle equivocal groupings of haplotypes. To measure the association of geography with the hierarchical structure in the gene network, within-clade and nested-clade geographical distances were calculated from the latitude and longitude coordinates for each collection locality using GEODIS v. 2.0 (Posada *et al.*, 2000). Statistical significance was calculated by comparison with a null distribution generated from 100,000 random permutations of clades against sampling localities. To infer the most suitable population structure model and historical scenario for the observed geographical associations we used the Templeton (2004) inference key.

***Genetic structure***

Pairwise $\Phi_{ST}$ values were calculated among all geographic populations as an estimate of genetic differentiation. To assess the significance of genetic differentiation, an analysis of molecular variance (AMOVA) was performed as described by Excoffier *et al.* (1992). The population structure of *X. variata* and *A. robustus* mtDNA was explored by calculating the different fixation indices ($\Phi_{ST}$, $\Phi_{CT}$ and $\Phi_{SC}$) at different hierarchical rearrangements (e.g. biogeography, phylogenetic, NCA results and by basins). Statistical significance was assessed using 20,000 permutations. In order to elucidate if a scenario of isolation by distance influenced in the same way to both species, a Mantel test (100,000 permutations) was conducted for populations within both species (Mantel, 1967). Linear geographic distances matrix (km) were generated with the program GEODIS v. 2.4 (Posada *et al.*, 2000), whereas the maximum composited likelihood genetic distances were generated in MEGA 4.0. All analyses were performed using ARLEQUIN v. 3.1. (Excoffier *et al.*, 2005).

***Diversity indices and demographic history***

Standard indices of genetic variation, such as the number of haplotypes, nucleotide diversity ($\pi$; Nei, 1987), haplotype diversity ($H_d$; Nei, 1987), number of polymorphic sites (S), and the average number of pairwise nucleotide differences ($k$; Tajima, 1983), were

calculated for the whole data set, clade, basin and each sampling location using DnaSP v 4.0. (Rozas *et al.,* 2003).

Past population dynamics among the whole data set for *Alloophorus robustus* and the main mitochondrial lineages for *Xenotoca variata* were examined with a Bayesian skyline plot (BSP) model using BEAST v. 1.4.7 (Drummond *et al.,* 2005; Drummond and Rambaut, 2007). The Bayesian skyline plot model generates a posterior distribution of effective population size through time using a Markov chain Monte Carlo (MCMC) sampling and does not require a specified demographic model (e.g, constant size, exponential growth, logistic growth, or expansive growth) prior to the analysis. The method was employed with relaxed molecular clock model (Drummond *et al.,* 2006). The parameter *m* that represents the number of grouped intervals was set to 10. The MCMC analysis was run for at least three times for 50 x $10^6$ generations. The substitution model used was HKY+I. All the other parameter were used as default options and were changed as recommended by the BEAST output file. We checked for effective sample size (ESS) convergence and stationarity of the different analyses in Tracer 1.4 and combined the results in the BEAST module LogCombiner 1.4.4 (Drummond and Rambaut, 2007), after removing the 10% generations from each analysis as "burn-in", the ESS exceed 210 for all parameters. Additionally, we used these analyses to estimate the root height and the time to the most recent common ancestor (TMRCA) in the different lineage and clades identified in the phylogenetic and NCA analyses, in this case, divergence times and their credibility intervals were estimated by using a relaxed clock model with branch rates drawn from an uncorrelated lognormal distribution (Drummond *et al.,* 2006), the substitution rate of the branch lengths being sampled from a prior normal distribution with a mean value of 0.009 and a standard deviation of 0.001 (Doadrio and Domínguez, 2004). Because we found two divergent lineages in *Xeonotoca variata*, in a second analyses we used a Yule tree prior to date the cladogenetic event, the sequences of the two lineages were grouped in two data sets and constraining each lineage to be monophyletic; three runs for 50 x $10^6$ generations was conducted.

To provide other estimation of population size change, we investigated the demographic signature of previously identified divergent mtDNA groups of haplotypes, throughout a pairwise mismatch distribution analysis (MMD) of pairwise differences to test

for population expansion (Rogers and Harpending, 1992). Contrasting plots of observed and theoretical distributions of site differences yields insight into past population demographics. The mismatch distribution of a recently expanded population is unimodal and smooth; the wave shaped curve is centered nearer the y-axis the more recent the expansion, moving away as the number of mismatches increases. Multimodal (including bimodal) mismatch distribution indicates diminishing population sizes, demographic equilibrium or structured size, and a ragged distribution suggests that the lineage was widespread (Rogers and Harpending, 1992; Excoffier *et al.,* 1992; Rogers *et al.,* 1996; Excoffier and Schneider, 1999). The multimodal distribution may also indicate that the population is influenced by migration, is subdivided, and/or has undergone historical contraction (Marjoram and Donnelly, 1994; Bertorelle and Slatkin, 1995; Ray *et al.,* 2003). For graphical representation, the mismatch distribution was also calculated with Roger's method of moments (Rogers, 1995), using the program DnaSP 4.0 (Rozas *et al.,* 2003). We use two statistics to test for significance of these distributions against a null distribution of recent population expansion; 1) Harpending's raggedness index (*Hri*; Harpending, 1994) which use information of the pairwise sequence difference, and 2) R2 statistics (Ramos-Onsins and Rozas, 2002) which use information of the mutation (segregatin site) frequency. The first was test using 10000 bootstrap replicates in Arlequin 3.1 (Excoffier *et al.,* 2005) and the second one was tested in the programa package DnaSP 4.0 (Rozas *et al.,* 2003).

Alternatively, Tajima's D (Tajima, 1993), Fu and Li's D* and F* (Fu and Li, 1993), and Fu's Fs (Fu, 1997) neutrality statistics were conducted in order to examine whether samples belonging in the different linageas and clades found in NCA are at equilibrium with respect to mtDNA. The null hypothesis of neutrality may be rejected when a population has experienced demographic expansion, bottlenecking or a heterogeneous mutation rate (Slatkin and Hudson, 1991; Tajima, 1996; Schneider and Excoffier, 1999). Significance of this statistics was examined using 10,000 permutations using ARLEQUIN version 3.1 (Excoffier *et al.,* 2005) and DnaSP 4.0 (Rozas *et al.,* 2003).

In order to indentify the differences in the migration rate between the two species, the migration rates between the different groups within each species were calculated using MIGRATE 2.3.1 (Beerli, 1997–2002). Analyses were repeated 5 times to ensure stability of parameter estimates. This method gives a maximum-likelihood approximation based on

coalescence theory and employs a Metropolis–Hastings MCMC algorithm to estimate migration rates. Starting theta and *M* values were generated from $F_{ST}$ calculations, and a migration matrix model with variable theta was employed under the following conditions: 10 short chains with 100 000 trees sampled, three long chains with 1 000 000 trees sampled with a burn-in of 100 000 trees. We applied an exhaustive search using 4 heated chains (1, 1.5, 2.5 and 3) and an interval between swapping trees of 1.

To investigate if both species have any differences in the effective population size after expansion ($\theta_1$), we conducted a historical demography test for the whole data set and the main clades within each species based on the coalescent-based method of Kunher *et al.* (1998). This method calculate maximum likelihood estimates of theta ($\theta_{ML}$), where $\theta = 2Ne\mu$ and exponential growth parameter (*g*) as implemented in the software Fluctuate 1.4 (Kuhner *et al.,* 1998). Each Markov chain Montecarlo run consisted of 10 short chains and 10 long chains. A neighbor-joining tree based on HKY+I was used as a starting tree for both species.

## RESULTS

### *Sequence variation and phylogenetic reconstruction*

By sequencing 1000 bp of the cytochrome b gene in 128 specimens of *X. variata* from 14 populations, 53 haplotypes were identified (Table 2). One hundred eighteen characters were variable (18 %) and 68 were parsimony informative. Third codon position was the most variable (55), follow by first position (11) and finally the second position (2). The average ratio of non-synonymous/synonymous substitutions was dN/dS = 0.18 (95% CI = 0.13-0.24. For the dataset of 95 specimens of *A. robustus* from 12 populations, 21 haplotypes were identified (Table 2). Thirty-seven characters were variable (3.7 %) and 19 were parsimony informative. Third codon position was the most variable (10), followed by the first position (5), and the second position (4). The average ratio of non-synonymous/synonymous substitutions dN/dS = 0.18 (95% CI = 0.11-0.29).

For both species the three methods of phylogenetic reconstruction (NJ, ML and BI) produced largely congruent tree topologies, with similar branch support for the major lineage and populations (Figs. 2 and 3). For *X. variata* two main divergent lineages were recovered, lineage I grouping 36 haplotypes of specimens collected in ten locations of five

discrete regions and lineage II representing seventeen haplotypes (*Hn*) of the specimens analyzed for the four locations sampled in the Cuitzeo Basin (Fig. 2). Genetic distances between the two lineages was $\bar{D}_{ML}$ = 5.4 % and $\bar{D}_p$ = 5.08%, when the genetic distance within groups was $\bar{D}_{ML}$ = 0.8% (SE = 0.05%) and $\bar{D}_p$ = 0.8% (SE = 0.2%) for lineage I and $\bar{D}_{ML}$ and $\bar{D}_p$ = 0.2% (SE = 0.05%) for lineage II (Table 3). Within the lineage I four major clades were identify, clade 2.1 comprising eight haplotypes from Zacapu Lake basin samples, clade 2.7 comprising five haplotypes for the samples locality from Chapala Lake basin, clade 3.2 comprising seventeen haplotypes for the six localities from Panuco and Middle Lerma Basins and clade 2.3 comprising six haplotypes for the two localities from Aguanaval River Basin (Fig. 2). The mean genetic distances between clades ranged from $\bar{D}_{ML}$ = 1.5% and $\bar{D}_p$ = 1.48% when compared Middle Lerma basin populations with respect to Aguanaval River basin populations to $\bar{D}_{ML}$ = 0.71% and $\bar{D}_p$ = 0.71% when compared Zacapu Lake basin population with respect to Chapala Lake basin population (Table 3).

In the case of *A. robustus* the phylogenetic tree showed the existence of two main lineages, but with low branch support. Lineage I comprises 18 haplotypes for the samples collected in the eleven populations belonging to nine discrete regions, including one population within the Middle Lerma Drainage. Lineage II comprises three haplotypes for the samples collected in the San Francisco del Rincon population, within the Middle Lerma Drainage (Fig. 3). The genetic distances between lineages was $\bar{D}_{ML}$ = 1.01% and $\bar{D}_p$ = 1% when the genetic distance within groups was $\bar{D}_{ML}$ and $\bar{D}_p$ = 0.55% = for lineage I and $\bar{D}_{ML}$ and $\bar{D}_p$ = 0.2% for lineage II. Within the lineage I, two clades were identified, clade 2.1 comprising twelve haplotypes for the samples collected in Patzcuaro, Zirahuen, Cuitzeo and Zacapu lakes basins, and clade 2.3 comprising six haplotypes for the samples collected in Chapala and San Juanico lakes basins and Lower Lerma, Middle Lerma and Balsas rivers basins. The mean genetic distances between clades was $\bar{D}_{ML}$ = 1.06% and $\bar{D}_p$ = 1.05%, when the genetic distance within clades weres $\bar{D}_{ML}$ and $\bar{D}_p$ = 0.11% for clade I and $\bar{D}_{ML}$ and $\bar{D}_p$ = 0.08% for clade II (Table 4).

**Nested clade analysis**

The statistical parsimony networks (Templeton *et al.*, 1992) for *X. variata* and *A. robustus* show a general congruence with the phylogenetic reconstructions (Figs. 4 and 5

respectively). For *X. variata* two unconnected clades were found (representing the lineages I and II in phylogenetic analyses), these clades were separated by 44 mutational steps, which exceed the maximum mutation steps connection justified for the 95% parsimony criterion (13 steps), the clade CUI-16 and ZAC-5 represent the haplotypes where the two clades were connected when enforced. The haplotype with the largest outgroup probability is the CUI-16 haplotype for the whole data set. Accordingly with these results, the two groups were treated separately. Clade 4.1 (lineage I) contained all the haplotypes distributed in nine sampled drainages (with the exception of Cuitzeo basin populations). Within clade 4.1, three 3 steps clades were identified, clade 3.1 is formed by the clade 2.1, represented by the eight haplotypes sampled in the Aguanaval river basin locations, and clade 2.3, represented by the six haplotypes sampled in the Zacapu Lake Basin. Clade 3.2 is formed by the 17 haplotypes sampled in the two locations of the Panuco River Basin and the four locations of the Lerma River Basin. Among them, the haplotype TQE-37 was the most abundant and is present in the six sampled locations, moreover this haplotype was the one with the largest outgroup probability (0.37) within clade 4.1. The clade 3.3 is represented by the five haplotypes sampled in the Chapala Lake basin population (Fig. 4). For the clade 4.1 the null hypothesis of the nested contingency analysis of no association between haplotype positions in the cladogram and their geographical locations was rejected ($P < 0.001$) (Table 5). Long distance colonization possibly coupled with subsequent fragmentation or past fragmentation followed by range expansion was inferred for this clade (Templeton, 2004). Clade 3.4 (lineage II) is represented by the 17 haplotypes sampled in the Cuitzeo Lake drainage. Within this clade, haplotype CUI-16 was the most common and was the haplotype with the largest outgroup probability (0.45) (Fig. 5).

For *Alloophorus robustus* three main clades nested in a 4 step clade were recovered. Clade 2.1 is composed by the six haplotypes sampled in the Balsas and Lower and Middle Lerma river basins, and Chapala and San Juanico lakes basins. Within this clade, the LUZ-11 was the most abundant and widespread haplotype, comprising most of the Chapala and Lower Lerma haplotypes and all of the Balsas basin samples, the 10 samples from the San Juanico Dam belong to one haplotype (COT-21). Clade 2.2 (lineage II) is represented by the four samples from San Francisco del Rincon location (within the Middle Lerma Basin). Clade 3.1 comprised 13 haplotypes sampled in Zirahuen, Patzcuaro, Zacapu and Cuitzeo

lake basin populations. Within this clade, ZAC-10 was the most widespread and abundant haplotype, represented by individuals collected in the three locations sampled in the Cuitzeo Lake basin and the Zacapu Lake population. The haplotype with the largest outgroup probability in the network (0.23) was PAT-4. Three clades showed no association between haplotype positions in the cladogram and their geographical locations, thus rejecting the null hypothesis of the nested contingency analysis ($P < 0.001$). For clade 1.1 past fragmentation and/or long-distance colonization and insufficient evidence to discriminate between long-distance movements of the organism, and the combined effects of gradual movement during a past range expansion and fragmentation was inferred. For clade 2.1 Contiguous range expansion was inferred and for clade 3.1 insufficient evidence to discriminate between long-distance movements of the organism was inferred (Templeton, 2004) (Table 5).

***Genetic structure***

The lowest $\Phi_{ST}$ not significant value obtained for *Xenotoca variata* for the comparison of all the populations ranged from 0.06 when the population of Salvador Escalante with respect to Belisario (both within the Cuitzeo basin) was compared and when the populations of Tierra Quemada with respect to Jesus Maria (both within the Panuco River basin) were compared. The highest $\Phi_{ST}$ significant value was obtained when comparing the populations of La Alberca (within the Chapala Lake basin) with respect to La Mintzita and Salvador Escalante (both within the Cuitzeo Lake basin) (Table 6). The only significant value for the comparisons of populations within the same basin was obtained between the populations of La Mintzita and San Cristobal, within the Cuitzeo Lake basin. Likewise, not significant values were obtained when compared the two populations sampled in the Panuco River basin with respect to the four populations from the Lerma River basin (Table 6). For *Alloophorus robustus* the lowest and not significant $\Phi_{ST}$ value was 0.001, was obtained when comparing the populations of Zacapu Lake with respect to Santa Maria (within the Cuitzeo Basin), whereas the highest $\Phi_{ST}$ significant value was 1 when comparing the populations of San Juanico and Uruapan (within the Balsas river basin) with respect to Zacapu and Santa Maria, and when comparing Cotija with respect to the Uruapan populations (Table 7). Low $\Phi_{ST}$ not significant values for comparisons of populations between drainages were obtained for the comparisons of Zacapu with respect to

Cuitzeo populations and for the comparisons between the populations of Lower Lerma, Chapala and Baslsa basins populations (Table 7).

For both species, *Xenotoca variata* and *Alloophorus robustus* the AMOVA analysis revealed significant structure among populations ($\Phi_{ST}$ = 0.95714, $P < 0.001$ and $\Phi_{ST}$ =.87927, $P < 0.001$, respectively). For the subsequent analyses, populations were grouped in different hierarchical arrangements according to previous biogeographic information (Domínguez-Domínguez *et al.,* 2006a) and to uncover groupings obtained in the previous analyses (e. g. phylogenetic and NCA) (Table 8). For *Xenotoca variata* significant values were obtained when the biogeographic arrangement of basins was considered ($\Phi_{CT}$ = 0.96057, $P < 0.001$). Division of the populations into the groups suggested by the phylogenetic and NCA analyses maximized among-group variance 97% ($\Phi_{CT}$ = 0.96338, $P < 0.001$), supporting statistically the structure suggested by the previous analyses conducted in the present study.

In the case of *Alloophorus robustus* significant values were obtained when the biogeographic arrangement of basins was considered ($\Phi_{CT}$ = 0.88711, $P < 0.001$) and was the arrangement that maximized among-groups variance (88.71%). The division of populations according to the phylogenetic results (two gene pools and Phylogenetic arrangement) showed significant values for the $\Phi_{CT}$ results ($\Phi_{CT}$ = 0.77266, $P < 0.005$; $\Phi_{CT}$ = 0.87471, $P < 0.001$, respectively), but explained lees of the among-groups variance (77.27% and 87.71% respectively).

The Mantel test shown not significant correlation between genetic distances and geographic distances for *Xenotoca variata* ($r^2$ = 0.155), whereas a significant correlation was found for *Alloophorus robustus* ($r^2$ = 0.588, P < 0.005).

***mtDNA variation.***

For the 128 sequenced specimens of the species *Xenotoca variata* 53 haplotypes were identified (Table 2). Most of the haplotypes were found in a single basin. Only the populations of the Middle-Lerma and Panuco river basins shared haplotypes among them (Haplotype 16). In all cases in which more than one location was sampled within the basin (e. g. Cuitzeo) at least one haplotype was shared among them (e. g. Haplotype 32 and 16 in Fig. 2). The overall gene diversity was *Hd* = 0.912 and nucleotide diversity $\pi$ = 0.2495 (Table 2). The level of gene diversity within basin ranged from *Hd* = 0.386 in the case of

Chapala lake basin to 0.933 for Zacapu Basin, and the nucleotide diversity ranged from $\pi$ = 0.00050 for Aguanaval basin to 0.00201 for the Cuitzeo basin samples. At the population level, gene diversity ranged from *Hd* = 0.333 for San Francisco del Rincon and Zacatecas populations to 0.933 for Zacapu and 0.844 for La Mintzita populations. The mean number of pairwise differences overall (*K*) was *K* = 24.95 (Table 2).

For the 95 specimens sequenced of the species *Alloophorus robustus* 21 haplotypes were identified (Table 2). Within the lineage I, most of the sampled locations within clades 2.1 and 3.1 shared haplotypes, except for San Juanico which contain a unique and private haplotype. For lineage II three private haplotypes were identified (Fig. 3). The overall gene diversity was *Hd* = 0.770 and nucleotide diversity $\pi$ = 0.00585 (Table 2). The level of gene diversity within basin ranged from *Hd* = 0.000 in the case of Zacapu, San Juanico and Balsas basin locations to *Hd* = 0.9011 for Patzcuaro Basin location, and the nucleotide diversity ranged from $\pi$ = 0.000 for Zacapu, San Juanico and Balsas basin locations to 0.00213 for the Patzcuaro basin samples. At the population level, the high gene diversity was *Hd* = 0.9011 for Patzcuaro population. The mean number of pairwise differences overall (*K*) was *K* = 5.852 (Table 2).

***Demography***

Estimation of Bayesian skyline plot (BSP) for lineage I in *X. variata* revealed a slightly past population decline between 250,000 to 100,000 years before present (yr BP), but between 100,000 to 75,000 Yr BP the population decline was more pronounced, with a exponent growth starting around 60,000 Yr BP, reaching the stability around 35,000 Yr BP (Fig. 6A). The BSP for lineage II (Cuitzeo populations) show somewhat population growth during the last 60,000 Yr BP. The mismatch distribution (MMD) for all the data set was multimodal (Fig. 7). This could be indicative of geographic subdivision, the raggedness index (*Hri*) was significant, but the *R2* index was not. Also a multimodal MMD was found for lineage I, but in this case both indices (*Hri* and *R2*) were not significant (Fig. 7 and Table 9). The MMD for lineage II (Cuitzeo populations) was unimodal or slightly bimodal, in this case the *Hri* was not significant, but the *R2* was significant (Fig. 7and Table 9). In all the other clades obtained within the lineage I the MMD were unimodal, in all the cases the *Hri* were not significant, but in the Lerma-Panuco populations (clade 3.2) shown significant results for the *R2* statistics. When the two basin populations within the clade 3.2 (Lerma

and Panuco) were tested independently, the MMD were unimodal to slightly bimodal and the *Hri* and *R2* indices were not significant (not shown).

The Tajima's D-statistic was significantly negative, except when all the populations were tested together. The Fu's statistic was significantly negative for all the groups tested, supporting the expansion model. The MIGRATE results showed no migration between lineage I and lineage II (Cuitzeo populations). A relative low value of migration rate were found between clades within lineage I ( $\bar{M}$ = 68, SD = 15). Although the five populations sampling for clade 3.2 come from two different basins, the migration rate was 42 orders of magnitude more ( $\bar{M}$ = 2852, SD = 866 for Panuco *vs* Middle Lerma) than contiguous connected basins belonging to different clades (e. g. $\bar{M}$ = 68, SD = 15 for Zacapu *vs* Middle Lerma). For populations within clades the migration rate was higher, showing $\bar{M}$ = 5498, SD = 2243 for the three populations within the Middle Lerma basin and $\bar{M}$ = 4014, SD = 2457 for the four populations within the Cuitzeo basin. Estimated effective population size after expansion was calculated in $\theta 1$ =88.76.

Estimation of Bayesian skyline plot (BSP) for all the data set in *A. robustus* showed a faster past population growth around 40,000 Yr BP, with more recent stabilization (Fig. 6C).

The MMD in all the data set and for lineage I were bimodal (Fig. 8). When the two clades within the lineage I were tested independently, unimodal distribution of the MMD was obtained (slightly bimodal for clade 3.1). In all the cases the *Hri* were not significant and with the exception of the clade 3.1, all the results for the *R2* statistics were also not significant (Fig. 8 and Table 9).

The Tajima's D-statistic and Fu's statistic were not significant, rejecting the null hypothesis of population expansion, except for the lineage 3.1, were both statistics shown negative significant values, supporting the expansion model. The MIGRATE results shown a relative low migration rate between the two clades $\bar{M}$ = 81, SD = 15. A relative high migration rate was found between contiguous not connected basins of Cuitzeo and Zacapu ( $\bar{M}$ = 21737, SD = 3375) and between the connected contiguous basins of Lower Lerma and Chapala ( $\bar{M}$ = 80591, SD = 11962). Between the four sampled localities within the Cuitzeo basin the migration rate was estimated in $\bar{M}$ = 40795, SD = 13566. Estimated effective population size after expansion was calculated in $\theta 1$ = 8.1.

***Divergence time estimates***

The root height for the entire data set in *X. variata* was estimated in a mean of 7 Ma (95% HDP = 4.71-9.64), whereas the time for the most common recent ancestor (TMRCA) was estimated in *ca.* 5.9 Ma (95% HDP = 3.57-8.66) for lineage I and *ca.* 3.09 Ma (95% HDP = 1.67-4.75) for lineage II. For the clades within the lineage I, the TMRCA varied between *ca.* 1.14 Ma (95% HDP = 2.1-0.5) for clade 3.2 (Lerma-Panuco populations) and *ca.* 0.87 Ma (95% HDP = 0.4-1.6) for clade 2.1 (Zacapu populations). For *Alloophorus robustus* root height for the entire data set was estimated in *ca.* 5.2 Ma (95% HDP = 2.2-11), whereas for the two main clades within the lineage I the TMRCA was *ca.* 1.9 Ma (95% HDP = 0.5-3.7) for clade 3.1 and 1.8 Ma (95% HDP = 0.4-3.7) for clade 2.1.

**DISCUSION**

Freshwater fishes are usually restricted to water bodies which are surrounded by biogeographic barriers (land areas). As a result, it is possible to invoke an island-like biogeographic model to explain the possible exchange of biotic components among “islands” (water bodies). This exchange is only possible when a physical connection occurred between them (e. g. river capture, water bodies connection). In this way, it has been argued that these connection events, mainly induced by geologic forces or climatic changes, influenced in the same way the exchange of fish fauna along the new dispersion route (Lumberg, 1993; Bermingham and Martin, 1998). Comparative phylogeography test for consistencies in the evolutionary and distributional history of taxa with respect to the particular geographical and ecological setting of a region, the time scale of phylogeographic events, intrinsic characteristics of taxa, and ages of populations (Avise *et al.* 1987; Bernatchez and Wilson, 1988; Joseph *et al.*, 1995; Bermingham and Martin, 1998;). The existence of forces such as ecological or biological characteristics of the organisms and sexual selection may contribute to rapid morphological, genetic and behavioral divergence in allopatric or parapatric populations (Uy and Borgia, 2000; Yeh, 2004). In most of the phylogeography published works, the main explanation for phylogeographic patterns is focused in the geologic or climatic hypotheses (Edwards *et al.*, 2005). In this case, our study provides contradictory results in the comparison of evolutionary history of the two species analyzed, *X. variata* resulted in a structured species with important divergences between populations and drainages, and with a high genetic

diversity, whereas *A. robustus* is shown as a poorly structured species exhibiting low genetic diversity. Even though the information on the ecology, natural history, and behavior of both species of goodeids is very scarce, the differences in phylogeographic patterns shed some light into the possible influence that this differences, combined with the effects of biogeographic barriers, may have in the evolutionary history of the fresh water fish fauna of a complex geological and climatic area, as the Mexican Plateau.

***Comparaive phylogeography and demography***

The historical biogeography of the fresh water ecosystems of Central Mexico has been addressed by studying different taxa such as Goodeids (Gesundheit and Macías-García, 2003; Domínguez-Domínguez *et al.,* 2006a; 2008b) Poeciliids (Mateos *et al.,* 2002; Mateos, 2005), Helmints (Pérez-Ponce de León, 2003; Aguilar-Aguilar *et al.,* 2003; Pérez-Ponce de León and Choudhoury, 2005; Mejia-Madrid *et al.,* 2007; Rosas-Valdéz *et al.,* 2007), snakes (Contant, 2003), frogs (Zaldívar-Riverón *et al.,* 2004), ambystomatids (Weisrock *et al*., 2006), and a combination of different groups (Huidobro *et al.,* 2006). These studies revealed several vicariant events that produced congruent biogeographic histories shared by different codistributed taxa. In contrast to the hypothesis of shared histories of codistributed species, in this study we found that the evolutionary history of *X. variata* and *A. robustus* not shared the same biogeographic scenario in the same geographical area. *Xenotoca variata* is a species genetically well structured, with five independent evolutionary groups showing high genetic divergences among them, significant $\Phi_{ST}$ values when comparisons between basins are made, relative low migration rate, high effective population size and high genetic diversity within populations. Instead, *A. robustus* resulted to be a species with low genetic structure, with at least two independent evolutionary groups exhibiting low divergences between them, significant $\Phi_{ST}$ values found in the comparisons between clades, but not when basins within clades where compared, high migration rates, low population size and low genetic diversity within populations.

*Xenotoca variata*

In the case of *Xenotoca variata,* a strong support for the separation of the populations in two highly divergent lineages was found with all the analyses conducted (see Table 6; Figs. 2 and 4). One of this lineages comprised populations with a wide

distribution, with distances of 450 km between some localities, while lineage II is composed only by the populations belonging to the Cuitzeo lake, which represents a contiguous basin with respect to most of the other population within lineage I. The Fu's *Fs* and Tajima's *D* for the whole data set were negative and not significant (Table 9). Although various factors such as background selection, effective population size, population growth and selective sweeps may account for deviations from neutrality, they can be distinguished from one another by evaluating the significance of different analyses. Fu (1997) $F_S$ is particularly sensitive to population growth and selective sweeps relative to Fu and Li (1993) D* and F*, whereas D* and F* are more sensitive to background selection than $F_S$. Therefore, a pattern of significant $F_S$ with not significant F* and D* indicates population expansion or selective sweep and the opposite pattern would indicate effects of background selection (Peck and Congdon, 2004). Our analyses support the idea of background selection for the whole data set, since the D* and F* were significant and *Fs* was not (Table 9). Other data as the low migration rate, high genetic diversity within each lineage (Table 2) and the multimodal mismatch distribution (Fig. 7A), indicates that the species is subdivided (Excoffier *et al.,* 1992; Ray *et al.,* 2003). In this case, the two lineages could represent independent demographic histories. Moreover, the number of mutational steps exceeded the connection steps of the 95% confident interval for the parsimony criterion in the NCA (Fig. 4). Although the phylogenetic and parsimony network showed the subdivision of *Xenotoca variata* in two highly divergent lineages and four caldes within lineage I, the mantel test did not show a correlation between genetic distances and geographic distances, not supporting the isolation by distance scenario. Moreover, the biogeographic arrangement in the AMOVA analyses do not explain the large variation among groups (Table 8). Since deep structure may bias the Mantel test, analyses were repeated without the samples belonging to lineage II (Cuitzeo populations) and a strong significant correlation was found ($r^2 = 0.8665$, $P < 0.001$). These results indicate than forces other than the formation and disappearing of biogeographic barriers, or stepping stone model, could have influenced the cladogenesis of this two divergent lineages within *Xenotoca variata*, one which is found in Zacapu, Lerma, Panuco, Chapala and Aguanaval basins, whereas the other one is restricted to the Cuitzeo basin.

In other studies using molecular data for phylogenetic reconstructions in Goodeids (Doadrio and Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008a) and Cyprinids (Domínguez-Domínguez *et al.*, 2007a), and other studies using cladistic biogeography (Domínguez-Domínguez *et. al.*, 2006a; 2008b), a close relationship was found between populations of different co-distributed species of Cuitzeo basin and Zacapu and Lerma River basin, supporting the hypothesis of faunal exchange between Cuitzeo and Zacapu-Lerma, an event that occurred between 1 to 0.5 Ma. These information is also in agreement with geological and paleoenvironmental data (diatomits distribution), which support the existence of a connection between Zacapu and Cuitzeo lakes promoted by the activity of the Northeast-Southwest fault system of the area, which was disrupted by the activity of the Ventanas Volcano, dated at *ca.* 0.7 to 0.5 Ma (Israde-Alcántara, 1999; Moncayo *et al.*, 2001). The historical connection among these areas has been also mentioned by other authors who based their conclusions in the distribution of fishes (e. g. Tamayo and West, 1964; Barbour, 1973; Smith, 1980). Moreover, the populations of *Zoogoneticus quitzeoensis* (Domínguez-Domínguez *et al.*, 2008a) and *Alloophorus robustus* (see below) possess haplotypes that are shared between Cuitzeo and Zacapu-Lerma areas. Instead, the populations of *X. variata* did not share haplotypes between these two areas, furthermore the genetic distance was calculated in $D_{ML}$ = 4.72% and $\bar{D}_p$ = 4.95%, this deep divergences demonstrate that the two populations of *Xenotoca variata* have a long history of isolation (Table 3). This divergence value is high in comparison with general results reported for cyt *b* genetic differentiation between species in vertebrates. Indeed, Avise *et al.* (1998) reported values ranging from 2.2%, 2.4%, 2.6% to 3.1% within bird, mammal, fish and reptile species, respectively, whereas for the Goodeinae a minimum value for uncorrected ‘‘p’’ distances of 1.7% was found for most of the comparisons among species (Doadrio and Domínguez, 2004).

Considering all this information, there is not doubt that a recent connection between these areas (Cuitzeo and Zacapu-Lerma), and the subsequent exchange of fish species between them was expected to occur. In such case, all this data reinforce the idea that forces other than the formation and disappearing of biogeographic barriers such as behavior, ecological segregation, etc., could have influenced *Xenotoca variata* populations after and/or before the isolation event. Behavioral and sexual selection studies in the

Goodeinae and particularly in *X. variata* , support the existence of strong male secondary sexual characters which related to female choice (i.e., terminal yellow band in caudal and dorsal tail, differences in dark patch in flanks, fin size, etc.). It is also largely recognized the role of sexual selection in the Goodeinae and there is substantial interspecific variation in sexual dimorphism and body morphology, with males not capable to inseminate females without female co-operation due to the lack of an intromittent gonopodium (Bisazza, 1997; Moyaho *et al.*, 2004; 2005; Ritchie *et al.*, 2005, Macías-García and Ramirez, 2005). Moreover, substantial phenotypic plasticity and differences in sexual dimorphism between populations, mainly in fin size and body height (which is one of the most important targets in female choice in Goodeids) was found in *X. variata* (Fitzimons, 1972; Macías-García *et al*., 1994, 1998) and in the color of terminal band between populations.

The role of sexual selection in the diversification of different animal groups has been widely recognized, including fish (Ryan *et al.*, 1992; Seehausen and van Alphen, 1999; Korol *et al.*, 2000; Irwin *et al*., 2001; Panhuis *et al.*, 2001). Sexual selection has the potential to produce a rapid divergence between populations and it is predisposed to generate reproductive isolation because of its direct effect on traits involved in mate recognition (Panhuis *et al.*, 2001). However, sexual selection promotes allopatric divergence rather than sympatric (Price, 1998; Via, 2001), but whether these changes lead to speciation, depends on the evolution of reproductive isolation before or after secondary contact (Ryan *et al*., 1992). In such case, although sexual selection might promote speciation, ecological processes are more important in determining the numbers of species that can coexist and, over longer time scales, might obscure the effect of increased speciation rate (Panhuis *et al.*, 2001; Rüber *et al.*, 2003). In Goodeids, a study carried out to test the relationship between sexual dimorphism and speciation rate (Ritchie *et al*. 2005), support the hypothesis of an early expansion of the group, perhaps due to an early radiation influenced by the key innovation of livebearing, or the existence of Miocene volcanism. Comparative analyses failed to demonstrate a significant association between sexual dimorphism and speciation rate, supporting the idea that vicariance biogeography and adaptive radiation are more important than sexual selection in the Goodeinae.

In this case, we propose that an initial vicariant event (see biogeographic section below) separated the ancestor of the two divergent lineages of *X. variata* but while this

event occurred, the strong sexual dimorphism and sexual selection displayed by this species promoted the rapid divergence of sexual trait once populations were isolated. This sexual differentiation prevented the two lineages to be interbreed in a secondary contact (precygotic isolation). This explanation contrasts with other species of goodeids showing an active movement in both directions between Cuitzeo and Zacapu-Lerma area (e. g. *Z. quitzeoensis* and *A. robustus*). Since faunal exchange is largely recognized among these areas in recent geological times (less than 1 Ma) and since *X. variata* is one of the most widespread species of Goodeine and our results shows demographic expansion in all clades within lineage I, we support the idea that the contact between the two lineages after isolation event was likely to occur, but the presence of the two divergent lineages occurring in sympatry was prevented by competition (ecological equivalence). Even though this is just a possible explanation for *X. variata,* other species of freshwater fishes, including goodeines, also exhibit equivalent species or lineages that do not overlap their distributional ranges, in spite that they inhabit the same hydrological system after secondary contact, as is the case of *Zoogoneticus quitzeoensis* (Domínguez-Domínguez *et al.*, 2008a,d), *Notropis calientis* (Domínguez-Dominguez *et al*., 2008e) and the species pair *Skiffia multipunctata-S. lermae* (Doadrio and Domínguez, 2004).

In the case of lineage I within *X. variata*, four clades highly supported and well structured were found (Figs. 2 and 4). This topology follows a basin arrangement, since each lineage is found in one particular river basin (with the exception of Lerma-Panuco populations). These well defined populations are separated from ≈72 (Zacapu-Lerma) to ≈500 km (Aguanaval-Zacapu) of distance between them, and a relative low migration rate was found in the comparisons between clades. Moreover, we found that Tajima's *D* was not significant, but the other demographic parameters (Fu's *Fs* and Fu and Li's D* and F*) were significant (Table 9). Since the Tajima *D* is the most conservative of the demographic parameters, and Fu's $F_S$ has been proved to be the most powerful test to detect population growth (Ramos-Onsins and Rozas, 2002), our data support the population expansion scenario for the entire data set of lineage I. A multimodal MMD was found for lineage I suggesting that the population is subdivided (Fig. 7B), representing the four clades found in the phylogenetic tree and NCA (Fig. 4).

All the clades within lineage I show a star-like phylogeny and network (Figs. 2 and 4), and negative and significant demographic parameters (Fu's *Fs*, Tajima's *D* and Fu and Li's D* and F*)(Table 9), indicating that the different groups analyzed are in mutation–migration–drift genetic disequilibrium with respect to mtDNA alleles, which is expected in a demographic expansion scenario. Instead, the null hypotesis of stationary was not rejected by the raggedness test and $R^2$ statistics in most of the clades (Table 9). This discrepancies can be attributed to the low power of mismatch distribution based tests, compared with methods based on the mutation frequency (e.g. Tajima's *D* and D* and F*) or haplotype distribution (e.g. Fu's $F_S$), as was previously explained for the Fu's $F_S$ statistics (Ramos-Onsins and Rozas, 2002). Moreover, the lineage I and the four clades analyzed display relative high haplotypic diversity with respect to the low levels of nucleotide diversity (Table 2). Accumulation of haplotypes suggests that the clades experienced bottlenecks during their origins, followed by major population demographic expansion (Grant and Bowen, 1998; Avise, 2000). In general, the observed levels of variability in most of the tested groups should indicate a recent population expansion, as shown by the BSP analysis (Fig. 6A), and suggest the persistence of large populations after expansion, as it is corroborated in our effective population size results, without significant bottlenecks. This information supports the findings of high genetic diversity in *Zoogoneticus quitzeoensis* (Domínguez-Domínguez *et al.*, 2007a; 2008a) and the expansion scenario found for this species in some of the basins studied herein.

Although this scenario applies in general for all the clades and most of the populations, the low intrapopulation mean genetic distances within clade 2.3 (Aguanaval populations) and 2.7 (Chapala population) could indicate strong bottleneck effect (Table 3), as the origin of those populations through a small number of founders (Wang *et al.*, 1999; Wang *et al.*, 2004) or by a more recent expansion. In the other hand, the low genetic diversity found in some populations (e.g. San Francisco del Rincon, Zacatecas, Chapala population) suggests a small population size or recurrent bottleneck after expansion.

*Alloophorus robustus.*

The whole data resulted in two lineages, but in this case, due to the low sample size for lineage II and the low support found for the two lineage relationship in the phylogenetic studies, we will discuss just the structure and demographic history uncovered in the two

clades within lineage I (Figs. 3 and 5). This species show no structure when river drainages are considered, and exhibit a different pattern with respect to *X. variata* (Table 8). One clade (Clade 3.1) is represented by the populations from Patzcuaro, Zirahuen, Cuitzeo and Zacapu basins, whereas the other clade (clade 2.1) is composed by populations distributed in the Middle and Lower Lerma, Chapala, Balsas and San Juanico basins. No internal genetic structure was found for both lineages (Fig. 5; Table 7).

Populations within lineage I show a very low genetic diversity values (Table 2)(except the Patzcuaro population belonging to clade 3.1) irrespective of the high migration rate found for populations within clades. The Fu's *Fs* and Tajima's *D* for the whole data set and for all samples within lineage I were negative and non-significant, whereas the Fu and Li's F* and D* statistic were negative and significant (Table 9). According to Frankham *et al.* (2002), the genetic diversity and differentiation in populations depend on migration (gene flow), breeding system, current and historical *Ne* (demographic events), and mutation rate.

Although the biological, ecological and ethological studies within this species are very scarce, available data reveal that this species is in the top of the food chain since is a piscivorous species, with a low mean production of offspring with respect to other goodeids and one brood per year while in other goodeids there is more than one brood per year (Mendoza; 1962; Moncayo-Estrada, 1996; Salazar-Tinoco, 2007), low ecological effective population size (Moncayo-Estrada. 1996), high sensitivity to environmental degradation (Mercado-Silva *et al.*, 2002, 2006), no apparent secondary sexual dimorphic characters (i.e. terminal yellow band) and low sexual dimorphism score (Macías-García and Ramirez, 2005; Ritchie *et al.*, 2005). In the case of *A. robustus*, we consider that aspects of ecology and life history are affecting the differences in the neutrality test. Even though phylogeographical data sets are not appropriate to estimate *Ne* because they cannot be considered as a single population from an extended temporal perspective, in a phylogeographical context, *Ne* does not depend on the current intrinsic diversity but rather on the species demographic history on evolutionary time (Avise, 2000). In such case, the small effective population size (*Ne*) found in our study (at least eleven orders of magnitude lees with respect to *X. variata*), as is expected for a species situated in the top of the food

web, and the very low genetic diversity may affect the neutrality test, and sexual selection seems not to play an important role as in *X. variata*.

The lack of structure within clades could be explained by the high migrtaion rate between populations, which is corroborated by the $\Phi_{ST}$ values (Table 7). *Alloophorus robustus* is one of the largest species of Goodeines, and this may influence its high migration rate with respect to *X. variata*, thus promoting the homogenization of the population and the lack of structure within clades. In contrast, a relative very low migration rate and lack of mixed haplotipes among clades was found (Fig. 3), even though the Zacapu area (clade 3.1) and Lerma River System (clade 2.1) are now part of the same hydrological system. These results can be explained by the formation of a physical barrier between Zacapu and Lerma System (see below) that prevent the current migration from Lerma River System to the Zacapu Lake area. Results on migration support this idea since only a unidirectional migration rate was found, showing a low value only from Zacapu to the Lerma river populations, which was also found in *X. variata* (not shown).

When clade 3.1 and calde 2.1 were tested independently, the analysis suggests two distinct demographic histories. The diversity values *Hd* were similar for both clades, whereas the $\pi$ was much lower for clade 2.1 than clade 3.1, and, although these two clades have similar mismatch distributions (Table 2; Figs. 8B and C), the neutrality test was rejected only for Clade 2.1 (Table 9). The TMRCA indicate that both clades have evolved in a similar time frame, approximately 1.9 Ma for clade 3.1 and 1.8 Ma for clade 2.1 (Table 9), indicating that differences among clades are not related with time after origin.

Although mismatch distributions generated by sudden expansion or by long bottlenecks are virtually indistinguishable (Rogers and Harpending, 1992), the unimodal MMD for clade 2.1, and the low nucleotide diversity, combined with the fact that statistical tests of neutrality provide strong support for non expansion scenario in this clade, indicate that this clade has been affected by a long term and recurrent bottleneck. Additionally, gene diversity could be maintained by a high migration rate between populations. This information corroborate the findings made in another species of goodeid, *Zoogoneticus quitzeoensis*, where the populations for the Lower-Lerma and Chapala regions show a low genetic diversity as a result of bottlenecks, significant inbreeding, low effective population size, and genetic drift (Domínguez-Domínguez *et al.*, 2007a, 2008a).

For the clade 3.1 all the neutrality statistics shows negative but significant values, supporting the expansion scenario (Table 9). Although gene diversity is slightly lower than in clade 2.1, nucleotide diversity is higher indicating a more stable population trough time (Table 2). But this information need to be take with caution, since all the populations from Cuitzeo and Zacapu Lake basins exhibit low number of haplotypes and low genetic diversity, and only the Patzcuaro populations show high levels of genetic diversity. These results could be related with more stable environmental conditions of Patzcuaro lake since it was originated. Apparently this lake has not experimented severe dry conditions in its recent history (Bradbury, 2000), and this stability could support a high effective population size through time avoiding bottleneck effects. Likewise, the low diversity found in Cuitzeo Lake (three haplotypes) and Zacapu Lake populations (one haplotype) may result fro two independent processes: A recent establishment of the populations of Zacapu and Cuitzeo lakes by a very low number of founders from Patzcuaro Lake (see Fig. 5), or a high reduction of the population and a long term an recurrent bottleneck effects. The expansion and founder event of Cuitzeo and Zacapu areas from Patzcuaro population seems to be supported by the population expansion scenario give by the neutrality statistics (Table 9). Instead, a considerable instability of Cuitzeo Lake has been reported trough time, and the evolution of the lacustrine zone indicate repeated episodes of high contractions and expansions in the last 5.5 Ma (Isrdae-Alcantara and Garduño-Monrroy, 1999), with the same fluctuations reported during the later Pliocene-Holocene (Israde-Alcántara *et al.,* 2000; Ortega *et al.,* 2002). It has even postulated that this lake was totally dry during geological times, and apparently was completely drained in 1941 by human causes (De Buen, 1943). Although no long term record of paleoenvironmental conditions are reported, information for Late Pliocene-Holocene indicates similar patterns of instability and contraction-expansion events in Zacapu (Ortega *et al.,* 2002). Moreover, the lacustrine area was dramatically drained from a original marshy area of 15,000 ha to only 32 ha in 1907 for agricultural purposes (Guzmán, 1985). In this case, the two scenarios could be feasible, the extinction or very high reduction of the populations of *A. robustus* from Cuitzeo-Zacapu area, and a second colonization of founders from Patzcuaro to Cuitzeo-Zacapu after formation of Patzcuaro and isolation of its populations (see below). This migration may have occurred throughout the ancient connection (around 38,000 to 25,000 yr BP), as is

also supported by the BSP results (Fig. 6C), however, this recent colonization is obscured by the absence of shared haplotypes between Patzcuaro and Cuitzeo-Zacapu populations (Fig. 3). The other possibility is that this second colonization event never occurred, and in such case the low genetic diversity in Cuitzeo-Zacapu populations could have resulted from recurrent bottlenecks due the instability of the lacustrine area in both lakes, and the reduction or dry events that occurred in the last century. This bottleneck could have a stronger effect in this species, since a small effective population size is expected for a piscivorous species, as was corroborated by our $\theta_1$ values. This information is supported by the low levels of genetic diversity found in *Zoogoneticus quitzeoensis* for the Zacapu population and some Cuitzeo populations, in which a low genetic diversity, bottleneck effects and inbreeding coefficient are reported for nuclear and mtDNA data (Domínguez-Domínguez *et al.*, 2007a, 2008a).

***Recent demographic history***

According with the BSP results, both of the species of goodeines considered in this study exhibit a recent demographic history. Our results support an important population growth in the last 60,000, larger in *X. variata* than in *A. robustus* (Fig. 6) as it could be expected according with their intrinsic biological characteristics. Population growth (that not necessarily means geographic expansion) for lineage I in *X. varaiata* (Aguanaval, Zacapu, Lerma-Panuco and Chapala populations) seems to have occurred around 65,000 yr BP, increasing exponentially around 55,000 yr BP. According to paleoenvironmental data, a wet period and the most humid conditions were found from 52 000 yr BP to *ca.* 39 000 yr BP in Zacapu Lake (Ortega *et al.,* 2002), which is approximately the period inferred for the BSP for the population stability. These humid period in the Zacapu Lake area has been also documented to occur in other lakes of central Mexico, as the Chalco Lake and Cuitzeo Lake whose higher water level was found at *ca*. 35,000 yr BP (Caballero and Ortega, 1998), and *ca.* 40,000 yr BP, respectively (Israde-Alcántara *et al.,* 2002). Lineage II for *X. variata* (Cuitzeo populations) documented some population growth during the last 60,000 Yr BP, but this growth seem to have been more intense between 65,000 and 35,000 yr BP, and this is congruent with the paleoenvironmental data. In addition to that, an expansion event also occurred in *A. robustus* in the last 40,000 yr BP, reaching a stability in around 10,000 (Fig. 6). This data seems to be contradictory, since most of the lakes around Central Mexico

show a dry and shallow period after 30,000 yr BP. In this case however, the BSP might have been affected by the Patzcuaro population which is the most diverse (representing ≈ 42% of the total haplotypes) and differentiated population (Tables 2 and 7). In this case, the apparent incongruence in expansion time between *X. variata* and *A. robustus* is a result of particular conditions found in Patzcuaro Lake. Paleoenvironmental records indicate that apparently this lake was the only in Central Mexico experiencing moist conditions through the last glacial periods, with the highest water level occurring between *ca.* 34 000 and 21 000 yr BP, and drier conditions found between *ca.* 13 000 and 9000 yr BP (Bradbury, 2000).

***Biogeographical scenario***

Being aware of possible mistakes due to limitations derived from the use of the molecular clock as an exact time of divergence, the use of a single gene for closely allied taxa, although the implementation of a relaxed molecular clock in a coalescent context, even with high confidence intervals (95% HPD), estimations can be inexact and must be interpreted judiciously (Hillis *et al.,* 1996; Graur and Martin, 2004). We believe that the evolutionary history of both species is related not only to geological or climatic events, but also to other forces that influenced the isolation of populations after secondary contact (e.g. ecological segregation or sexual selection) as mentioned previouisly. Our results are in agreement with diversification processes experienced by other fish taxa in Central Mexico (Barbour, 1973; Smith, 1980; Miller and Smith, 1986; Mateos *et al.,* 2002; Gesundheit and Macías, 2003; Mateos, 2005; Domínguez-Domínguez *et al.*, 2006a, 2008a,b). Next, we propose a possible biogeographic scenario for both species, discussing the link between the cladogenetic events, the time and space where they occurred and the geological and climatic data available for the region where these species are distributed.

Our data reveal that the ancestral lineage of both species of goodeines, *Alloophorus robustus* and *Xenotoca variata*, seems to have been originated in the Cuitzeo-Patzcuaro lakes area, with a first diversification event occurring at *ca.* 7 Ma (95% HDP = 4.71-9.64) for *X. variata* and *ca.* 5.2 Ma (95% HDP = 1.2-11) for *Alloophorus robustus*. The time for the cladogenetic event for *X. variata*, in the context of the root height estimated time, and the allopatric fragmentation suggested by the nested design, is in agreement with a vicariant event that produced the formation of Cuitzeo Lake around *ca* 8 Ma (Israde-Alcántara,

1999), and the evolution of ancient lacustrine zone which has evolved under an intense fault activity, associated with different episodes of lacustrine regression-transgressions and changes in water levels, as a result of tectonic activity at the late Miocene and early Pliocene, having it mayor extension and deepness between Late Miocene-Early Pleistocene (Israde-Alcántara and Graduño-Monroy, 1999; Silva-Romo *et al.*, 2002). The increase in extension and deepness of Cuitzeo Lake could have been also related with the humid conditions in the Early Pleistocene (*ca.* 5.2 a 3.6 Ma), a period related with the increase of oceanic levels and warmer temperatures in the sea surface and the increase of rain in a continental scale (Israde-Alcántara *et al.*, 2008). These episodes of tectonism and climatic change might have affected the cladogenesis of *A. robustus* and the two main lineages within *Xenotoca*, promoting episodes of connection and isolation among basins and the fish species inhabiting them.

Other events related with a possible expansion of lineages and posterior isolation seems to have occurred through two episodes of climatic change and tectonic activity in the area. One is the TMRCA for the two clades (3.1and 2.1) within *Alloophorus robustus* at *ca.* 1.8-1.9 Ma. This episode is related with a dry period associated with the development of lacustrine faces, and low deep water levels that occurred around *ca.* 3.5 a 1.8 Ma (Israde-Alcántara *et al.*, 2008). Apparently during this period the activity of the so-called Tarascan Corridor was also intense and this could have promoted the isolation of the two main clades within *Alloophorus*, but also they may have influenced the populations of *Xenotoca variata* throughout the formation of a biogeographical barrier in the limits of Zacapu and Lerma River basins. In this case, the presence of a waterfall of around 6 m high is documented to have been formed in the Angulo river, in the mid way between Zacapu Lake and Lerma River, although there is no data on the time where this water fall was formed. This water fall could represents the explanation of the unidirectional migration rate described from Zacapu Lake to the Lerma-Chapala river basin populations.

Within lineage I of *Xenotoca variata*, clades showed similar results for the TMRCA, with a mean value ranging from 1.1 to 0.8 Ma. This indicates that all cades were affected at the same time responding to the same event dated around 1 Ma, which is in accordance with the estimate time when Goodeids experienced their highest diversification during a dry period that caused isolation by basin fragmentation in the Mesa Central of Mexico (Doadrio

and Domínguez, 2004; Domínguez-Domínguez *et al.*, 2008b), but also corresponding to diversification in other clades of freshwater fishes such as Cyprinids (Domínguez-Domínguez *et al.*, 2007b), Characids (Strecker *et al.*, 2004) and Poecilids (Mateos *et al.*, 2002). The AMOVA results also support this interpretation (Table 8); test for phylogenetic arrangements maximize the percentage of the variance explained among groups, indicating that the actual configuration of the drainages (biogeographic arrangement) does not explain the diversification and divergences in this lineage, also the NCA results (Table 5) suggest a long distance colonization coupled with subsequent fragmentation, as was expected for a common influence of wet period that promote the interchange of fauna at the same time and subsequent fragmentation induced for dry periods and disappearing of those connections.

For *Alloophorus robustus* three caldes show a statistic association between genetic distances and geographic distance. For the case of clade 3.1 (Fig. 5), the NCA was not able to discriminate between long-distance movements of the organism and the combined effects of gradual movement during a past range expansion and fragmentation, other result, as the neutrality test and BSP support the expansion model, the $\Phi_{ST}$ results for Patzcuaro population, the genetic diversity indices and the arrangement in the NCA indicate that the past range expansion and fragmentation is the most plausible scenario.

Two populations (Uruapan and San Juanico) showed unespected results. Although population of Uruapan contained only three samples and this may have biased the demographic interpretation, the fact that these samples corresponded to a single haplotype that is shared between La Luz and La Alberca samples, suggest a strong founder effect and a very recent connection between Uruapan area, in the Balsas Basin, and Chapala-Lower Lerma basins. No relationship between populations or species inhabiting Uruapan and Lower Lerma-Chapala have been established, moreover, the only sampled incorporated to the analysis from Zirahuen area belong to the clade 3.1, which also include populations from Patzcuaro and Cuitzeo basins. The only established connection of the Uruapan area with drainages of the Mesa Central is a recent connection (less than 1 Ma) between Zirahuen-Patzcuaro-Cuitzeo and Uruapan in the basis of the close relationship between the species *Allotoca diazi* (endemic to Patzcuaro), *A. meeki* (endemic to Zirahuen) and *A. catarinae* (apparently inhabiting Uruapan and Cuitzeo basin). Accordingly, we avoid to propose any biogeographic scenario for this statistical significant result of the NCA.

In the case of the San Juanico population, only one haplotype out of the 10 samples was found and was separated only by one mutational step with respect to the other members within the clade 2.1, indicating a recent formation of this population throughout a small number of individuals.

Three picks of tectonic activity of the Cotija graben have been documented; tectonic activity is proposed as the main cause of the isolation of the ancient San Juanico lake from the Chapala Paleolake, and this event is dated between *ca.* 9 to 3.8 Ma (Rosas-Elguera *et al.*, 2002, 2003). Not strong inference of this biogeographic scenario could be propose, although a recent activity in this graben and the impact that this could have in the geomorphology of the region has been also documented (Rosas-Elguera *et al.*, 2002, 2003), in such case, this activity could be favored the river piracy in the area and promote the unidirectional interchange of fauna, explaining the possible founder effect found in this populations and the contiguous range expansion inferred for the inference key for the NCA for clade 2.1. Other fish species (or their populations) between the Chapala-Lerma river basin and San Juanico support these connection, as was proposed for the first time by Álvarez del Villar (1963). This author mentioned that several species inhabiting the San Juanico Lake were related with the freshwater fish fauna of the Lerma River, such as *Poeciliopsis infans, Ictalurus dugesii*, *Chirostoma reseratum* and *C. melanocus*, however, molecular data for these species and populations is lacking.

***Implications for conservation***

The recognition of conservation units under the species level is a crucial task if we intend to avoid the loss of genetic diversity in species inhabiting threatened environments, such as the freshwater ecosystem of Central Mexico. Conservation biologists, however, have not agreed on the precise terms to be used in characterizing genetic variation. One widely used framework to distinguish units for conservation purposes has been that of evolutionary significant units (ESU), originally proposed by Ryder (1986), but further developed by different authors, and now the concept of ESU has several working definitions (e.g., Waples, 1991; Avise, 1994; Moritz, 1994a; Vogler and DeSalle, 1994, see Fraser and Bernatchez, 2001 for a review). However, the identification (and subsequent management) of distinct evolutionary significant units

(Moritz, 1994b) within species is far from simple, with emphasis being given to adaptive phenotypic diversity and/or historical isolation processes (reviewed in Moritz, 2002). Crandall *et al.* (2000) argued that the increased use of molecular data to determine ESUs excluding of other sources of information has reduced the relevancy of ESUs for conservation. For the conservation of freshwater fish diversity, Doadrio *et al.* (1996) introduced the operation conservation unit (OCU) concept, which is defined as "a continuous area limited by geographical boundaries and inhabited by one or more populations sharing the same genetic pattern". We are aware of the potential effects of evolutionary stochasticity and of sampling limitations in defining conservation units based exclusively on mtDNA, which is also maternally inherited and therefore does not reflect male-mediated gene flow (Moritz, 1994b; Avise, 1995). Although these limitation, comparative phylogeography has the potential to provide insights into the patterns and processes that determine and maintain species' distributions, and hence are informative for conservation decisions. Data provided herein support conservation management decisions by analyzing the pattern of geographical distribution of genetic variability within populations and the differentiation among populations. In order to optimize the management and conservation strategies in *X. variata* and *A. robustus*, it is necessary to identify distinct population units. Accordingly, next we include genetic and biogeographic information, in addition to ecological and biological data adopting the OCU's concept to propose particular populations to be preserved. We also incorporate to the OCUs concept the criteria of phylogeographical differentiation and reciprocal monophyly using to delineate ESU's, as well as the observed significant values of ΦST and AMOVA results.

The mtDNA data presented here suggest significant geographical structure of genetic variation across the range of *X. variata*, and the phylogenetic and phylogeographic analyses point out to a long-term historical isolation among the five geographical areas where this species occur. In such case, five main OCU's should be considered for *X. variata*: Zacapu, Lerma-Panuco, Chapala, Aguanaval and Cuitzeo. On the other hand, three genetically structured OCU's were found for A. *robustus*, corresponding to San Francisco del Rincon, Cuitzeo-Patzcuaro-Zacapu-Zirahuen, and Chapala-Cotija-Lower Lerma-Middle Lerma- Uruapan.

Accordingly, the management of Central Mexico freshwater ecosystems requires a great amount in information from organisms before decisions are taken. For instance, data provided in this study show that *Xenotoca variata* exhibit a strong differentiation between basins and that the conservation efforts should be addressed by considering populations in river drainages. Instead, for *A. robustus* dispersal among drainages seems to be important to maintain the genetic diversity and long-term subsistence of the species. The freshwater ecosystems in Central Mexico have been significantly affected by habitat degradation, introduction of alien species, desiccation of water bodies, drainage deforestation, overharvesting and water diversion (Soto-Galera *et al.,* 1998; Lyons *et al.*, 1998; Zambrano *et al.,* 1999; de la Vega-Salazar *et al.,* 2003; Domínguez-Domínguez *et al.,* 2005, 2007a, 2008c; Mercado-Silva *et al.,* 2006). In this sense, some species have drastically reduced its populations, and in some cases they were now extirpated from some areas. Distributional range of *A. robustus* has decreased by comparing historical points of occurrence with respect to new data on field samplings and this indicated that this carnivore is a sensitive species whereas *X. variata* is a widespread species that, although affected for anthropogenic activities, it is found in several localities along its distributional range and just a small reduction in occurrence points and basins has been reported (Soto-Galera *et al.,* 1998, 1999; Domínguez-Domínguez *et al.,* 2005, 2006a, 2008c; Mercado-Silva *et al.,* 2006), as is expected in a omnivorous and tolerant species. Although the patterns of genetic diversity found in the present study may not be related with present drainage degradation, and even though the use of other molecular markers are needed in order to elucidate the influences of present *vs* historical causes (e. g. microsatellites), the effects on the distribution is also evidenced by the diversity indices for both species, with *X. variata* having a considerable higher diversity than *A. robustus*.

For *Xenotoca variata,* the northern OCU identified in this study (Aguanaval populations), is distributed in arid environments, which are subjected to drastically seasonal changes. Moreover, this species cannot survive in temporal rivers of the area, and the two populations are maintained only in two small spring (lees that 5 $m^2$), one of which was found completely drained in a field trip conducted in 2005. Clearly, these populations show a low genetic variability and as a result, the maintenance of the water bodies and the permanence of springs where these populations inhabit is imperative for the long-term

survival of the population. Other identified OCU that maintain a low genetic diversity is represented by the Chapala populations. Even though these populations are only found in La Alberca, within the Chapala basin, these location maintain a good water quality and is large enough to maintain long term populations of these species, but the introduction of exotics such as *Oreochromis* spp, *Lepomis macrochirus, Micropterus salmoides* and *Cyprinus carpio,* could represent a threat to the survival of these population.

The genetic diversity at the mtDNA level of Zacapu population shown the highest genetic diversity indices, but these results need to be taken with caution, the Zacapu lake was drained in more than 99% of the original marshy area drained for agricultural purposes, and despite of the mtDNA not reveal this information, the use of other makers (as microsatellites) need to be carried out in order to know the effect of these desiccation in the genetic richness of these species. The genetic diversity found in this population is comparable to other studies conduct with the goodeid *Zoogoneticus quitzeoensis,* founding a high genetic diversity at mtDNA, but a low genetic indices, bottleneck effect and inbreeding at microstallites loci (Domínguez-Domínguez *et al.*, 2007a, 2008a), in these sense, our study support the status of Operational Conservation Unit of Zacapu as recommended by Domínguez-Domínguez *et al.*, (2007a), but recommend the use of faster markers in order to elucidate the genetic impact of a recent basin desiccation.

The Cuitzeo populations are the most differentiated within the *X. variata.* Accordingly, it represents an important OCU to maintain the genetic variability of the species as a whole, giving to these population an important role for a conservation purpose. This basin maintains an important number of historical populations of these species and is one of the most widespread species in the basin (Soto-Galera *et al.,* 1999; Domínguez-Domínguez *et al.,* 2008c). At this moment, this OCU seems not to be endangered, although the increase of water pollution, desertification level in the basin, and the introduction of alien species may represent a threat to this population in a near future.

Finally the Panuco-Lerma OCU maintain its presence in a important number of historical localities within these basins (Mercado-Silva *et al.,* 2006; Domínguez-Domínguez *et al.,* 2005, 2008c), although the permanence of this populations in both drainages apparently not suffer drastically reduction, the Lerma River is considered one of the most polluted river systems in the world (Soto-Galera *et al.,* 1998) and the upper

tributaries of the Panuco River receive the waste water of the highly populated City of Mexico, so the permanence of these population in these drainages need to be monitored in order to detect any negative change induced by the high environmental degradation.

*Alloophoporus robustus* provide a different scenario, the very low genetic diversity in all the studied populations (except for Patzcuaro population) shows a pessimistic scenario for this species. Moreover, our results evidenced that the genetic exchange and migration rate shown by the populations within clade are more important to maintain the genetic diversity of this species, accordingly, the long-term conservation genetics of at least two of the OCU's identified here seem to be more related with the permanence of the migration route between populations, which mean the maintenance of the water and habitat quality of rivers and streams that connected these localities. This seems to be a hard and very difficult task due the low tolerance of this species to environmental degradation, the feeding habits (carnivorous) that make it more sensitive to web chain alterations, and the level of degradation of water systems in Central Mexico. In this case it is not only important to maintain the habitat quality in the areas where the species still exist, maintaining the genetic diversity as in Patzcuaro Lake. Ecological restoration of these areas is urgent, and furthermore the restoration of corridor areas, as rivers and streams connecting basins should be the main effort for the conservation of these species.

**CONCLUSIONS**

This study demonstrates that the dynamic of genesis and destruction of the Mesa Central drainages, induced by the high tectonic and volcanic activity since the Miocene, coupled with the climatic change events, produced a complex pattern in the evolutionary and biogeographic history of the fresh water fish fauna of the Mesa Central of Mexico, promoting early diversification within the populations. But, our phylogeographic results show that *Xenotoca variata* and *Alloophorus robustus* not share their evolutionary history within the same geographic area. We argued that some of these differences in the evolutionary history of these two closely related species within the same geological and climatic complex area, could be affected not only by the effects of geological or climatic events, and other influences, as sexual selection, ecological egregation and biological and ecological intrinsic characteristics of each species could be affect the evolutionary history of these species after the early diversification promoted by events of geographic isolation.

These differences in populations subdivision and in the genetic variability within each species have strong implication for the management of Central Mexico freshwater ecosystems, and point out the importance to compare different species to make better decisions of conservation of these diverse area, not only focusing in the species level or within basin conservation, also taking in consideration genetic and ecological subdivision within species and between drainage and populations connectivity.

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**Figure 1.** Sample locations for: black, *Xenotoca variata;* red, *Alloophorus robustus*; and blue, both species. Numbers are related with table 1 locality: 1) Tierra Quemada Stream, 2) Jesus Maria Stream, 3) La Alberca Lake, 4) La Luz Spring, 5) Patzcuaro Lake, 6) Zirahuen Lake at Tembucharo, 7) San Francisco del Rincon Spring, 8) Yuriria Dam, 9) Ignacio Allende Dam, 10) Cuitzeo Lake at Belisario, 11) San Cristobal Spring, 12) La Mintzita Spring, 13) Cuitzeo Lake at Salvador Escalante, 14) Santa Maria Stream, 15) Outflow Stream of f Zacapu Lake, 16) Spring nearZacatecas, 17) La Valenciana Spring, 18) San Juanico Dam, 19) Stream at Uruapan Urban Park. Each biogeographic discrete region appears in different color in the map.

**Figure 2.** Neighbor-joining phylogram of composite Cyt b haplotypes of X. variata constructed using the ML distances. Values above branches represents BI, ML and NJ branch support. The name of the haplotypes is related with the name in the network (Fig4). In parenthesis is the number of individuals per population within the haplotype, abbreviations are refereed in table 1.

**Figure 3.** Neighbor-joining phylogram of composite Cyt b haplotypes of *Alloophorus robustus* constructed using the ML distances. Values above branches represents BI, ML and NJ branch support. The name of the haplotypes is related with the name in the network (Fig4). In parenthesis is the number of individuals per population within the haplotype, abbreviations are refereed in table 1.

**Figure 4.-** Statistical Parsimony haplotype network (95%) and nesting design for cyt *b* haplotypes of *Xenotoca variata.* The 44 mutation steps between clades 3.4 and 4.1 exceeded the maximum number of mutational connections justified by the 95% parsimony criterion (13 mutational steps). Stars represent the haplotype were the two clades were connected, the haplotype with the largest outgroup probability of the whole network was haplotype CUI 16. Open cicles represent inferred, undetected internal haplotypes, each line represents one mutation change. Number inside the boxes represents the step-clade in the nested design.

**Figure 5.-** Statistical Parsimony haplotype network (95%) and nesting design for cyt *b* haplotypes of *Alloophorus robustus.* The haplotype with the largest outgroup probability of the whole network was haplotype PAT 4. Open circles represent inferred, undetected

internal haplotypes, each line represents one mutation change. Number inside the boxes represents the step-clade in the nested design.

**Figure 6.** Bayesian skiline plots for *Xenotoca variata*: A) lineage I; B) lineage II and *Alloophorus robustus*: C) All data set.

**Figure 7.** MMD for the different lineages and clades identified in the NCA. For *Xenotoca variata* A) all groups together; B) Lineage I; C) Lineage II (Cuitzeo population); D) Zacapu clade; E) Aguanaval clade; F) Lerma-Panuco clade; G) Chapala clade.

**Figure 8.** MMD for the all data set (A) and the two clades identified in the NCA for *Alloophorus robustus*; B) clade 3.1; C) clade 2.1.

Table 1. List of sampled localities with geographic coordinates and number of individuals analyzed for each species. The numbers in locality refers to umbers in Fig. 1.

| Discrete region | Locality (abbreviation) | *N* | Coordinates | GenBank Acc |
|---|---|---|---|---|
| Panuco | 1 Tierra Quemada (Tqe) | 8/-- | 21º42’53.2” N 100º41’13.7” W | |
| | 2 Jesus Maria (JMa) | 12/-- | 21º55’30.5” N 100º53’57.6” W | |
| Chapala Lake | 3 La Alberca (Alb) | 19/6 | 20º03’32.9” N 102º36’33.1” W | |
| Lower Lerma River | 4 La Luz (Luz) | --/14 | 19º56’08” N 102º18’02.1 W | |
| Patzcuaro Lake | 5 Patzcuaro (Pat) | --/14 | 19°37’59.7” N 101°37’58.8” W | |
| Zirahuen Lake | 6 Tembucharo (Zir) | --/1 | 19°26’16.5” N 101°44’31.8” W | |
| Middle Lerma River | 7 San Francisco del Rincon (SFR) | 6/4 | 21º02’ 47.3” N 101º50’9.3” W | |
| | 8 Yuriria (Yur) | 9/-- | 20°14’13.6” N 101°09’25.5” W | |
| | 9 Ignacio Allende Dam (IAD) | 8/1 | 20°53’11.4” N 100°47’24.2” W | |
| Cuitzeo Lake | 10 Belisario (Bel) | 11/-- | 19º53’42.41” N 101º04’16.8” W | |
| | 11 San Cristóbal (SCr) | 8/9 | 19º57’42” N 101º18’6” W | |
| | 12 La Mintzita (Min) | 10/10 | 19º38’40” N 101º16’28” W | |
| | 13 Salvador Escalante (SEs) | 7/-- | 19º57’57.4” N 101º03’19.2” W | |
| | 14 Santa María (SMa) | --/8 | 19º47'25.4" N 101º13'39.6" W | |
| Zacapu Lake | 15 Zacapu (Zac) | 10/15 | 19º49'35" N 101º47'10" W | |
| Aguanaval | 16 Zacatecas (Zat) | 6/-- | 23º33'33.8" N 103º15'50.3" W | |
| | 17 Valenciana | 14/-- | 24º10'25.3" N | |

| | | | |
|---|---|---|---|
| | (Val) | | 103º17'33.5" W |
| San Juanico Dam | 18 San Juanico | --/10 | 19º50'10.5" N |
| | (SJD) | | 102º40'10" W |
| Balsas-Cupatitzio | 19 Uruapan Urban Park | --/3 | 19º23'23.5" N |
| | (Uru) | | 102º00'52.4" W |

*N* = Number of samples (for *Xenotoca variata* / *Alloophorus robustus*).

Table 2. Genetic diversity and descriptive statistics of studied populations of *Xenotoca variata* and *Alloophorus robustus*.

| Basin (clade) | Population | *N* | *Hn* | *S* | $H_d$ ± SD | π ± SD | *K* |
|---|---|---|---|---|---|---|---|
| ***Xenotoca variata*** | | | | | | | |
| | All populations | 128 | 53 | 118 | 0.912±0.014 | 0.02495±0.00141 | 24.95 ±11.02 |
| Lineage I | | 92 | 36 | 58 | 0.865±0.024 | 0.00792±0.00044 | 7.919±0.049 |
| Zacapu (clade 2.1) | Zacapu | 10 | 8 | 8 | 0.933±0.077 | 0.00160±0.00032 | 1.6±1.033493 |
| Lerma-Panuco (clade 3.2) | | 43 | 17 | 24 | 0.611±0.090 | 0.00112±0.00025 | 1.1±0.860 |
| Panuco | | 20 | 9 | 12 | 0.653±0.122 | 0.00120±0.00039 | 1.168±0.81 |
| | Tierra Quemada | 8 | 4 | 4 | 0.643±0.184 | 0.0010±0.00085 | 1.0±0.7480 |
| | Jesus Maria | 12 | 6 | 8 | 0.682±0.1482 | 0.00133±0.0010 | 1.33±0.888 |
| Middle Lerma | | 23 | 9 | 12 | 0.585±0.122 | 0.00104±0.00030 | 1.0434 +/-0.72 |
| | Yuriria | 9 | 5 | 5 | 0.722±0.159 | 0.0011±0.00090 | 1.11±0.796 |
| | Ignacio Allende Dam | 8 | 4 | 5 | 0.643±0.184 | 0.00125±0.00047 | 1.2±0.6212 |
| | San Francisco del Rincon | 6 | 2 | 2 | 0.333±0.2152 | 0.000667±0.00068 | 0.666±0.5868 |
| Chapala (clade 2.7) | La Alberca | 19 | 5 | 5 | 0.386±0.139 | 0.00053±0.00023 | 0.5263 +/-0.46 |
| Zacatecas (clade 2.3) | | 20 | 6 | 5 | 0.447±0.137 | 0.00050±0.00017 | 0.852 +/-0.534 |
| | Valenciana | 14 | 5 | 4 | 0.5055±0.1581 | 0.00057±0.00055 | 0.5714±0.4917 |
| | Zacatecas | 6 | 2 | 1 | 0.333±0.2152 | 0.00033±0.00044 | 0.333±0.30 |
| Cuitzeo (Lineage II) | | 36 | 17 | 24 | 0.754±0.079 | 0.00201±0.00038 | 2.00±1.159 |
| | Belisario | 11 | 6 | 11 | 0.7273±0.144 | 0.002±0.00137 | 2.0±1.2188 |
| | San Cristobal | 8 | 5 | 7 | 0.7857±0.1508 | 0.00229±0.00159 | 2.286±1.398 |
| | La Mintzita | 10 | 6 | 7 | 0.844±0.1029 | 0.001711±0.00123 | 1.711±1.088 |
| | Salvador Escalante | 7 | 3 | 5 | 0.667±0.16 | 0.0029±0.00058 | 2.0±1.276 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Alloophorus robustus*** | | | | | | | |
| | All populations | 95 | 21 | 37 | 0.770±0.035 | 0.00585±0.00312 | 5.852±2.821 |
| Lineage I | | 91 | 18 | 30 | 0.749±0.037 | 0.00549±0.00027 | 5.492±0.24 |
| Clade 3.1 | | 57 | 12 | 15 | 0.504±0.080 | 0.001367±0.00095 | 1.367±0.8565 |
| Patzcuaro | Patzcuaro | 14 | 9 | 12 | 0.9011±0.0624 | 0.00213±0.001415 | 2.1318±1.2604 |
| Zacapu | Zacapu | 15 | 1 | 0 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| Cuitzeo | | 28 | 4 | 13 | 0.2063±0.1005 | 0.00093±0.00073 | 0.9285±0.6594 |
| | San Cristobal | 10 | 2 | 2 | 0.200±0.1541 | 0.00045±0.0004 | 0.44±0.40 |
| | La Mintzita | 11 | 3 | 11 | 0.3455±0.1722 | 0.0020±0.001375 | 2.00±1.2189 |
| | Santa Maria | 7 | 1 | 0 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| Clade 2.1 | | 34 | 6 | 8 | 0.581±0.070 | 0.00084+/-0.0006 | 0.8395 +/-0.612 |
| Chapala | La Alberca | 6 | 2 | 2 | 0.333±0.2152 | 0.00067±0.00066 | 0.666±0.5668 |
| San Juanico Dam | San Juanico Dam | 10 | 1 | 0 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| Balsas | Uruapan Urban Park | 3 | 1 | 0 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| Lower Lerma | La Luz | 13 | 3 | 2 | 0.2949±0.1558 | 0.00038±0.00030 | 0.338±0.307 |
| Lineage II | San Francisco del Rincon | 4 | 3 | 4 | 0.833±0.222 | 0.0020+/-0.00168 | 2.00 +/-1.405 |

N=sample size, *Hn*=number of haplotypes, *S*=number of polymorphic sites, $H_d$=gene diversity, $\pi$=nucleotide diversity (Nei, 1987), *k*=mean number of pairwise differences (Tajima, 1993)

Table 3. Maximum likelihood and uncorrected p distances between lineages and clades for *X. variata*.

| Clade (group) | Lineage I | Lineage II (CUI) | Clade 2.7 (CHA) | Clade 2.1 (ZAC) | Clade 2.3 (ZAT) | PAN | MLE | Clade 3.2 (PAN-MLE) |
|---|---|---|---|---|---|---|---|---|
| Lineage I | (0.8/0.8) | 5.08 (0.64) | -- | -- | -- | -- | -- | -- |
| Lineage II (CUI) | 5.36 (0.78) | (0.2/0.2) | 5.37 (0.78) | 4.95 (0.74) | 5.69 (0.81) | 5.3 (0.76) | 5.3 (0.77) | 5.3 (0.80) |
| Clade 2.7 (CHA) | -- | 5.09 (0.66) | (0.05/0.05) | 0.71 (0.24) | 1.42 (0.36) | 0.79 (0.27) | 0.78 (0.26) | 0.78 (0.26) |
| Clade 2.1 (ZAC) | -- | 4.72 (0.65) | 0.71 (0.24) | (0.16/0.16) | 1.42 (0.37) | 0.85 (0.25) | 0.84 (0.25) | 0.84 (0.26) |
| Clade 2.3 (ZAT) | -- | 5.39 (0.72) | 1.35 (0.35) | 1.4 (0.37) | (0.05/0.05) | 1.49 (0.39) | 1.5 (0.4) | 1.49 (0.40) |
| PAN | -- | 5.03 (0.67) | 0.79 (0.26) | 0.84 (0.27) | 1.46 (0.38) | (0.12/0.12) | 0.11 (0.02) | -- |
| MLE | -- | 5.02 (0.67) | 0.77 (0.25) | 0.83 (0.27) | 1.46 (0.38) | 0.11 (0.02) | (0.10/0.10) | -- |
| Clade 3.2 (PAN-MLE) | -- | 5.02 (0.67) | 0.78 (0.26) | 0.84 (0.27) | 1.47 (0.39) | -- | -- | (0.11-0.11) |

Above diagonal, uncorrected *p* distances; below diagonal, maximum composited likelihood distances (ED); diagonal, within clade mean distance (ML /*p*).

All is represented in %. CUI, Cuitzeo; CHA:,Chapala; ZAC, Zacapu; ZAT, Zacatecas; PAN, Panuco; MLE, Middle Lerma.

Table 4. Maximum likelihood and uncorrected *p* distances between lineages and clades for *Alloophorus robustus*.

| Clade (Group) | Lineage I | Lineage II (SFR) | Clade 3.1 | Clade 2.1 |
|---|---|---|---|---|
| Lineage I | (0.55/0.55) | 1.01 (0.027) | --- | --- |
| Lineage II (SFR) | 1.0 (0.27) | (0.2/0.2) | 0.91 (0.03) | 1.14 (0.03) |
| Clade 3.1 | --- | 0.92 (0.02) | (0.11/0.11) | 1.05 (0.03) |
| Clade 2.1 | --- | 1.16 (0.03) | 1.06 (0.03) | (0.08/0.08) |

Above diagonal, uncorrected *p* distances; below diagonal, maximum composited likelihood distances (ED); diagonal, within clade mean distance (ML /*p*). All is represented in %. SFR, San Francisco del Rincon.

Table 5. Inference chain for all clades showing a significant association in the nested clade analysis results for *X. variata* and *Alloophorus robustus* (Provide in Figs. 4 and 5 respectively).

| Clade number | Populations included | Statistics | Chain | Inference |
|---|---|---|---|---|
| ***Xenotoca variata*** | | | | |
| Clade 4.1 (Lineage I) | Middle Lerma, Zacapu, Panuco, Zacatecas and Chapala. | $\chi^2$=184.00 *P*=0.0000 | 1NO-2YES-3YES-5N0-6YES-13YES | Long Distance Colonization Possibly Coupled with Subsequent Fragmentation or Past Fragmentation Followed by Range Expansion. |
| ***Alloophorus robustus*** | | | | |
| Clade 1.1 | Chapala, Balsas, Lower Lerma and Cotija. | $\chi^2$=26.3158 *P*=0.0000 | 1NO-2YES-3YES-5YES-15-NO-21-NO | Past fragmentation and/or long-distance colonization. insufficient evidence to discriminate between long-distance movements of the organism and the combined effects of gradual movement during a past range expansion and fragmentation. |
| Clade 2.1 | Chapala, Balsas, Lower and Middle Lerma and Cotija. | $\chi$2=38.7813 *P*=0.0160 | 1NO-2N0-11YES-12NO | Contiguous range expantion. |
| Clade 3.1 | Patzcuaro, Zacapu, Cuitzeo and Zirahuen | $\chi$2=57.6264 *P*=0.0000 | 1NO-2YES-3YES-5NO-6YES-13NO-14NO-21NO | Insufficient evidence to discriminate between long-distance movements of the organism and the combined effects of gradual movement during a past range expansion and fragmentation. |

Table 6. Estimate pairwise ΦST values for 1000 bp of the cytochrome b sequences between sampled populations of *X. variata.*

| Population | Zacapu | La Alberca | S. Escalante | Belisario | Mintzita | S. Cristobal | Zacatecas | Valenciana | Yuriria | Allende | S. Francisco | T. Quemada |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| La Alberca | 0.873*** | 0 | | | | | | | | | | |
| M. Escobedo | 0.964*** | 0.983*** | 0 | | | | | | | | | |
| Belisario | 0.963*** | 0.980*** | -0.006 | 0 | | | | | | | | |
| Mintzita | 0.966*** | 0.983*** | 0.096 | 0.023 | 0 | | | | | | | |
| SCR | 0.961*** | 0.981*** | -0.092 | -0.011 | 0.075* | 0 | | | | | | |
| Zacatecas | 0.9120*** | 0.965*** | 0.978*** | 0.975*** | 0.979*** | 0.974*** | 0 | | | | | |
| Valenciana | 0.930*** | 0.960*** | 0.982*** | 0.979*** | 0.981*** | 0.979*** | -0.02414 | 0 | | | | |
| Yuriria | 0.837*** | 0.909*** | 0.971*** | 0.970*** | 0.972*** | 0.968*** | 0.946*** | 0.948*** | 0 | | | |
| Allende | 0.810** | 0.907*** | 0.962*** | 0.962** | 0.967*** | 0.958*** | 0.947* | 0.950*** | 0.07793 | 0 | | |
| SFR | 0.847*** | 0.927*** | 0.973*** | 0.970*** | 0.974*** | 0.969*** | 0.966** | 0.959*** | -0.01419 | 0.10712 | 0 | |
| TQE | 0.840*** | 0.915*** | 0.972*** | 0.970*** | 0.973*** | 0.969*** | 0.950*** | 0.951*** | -0.00061 | 0.08655 | -0.00853 | 0 |
| JMA | 0.829*** | 0.894*** | 0.970*** | 0.969*** | 0.971*** | 0.968*** | 0.932*** | 0.938*** | -0.00256 | 0.05909 | -0.02717 | -0.00611 |

* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.001$.

Table 7. Estimate pairwise ΦST values for 1000 bp of the cytochrome b sequences between sampled populations of *A. robustus.*

| Population | Patzcuaro | Zacapu | Mintzita | Santa Maria | San Cristobal | Ignacio Allende | La Luz | La Alberca | San Juanico |
|---|---|---|---|---|---|---|---|---|---|
| Zacapu | 0.513*** | 0.000 | | | | | | | |
| Mintzita | 0.298*** | 0.029 | 0.000 | | | | | | |
| Santa Maria | 0.406*** | 0.001 | -0.046 | 0.000 | | | | | |
| San Cristobal | 0.425*** | 0.043 | -0.006 | -0.040 | 0.000 | | | | |
| Ignacio Allende | 0.771*** | 0.959*** | 0.785*** | 0.924** | 0.911*** | 0.000 | | | |
| La Luz | 0.878*** | 0.986*** | 0.882*** | 0.980*** | 0.966*** | 0.940*** | 0.000 | | |
| La Alberca | 0.840*** | 0.983*** | 0.830*** | 0.971*** | 0.953*** | 0.897*** | 0.040 | 0.000 | |
| San Juanico | 0.889*** | 1*** | 0.897*** | 1*** | 0.982*** | 0.958** | 0.850** | 0.811*** | 0.000 |
| Uruapan | 0.827*** | 1*** | 0.826* | 1*** | 0.968** | 0.893* | -0.190 | -0.154 | 1** |

* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.001$. n.s = No significative

Table 8. Genetic structure found with the AMOVA analyses for *X. variata* and *A. robustus*.

| Groups | $F_{ST}$ | $F_{CT}$ | $F_{SC}$ | % Among groups | % Among populations within groups | % Within populations |
|---|---|---|---|---|---|---|
| ***Xenotoca variata*** | | | | | | |
| One gene pool (Populations) | 0.95714*** | -- | -- | 95.71 | -- | 4.29 |
| Biogeographic regions (Zacapu)(Chapala)(Cuitzeo)(Middle Lerma)(Panuco)(Aguanaval) | 0.96180*** | 0.96057*** | 0.3120n.s | 96.06 | 0.12 | 3.82 |
| Phylogenetic arrangement Lineage I, (Clade 2.1)(Clade 2.7)(Clade 3.2)(Clade 2.3); (Lineage II) | 0.96435*** | 0.96338*** | 0.02658* | 97 | 0.10 | 2.89 |
| ***Alloophorus robutus*** | | | | | | |
| One gene pool (Populations) | 0.87927*** | -- | -- | 87.93 | -- | 12.07 |
| Biogeographic regions (Patzcuaro)(Zacapu)(Cuitzeo)(Middle Lerma)(Lower Lerma)(Chapala)(San Juanico)(Balsas) | 0.88648*** | 0.88711** | -0.00558** | 88.71 | 0.06 | 11.35 |
| Two gene pools (Clade 3.1)(Clade 2.1) | 0.9227*** | 0.77266** | 0.66015*** | 77.27 | 15.01 | 7.73 |
| Phylogenetic arrangement Lineage I, (Clade 3.1)(Clade 2.1); (Lineage II) | 0.9245*** | 0.87471*** | 0.39748*** | 87.47 | 4.98 | 7.55 |

* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.001$.

Tabla 9. Estimate of historical demographic parameters in *Xenotoca variata* and *Alloophorus robustus* from 1000 bp of the mtDNA cyt *b*.

| | Fu's Fs | Tajima's D | Fu and Li's D* | Fu and Li's F* | Hri ($R_2$) | TMRCA in Ma (95% HPD) |
|---|---|---|---|---|---|---|
| ***Xenotoca variata*** | | | | | | |
| All | -2.39n.s | 0.445n.s | -3.37** | -2.01** | 0.021* (n.s) | 7 (4.7-9.6) |
| Lineage I | -10.03* | -0.98n.s | -2.18* | -2.36* | 0.048n.s | 5.9 (3.6-8.6) |
| Lineage II (Cuitzeo) | -12.21*** | -2.24** | -3.74** | -3.83** | 0.036(n.s) * | 3 (1.7-4.7) |
| Zacapu | -6.03*** | -1.87* | -5.59** | -5.44** | 0.2n.s | 0.9 (0.4-1.6) |
| Lerma-Panuco | -5.79*** | -2.66*** | -3.46** | -3.63** | 0.040(n.s) * | 1.1 (0.5-2.1) |
| Panuco | -5.73*** | -2.31** | -3.70** | -3.84** | 0.072n.s | -- |
| Lerma | -5.96*** | -2.35** | -2.75* | -2.92* | 0.073n.s | -- |
| Chapala | -2.91*** | -1.96* | -2.81* | -2.97* | 0.15n.s | 0.9 (0.4-1.7) |
| Aguanaval | -4.62*** | -1.97* | -3.37** | -2.01** | 0.15n.s | 0.9 (0.04-1.6) |
| ***Alloophorus robustus*** | | | | | | |
| All | -1.685n.s | -0.59n.s | -2.98* | -2.44* | 0.074n.s | 5.2 (1.2-11) |
| Lineage I | -0.669n.s | -0.214n.s | -3.66** | -3.66** | 0.088n.s | -- |
| Cuitzeo | -6.97** | -2.01* | -3.26** | -3.24** | 0.141n.s (*) | 1.9 (0.5-3.7) |
| Chapala | -2.021n.s | -1.68n.s | -0.78n.s | -0.72n.s | 0.128n.s | 1.8 (0.4-3.7) |

* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.001$. n.s = No significative

Figure 1.

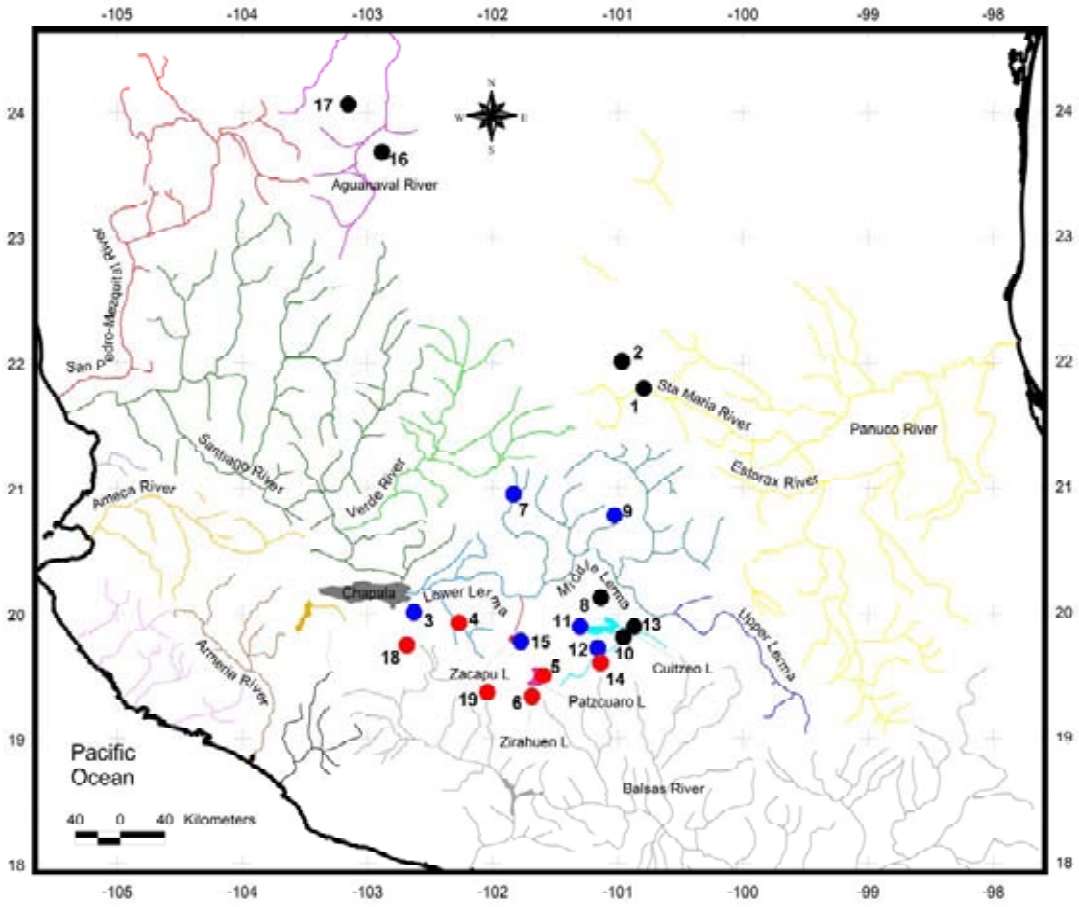

-105
-104
-103
-102
-101
-100
-99
-98
24
23
22
21
20
19
18
17
16
Aguanaval River
San Pedro-Mezquital River
Santiago River
Amica River
Verde River
2
1
Sta Maria River
Panuco River
Estorax River
7
9
Chapala
Lower Lerma
Middle Lerma
Upper Lerma
8
3
4
11
13
15
12
10
18
5
14
Zacapu L.
Cuitzeo L.
19
6
Patzcuaro L.
Armeria River
Zirahuen L.
Balsas River
Pacific
Ocean
40 0 40 Kilometers

Figure 2.

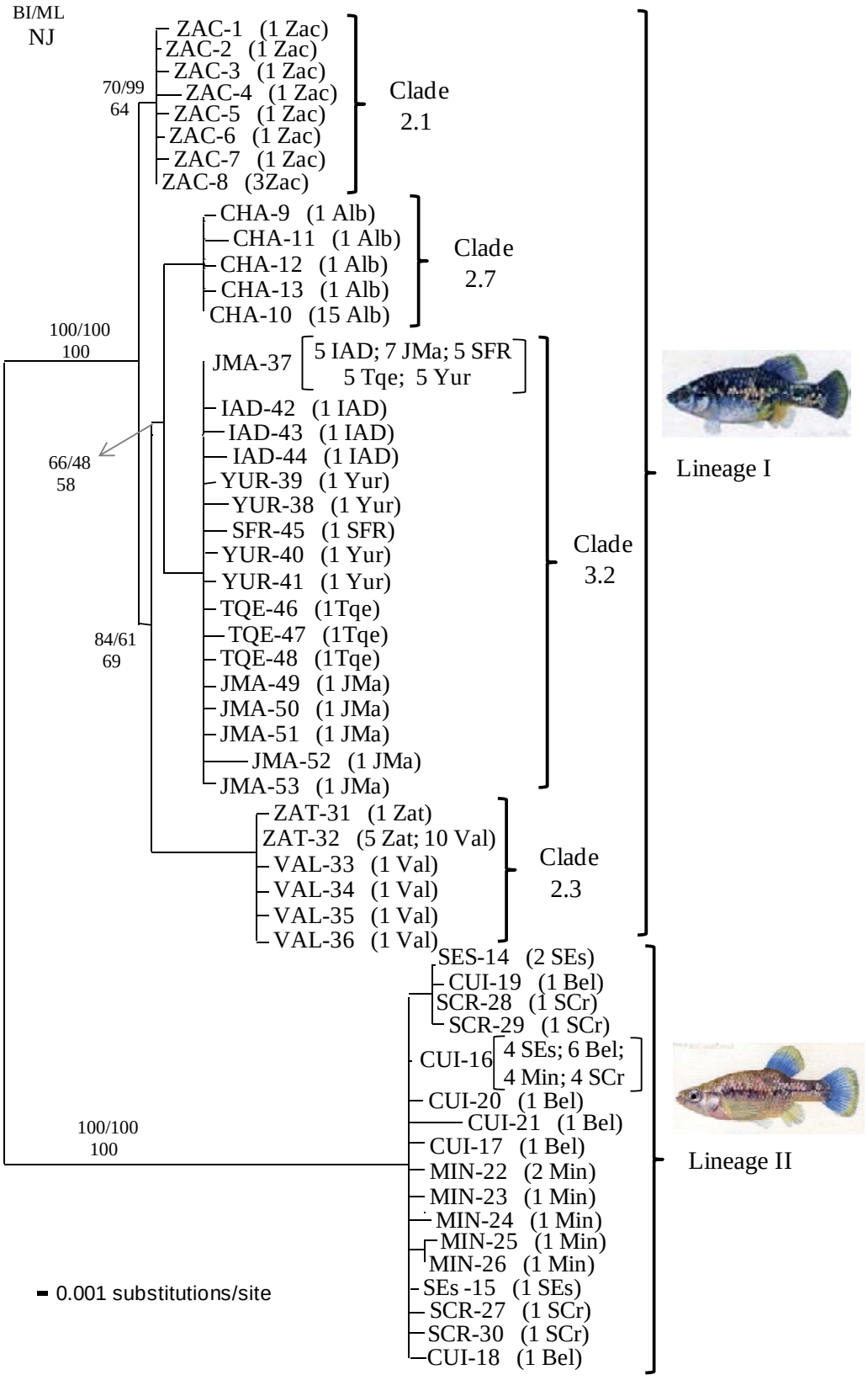

BI/ML
NJ
ZAC-1 (1 Zac)
ZAC-2 (1 Zac)
ZAC-3 (1 Zac)
ZAC-4 (1 Zac)
ZAC-5 (1 Zac)
ZAC-6 (1 Zac)
ZAC-7 (1 Zac)
ZAC-8 (3Zac)
70/99
64
Clade 2.1
CHA-9 (1 Alb)
CHA-11 (1 Alb)
CHA-12 (1 Alb)
CHA-13 (1 Alb)
CHA-10 (15 Alb)
Clade 2.7
100/100
100
JMA-37
5 IAD; 7 JMa; 5 SFR
5 Tqe; 5 Yur
IAD-42 (1 IAD)
IAD-43 (1 IAD)
IAD-44 (1 IAD)
YUR-39 (1 Yur)
YUR-38 (1 Yur)
SFR-45 (1 SFR)
YUR-40 (1 Yur)
YUR-41 (1 Yur)
TQE-46 (1Tqe)
TQE-47 (1Tqe)
TQE-48 (1Tqe)
JMA-49 (1 JMa)
JMA-50 (1 JMa)
JMA-51 (1 JMa)
JMA-52 (1 JMa)
JMA-53 (1 JMa)
66/48
58
84/61
69
Clade 3.2
ZAT-31 (1 Zat)
ZAT-32 (5 Zat; 10 Val)
VAL-33 (1 Val)
VAL-34 (1 Val)
VAL-35 (1 Val)
VAL-36 (1 Val)
Clade 2.3
Lineage I
SES-14 (2 SEs)
CUI-19 (1 Bel)
SCR-28 (1 SCr)
SCR-29 (1 SCr)
CUI-16
4 SEs; 6 Bel;
4 Min; 4 SCr
CUI-20 (1 Bel)
CUI-21 (1 Bel)
CUI-17 (1 Bel)
MIN-22 (2 Min)
MIN-23 (1 Min)
MIN-24 (1 Min)
MIN-25 (1 Min)
MIN-26 (1 Min)
SEs -15 (1 SEs)
SCR-27 (1 SCr)
SCR-30 (1 SCr)
CUI-18 (1 Bel)
100/100
100
Lineage II
0.001 substitutions/site

Figure 3.

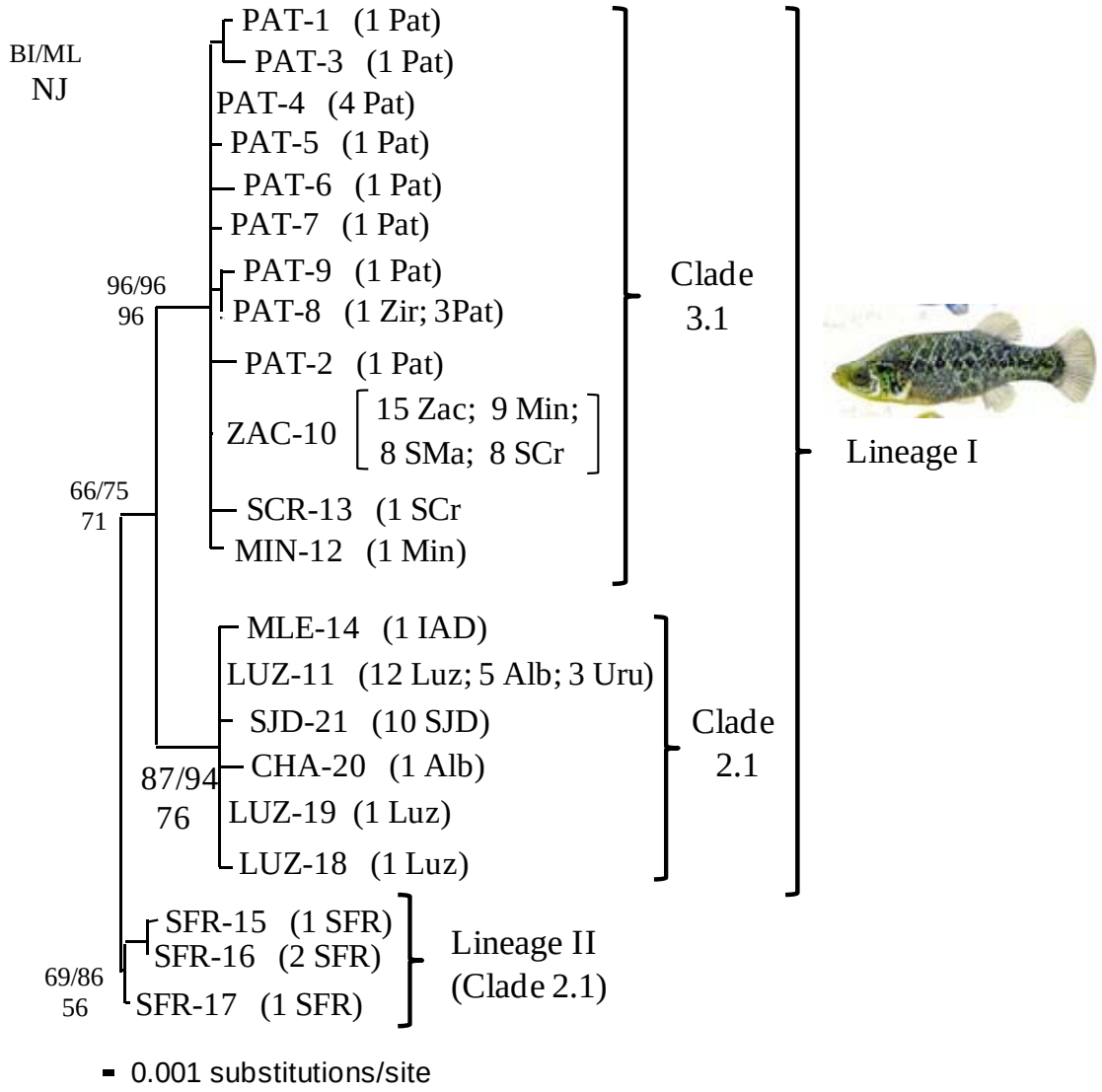

BI/ML
NJ
PAT-1 (1 Pat)
PAT-3 (1 Pat)
PAT-4 (4 Pat)
PAT-5 (1 Pat)
PAT-6 (1 Pat)
PAT-7 (1 Pat)
PAT-9 (1 Pat)
96/96
96
PAT-8 (1 Zir; 3Pat)
PAT-2 (1 Pat)
ZAC-10
15 Zac; 9 Min;
8 SMa; 8 SCr
66/75
71
SCR-13 (1 SCr
MIN-12 (1 Min)
Clade
3.1
MLE-14 (1 IAD)
LUZ-11 (12 Luz; 5 Alb; 3 Uru)
SJD-21 (10 SJD)
87/94
76
CHA-20 (1 Alb)
LUZ-19 (1 Luz)
LUZ-18 (1 Luz)
Clade
2.1
Lineage I
SFR-15 (1 SFR)
SFR-16 (2 SFR)
69/86
56
SFR-17 (1 SFR)
Lineage II
(Clade 2.1)
0.001 substitutions/site

Figure 4.

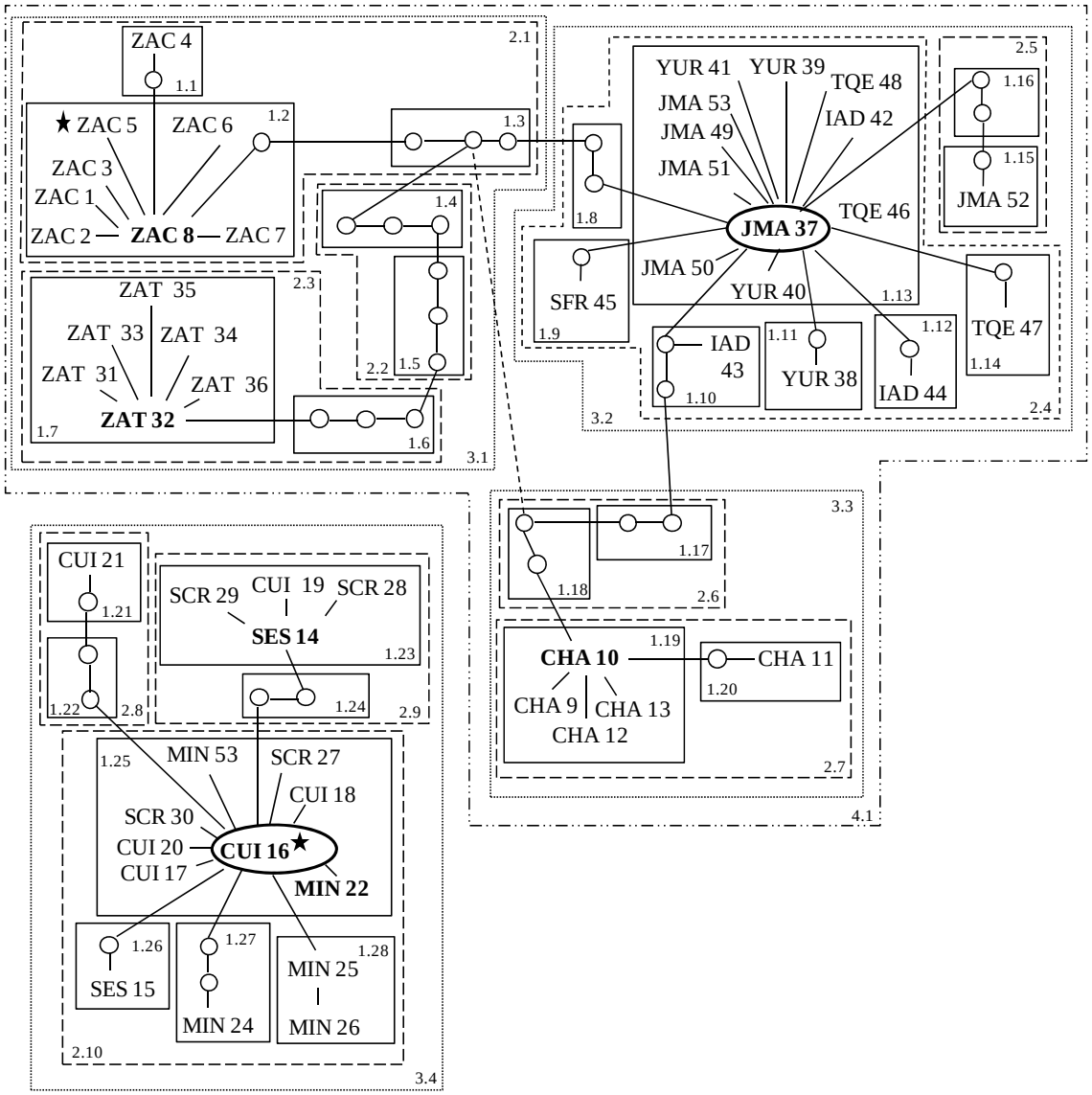

ZAC 4
1.1
2.1
★ZAC 5
ZAC 6
1.2
1.3
ZAC 3
ZAC 1
ZAC 2
ZAC 8
ZAC 7
1.4
2.3
ZAT 35
ZAT 33
ZAT 34
ZAT 31
ZAT 36
ZAT 32
1.7
2.2
1.5
1.6
3.1
1.8
YUR 41
YUR 39
TQE 48
JMA 53
JMA 49
IAD 42
JMA 51
JMA 37
TQE 46
2.5
1.16
1.15
JMA 52
JMA 50
YUR 40
1.13
SFR 45
1.9
IAD 43
1.10
1.11
YUR 38
1.12
IAD 44
TQE 47
1.14
2.4
3.2
3.3
1.17
1.18
2.6
CUI 21
1.21
SCR 29
CUI 19
SCR 28
SES 14
1.23
1.22
2.8
1.24
2.9
CHA 10
1.19
CHA 11
1.20
CHA 9
CHA 13
CHA 12
2.7
4.1
1.25
MIN 53
SCR 27
CUI 18
SCR 30
CUI 20
CUI 16★
CUI 17
MIN 22
1.26
SES 15
1.27
MIN 24
1.28
MIN 25
MIN 26
2.10
3.4

Figure 5.

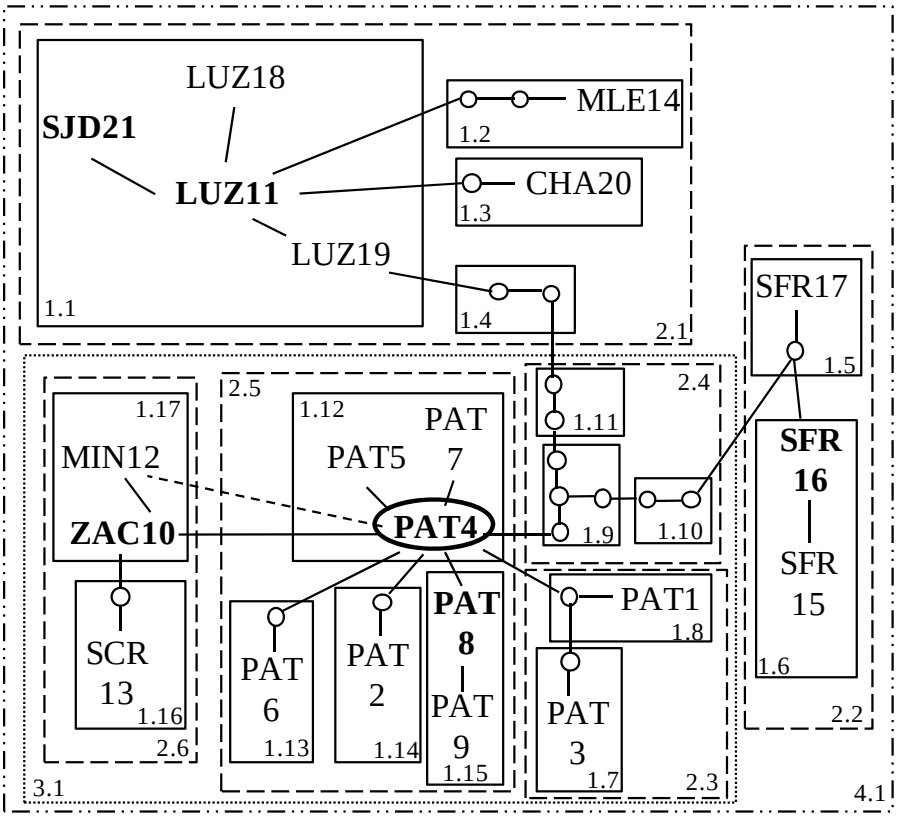

LUZ18
SJD21
LUZ11
LUZ19
1.1
MLE14
1.2
CHA20
1.3
1.4
2.1
SFR17
1.5
2.4
1.11
1.9
1.10
SFR
16
SFR
15
1.6
2.2
2.5
1.12
PAT
7
PAT5
PAT4
1.17
MIN12
ZAC10
SCR
13
1.16
2.6
PAT
6
1.13
PAT
2
1.14
PAT
8
PAT
9
1.15
PAT1
1.8
PAT
3
1.7
2.3
3.1
4.1

Figure 6.

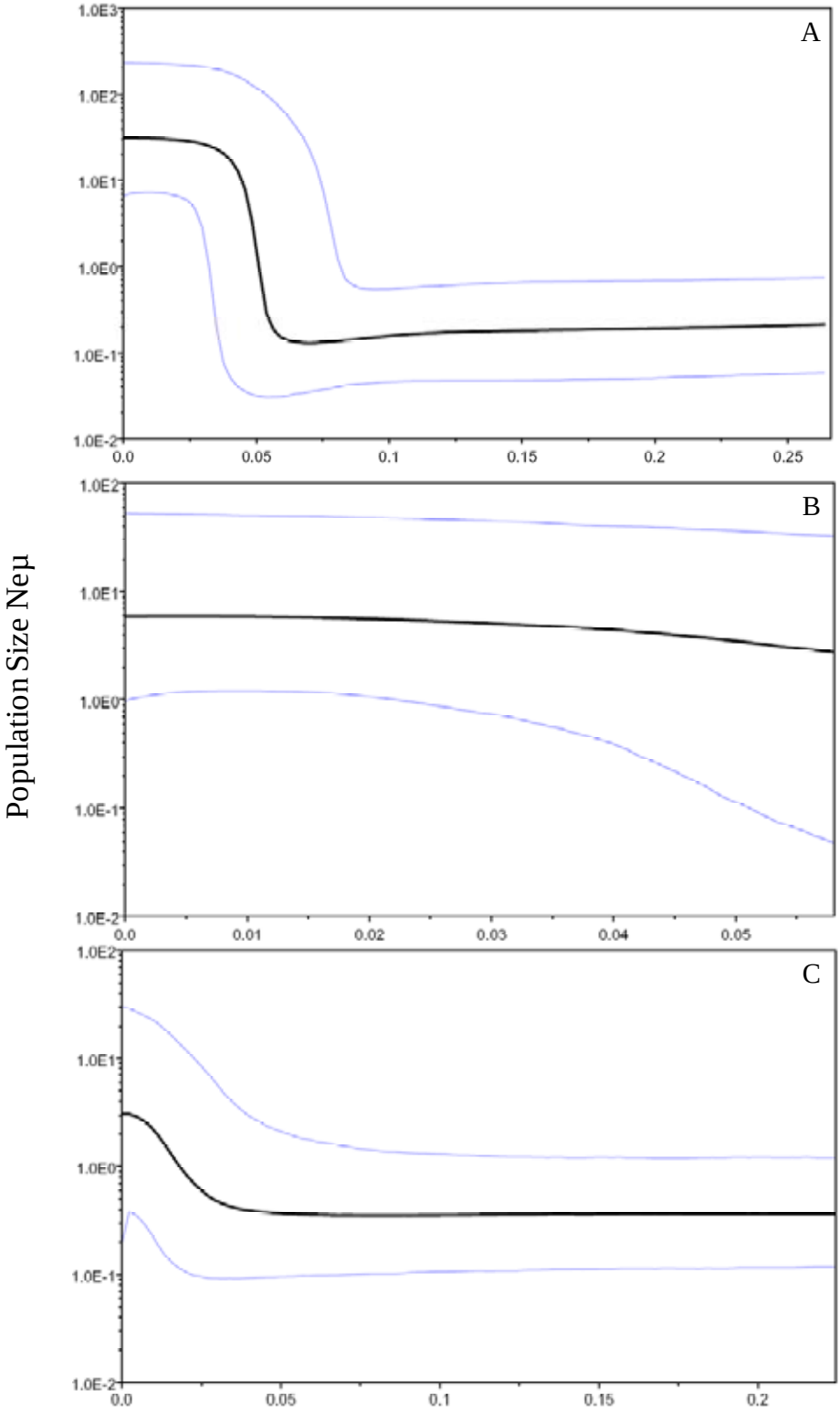

A
B
C
1.0E3
1.0E2
1.0E1
1.0E0
1.0E-1
1.0E-2
0.0
0.05
0.1
0.15
0.2
0.25
0.01
0.02
0.03
0.04
Population Size Neμ
Time since most recent comon ancestor (Ma)

Figure 7.

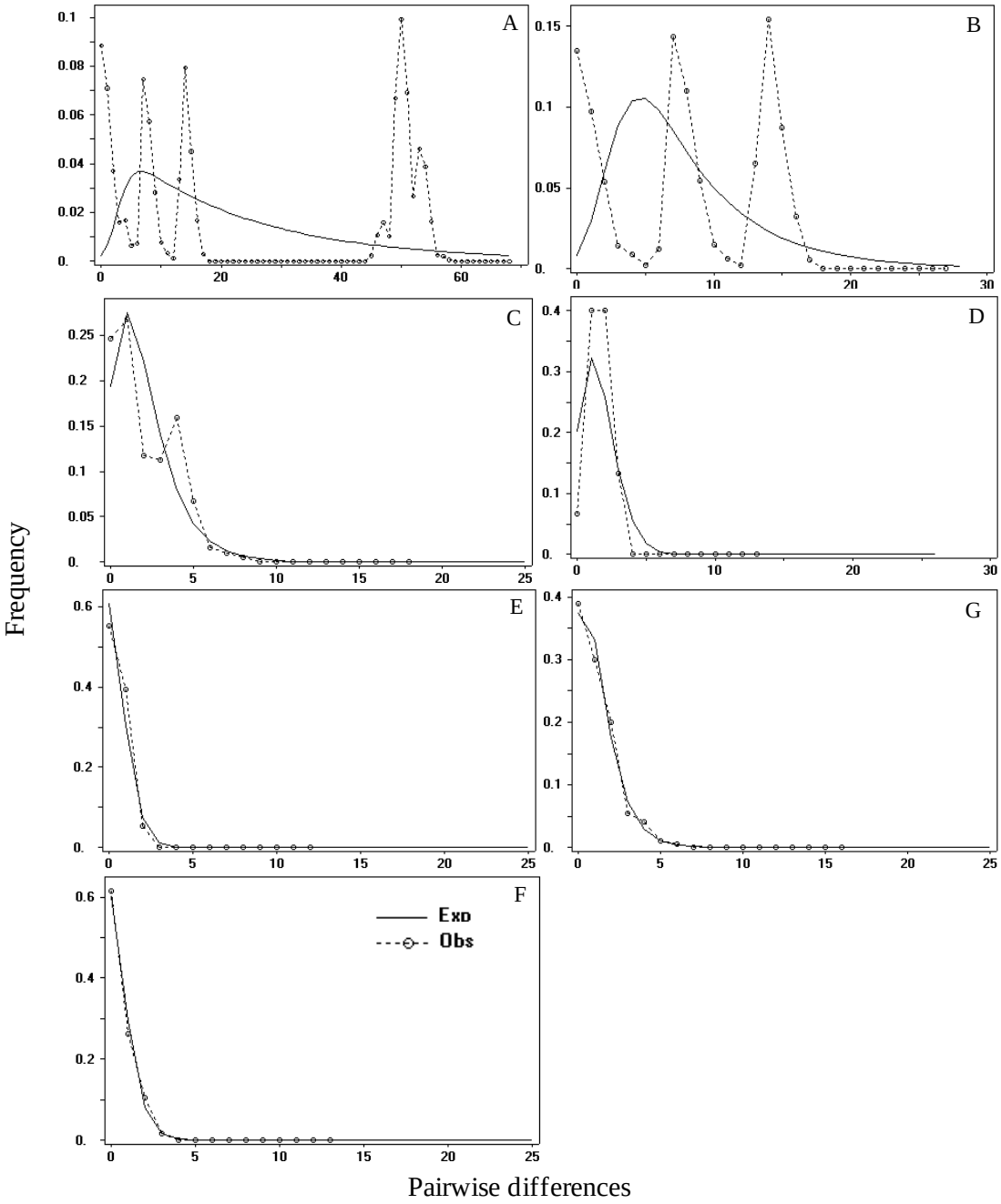
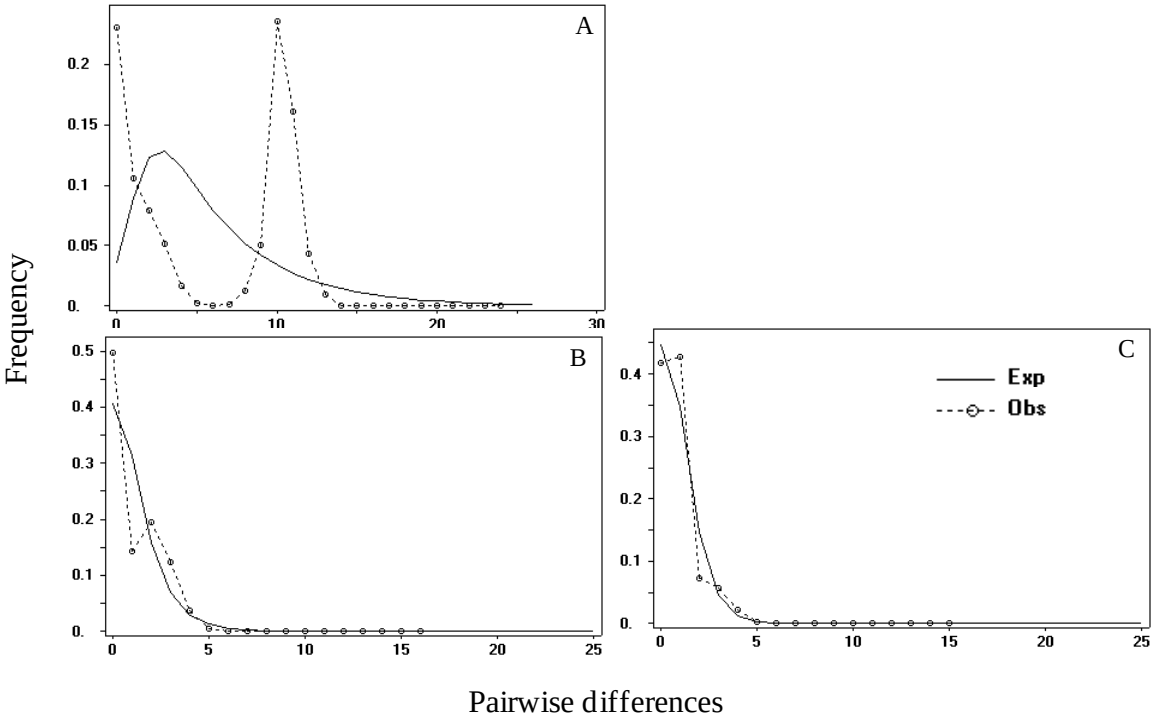
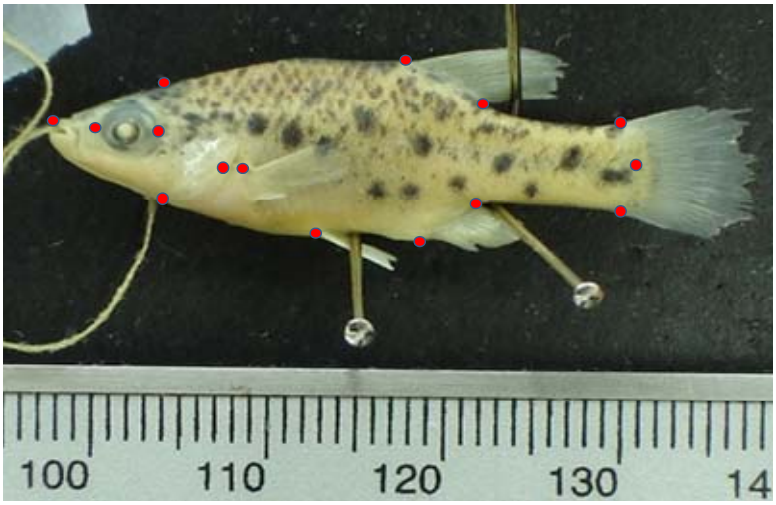


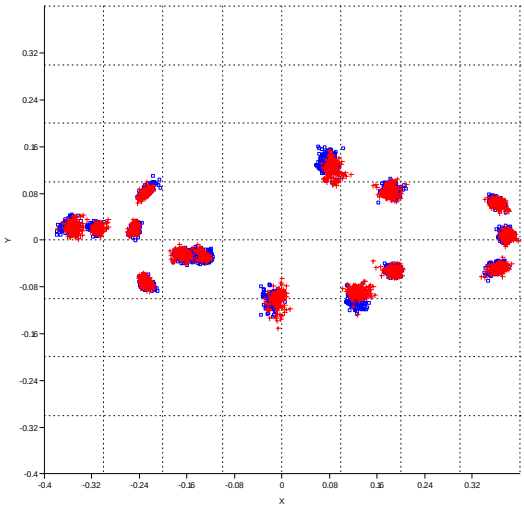
Figure 8.

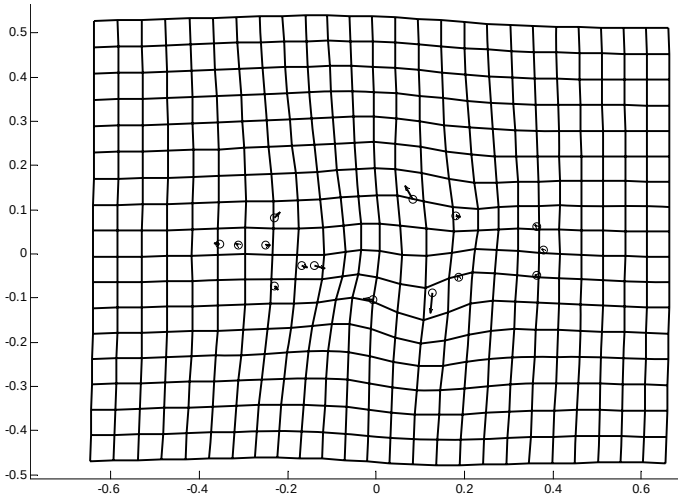


# TAXONOMÍA

## ARTICULO V



Para ser enviado a ZOTAXA

**A new species of *Xenotoca* Hubbs and Turner 1939 (Cyprinodontiformes: Goodeidae) from Cuitzeo Basin by using morphology and Cytochrome *b* gene sequences.**

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**Running header:** New species of *Xenotoca* from Central Mexico.

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**Abstract**

A new Goodeid species is described based on meristic, morphometric and genetic characters. The new species, *Xenotoca dibblenii* sp. nov. is endemic to the Cuitzeo Basin in the Mesa Central of Mexico. The new species is morphologically very similar to its sister species, *Xenotoca variata*, but differs in the following combination of characters: 14 rarely 13 or 15 branched rays in the dorsal fin (*vs.* 13, rarely 12 or 14, in *X. variata*); 21 rarely 17-20 or 22-24 gill rakers in the first arch (*vs* 14, rarely 12-13 or 15-16, in *X. variata*). Additionally, Cytochrome *b* gene sequences among the two species differ in terms of forty-five fixed nucleotide positions (molecular autopomorphies). Calculated genetic divergences when comparing individuals from all the populations of *X. dibblenii* sp. nov. with respect to those from all the populations of *X. variata* were $\overline{D}_{GTR}$ = 6.1% and $\overline{D}_{P}$ =5.5%. The speciation event that led to the formation of the new species seems to be associated with the lacustrine evolution of Cuitzeo Lake endorheic basin.

**Key words:** Mesa Central of Mexico, *Xenotoca dibblenii* sp. nov. Goodeidae. Cuitzeo Basin, Taxonomy, Biogeography, Cytochrome b.

## Introduction

The Goodeinae is an endemic subfamily of freshwater fishes represented by approximately 41 species restricted to Mesa Central of Mexico and adjacent areas (Domíguez-Domínguez et al., 2005). Goodeines are generally small fish inhabiting a wide range of ecosystems (e.g. rivers, lakes and springs), with wide variation of food habits (e.g. herbivores or carnivores) and exploiting different habitats (e.g. water column or bottom dwellers). The principal characteristics that unify this group of fishes are the special modification for internal fertilization and live birth; males exhibit a modification of the first 5 to 7 rays forming a lobe that helps in the transfer of the sperm package to the female. The close contact necessary for the copulation has driven to a wide range of sexual selection strategies that led to most of the species a strong sexual dimorphism (Moyaho et al., 2004; Ritchie et al., 2007). Another diagnostic trait is the matrotrophy. In this case the embryos develop in the ovarian lumen were the trophotaenial placenta serves in the transfer of nutrients and molecules between the female and the embryos. This placenta consists of a specialized structure developed in the perianal area of the embryo, the trophotaeniae, and a maternal component related with the internal ovarian as the septum and epithelium (Wourms, 1981; Lombardi and Wourms, 1988; Uribe et al., 2004).

The taxonomy and classification of the members of Goodeinae dates from the eighteenth century, with the description of a very peculiar cyprinodontiform belonging to an unnamed species of *Lucania* collected in the vicinity of Mexico City by Girard (1859). This species was later described as *Girardinichthys innonimatus* by Bleeker (1860). This finding was followed by the description of *Characodon lateralis* by Günther (1866) and *Goodea atripinnis* by Jordan (1880). During this period, the species of goodeines were recognized in accordance with their dentition and the length of their intestines, and were included in genera belonging to the families Cyprinodontidae and Poeciliidae, which also comprised the presently recognized families Profundulidae, Fundulidae, Rivulidae, Cyprinodontidae and Anablepidae (*sensu* Parenti, 1981).

The first recognition of the phyletic unit of the Goodeinae was made in the taxonomic revision by Meek (1902; 1904). This author recognized the viviparity and the shortening of the anterior rays of the males as a diagnostic trait characterizing the Goodeidae. Posterior classification of the Goodeids was made based in trophic-related

characters as the jaws, teeth form, and length of the intestine. Jordan (1923) divided the previous classified genera in two sub-families; Characodontinae and Goodeinae, which were later lumped together and Characodontinae was synonymized with Goodeinae (Hubbs, 1924). Hubbs and Turner (1939) reclassified the family Goodeidae based on characters associated with the viviparity, mainly using two structures of the trophotaenial placenta, the trophotaenia and the ovarian septum, recognizing four sub-families to accommodate the XX species described up to that time (Ataeniobiinae, Goodeinae, Characodontinae and Girardinichthynae).

Following this taxonomic classification of the Goodeidae, and considering that further taxonomic revisions of the family indicated that anatomical features of the trophotaenial placenta are highly variable among species and even within species, it was revealed that the trophotaenia could not be taken as a unique characters in the taxonomy of the group (Mendoza, 1965; Miller and Fitzimons, 1971; Fitzimons, 1972). Then other sources of characters started to be use to classify goodeids. Miller and Fitzimons (1971) used for the first time a combination of traditional morphological traits with karyotype information resulting in a considerable number of changes in the previous classification of the family, proposing several synonymies for genera and species and recommending more studies to elucidate if the designation of the previous subfamilies and what they called phyletic lines reflected a natural classification. Later, Uyeno et al. (1983) conducted a karyological study of 35 species of goodeines and proposed four provisional groups, however some recognized genera at that time such as *Characodon*, *Ilyodon* and *Allodontichthys* were not included. The most recent taxonomic revision of the group was made by Webb et al. (2004) by including molecular markers. These authors conducted a phylogenetic study of 34 species of goodeines and identified some important differences among sister-species relationships with respect to those found in previous papers, and recognizing that the family Goodeidae is composed by two subfamilies, Empetrichthyinae and Goodeinae, as was previously noted by Parenti (1981). Doadrio and Domínguez (2004) also conducted a phylogenetic study including 104 samples belonging to 41 species of Goodeidae. These authors recognized the monophyly of the subfamily Goodeinae and that of three different species groups within them, i.e. Characodontini, Ilyodontini and a group containing the remaining species, which they classified into the traditional species group

named Chapalichthyini, Girardinichthyini and Goodiini. In addition, they noted that the morphology of the trophotaenia appears to be homoplasious.

Within the genus *Xenotoca* Hubbs and Turner 1939, three species have been described thus far: *Xenotoca variata* (Bean, 1887), *Xenotoca eiseni* (Rutter, 1896), and *Xenotoca melanosoma* Fitzimons, 1972. There has been some controversy on the possible synonymy of *X. eiseni* with *X. variata* (Mendoza, 1965; Romero, 1967). The genus *Xenotoca* was erected in the revision by Hubbs and Turner (1939), placing *C. variatus* and *C. eiseni* as synonyms of *Xenotoca variata*. More recently, Fitzimons (1972) revised the genus *Xenotoca* by using morphological, karyological and behavioural data, and described *X. melanosoma* as new species. Although all this studies support the existence of three species within the genus *Xenotoca*, recent phylogenetic data does not support the monophyly of the genus, placing *X. melanosoma* and *X. eiseni* as a sister taxa as basal members of the so-called tribe Chapalichthyini, but the other species, *X. variata* occupies a more derived position within the group (Doadrio and Domínguez, 2004).

The species *X. variata* is considered to be the second widespread species within the Goodeinae, after *G. atripinnis*. Its distribution range is reported in upper tributaries of the Pánuco River in the Atlantic slope, in lakes Chapala and Zacapu, in the Verde-Santiago (above the falls at Juanacatlán, Jalisco) and Middle-Lerma river, which are part of the current Lerma-Chapala-Santiago river system in the Pacific slope of Mexico, but also it has been found in the interior drainages of the Aguanaval river in Zacatecas, and Lake Cuitzeo in Michoacán (Domínguez-Domínguez et al., 2005). This species is recognized as a medium sized goodeid (around 75 mm standard length), with a yellow terminal band in a dusky to black caudal fin and with a dark stripe extending along the mid-side of the opercle, body and caudal peduncle in males (Fitzimons, 1972). The males and females possess a body surface covered with black irregular spots with variable size, number and brightness in different populations. A ribbon-like trophotenia type and four distinctive male courtship displays also characterizes this species (Fitzimons, 1972).

Hubbs and Turne (1939) did not find important differences among specimens of *X. variata* from several localities, but proposed the analysis of a larger sample size to provide a more accurate description, whereas Fitzimons (1972), discovered some minor differences in a north-south clinal distribution of populations of *X. variata* (from Rio Verde to Cuitzeo

samples), particularly in the number of dorsal rays and vertebrae. More recently, Doadrio and Domínguez (2004) distinguishes high genetic divergence and differentiation among some populations of *X. variata*.

The purpose of this study, therefore, is to analyze the morphologic, meristic and genetic differences among populations of *Xenotoca variata* along its distributional range and to provide the description of a newly recognized species from Cuitzeo Basin, Michoacán. Based on different sources of evidence, populations of *X. variata* are re-analyzed in order to establish the distribution range of both, the new species and *X. variata*.

**Materials and Methods**

The specimens were collected using hand and seine net and an electroshoker. Most of the sampled specimens were fixed in 10% formalin and preserved at 70% ethanol, however, for molecular analysis some fin clips were preserved in 100% ethanol. Voucher specimens for genetic analyses are deposited in the Colección de Peces de la Universidad Michoacana (CPUM). The morphological analyses are based on 29 specimens from La Mintzita spring, Cuitzeo basin Coleccion de Peces de la Universidad Michoacana, CPUM-1529 and 1525; Museo Nacional de Ciencias Naturales de Madrid, MNCN-23562; and Colección Nacional de Pecesdel Instituto de Biologia, UNAM, IBUNAM-P-1989, 21 specimens from Jesús María stream CPUM - 1064, 1262 and 1280, Pánuco drainage, state of San Luis Potosí, 29 specimens from La Mintzita spring CPUM – 1125, 16 specimens from San Cristobal spring (CPUM - 1198), both in the Cuitzeo drainage, state of Michoacán (Fig. 1 and Table 1).

*Morphological analysis*

Twenty morphometric characters were measured with digital callipers (0.01 mm) and four meristic variables were recorded using a stereoscope. The abbreviations used for morphometric variables are: SL, standard length; HL, head length; PrOL, preorbital length; ED, eye diameter; InOW, interorbital width; BD, body depth; BLD, body least depth; PAD, origin of the pelvic fin to origin of the anal fin distance; PDD, origin of pelvic fin to origin dorsal fin distance; PODE, origin of the pelvic fin to dorsal fin posterior extent distance; DAD, origin of the dorsal fin to origin of the anal fin distance; DOAE, origin of the dorsal fin to anal fin posterior extent distance; DFL, dorsal fin base length; DEAO, dorsal fin

posterior extent to anal fin origin distance; AFL, anal fin base length; AEDE, anal fin posterior extent to dorsal fin posterior extent distance; EDUP, end of dorsal fin to upper extreme of caudal peduncle distance; EDLP, end of dorsal fin to lower extreme of caudal peduncle distance; EAUP, end of the anal fin to upper extreme of caudal peduncle distance; EALP, end of the anal fin- lower extreme of caudal peduncle distance. The abbreviations for meristic characters are: D, dorsal-fin rays; A, anal-fin rays; P, pectoral-fin rays; GR, gill rakers. All the measurements are in millimetres.

A two-way analysis of variance (ANOVA), comparing both morphometric and meristic characters, was conducted to test sexual dimorphism, variation between species and their interactions. Since sexual dimorphism was found for both types of characters, all the subsequent analyses were conducted using fish sex independently.

To identify the variables contributing most to the differences between the groups of the sampled populations, principal component analyses (PCA) were performed on the meristic and morphometric data collected from all the specimens examined by sex. The PCA results for the morphometric data (not shown) indicated that all weighted characters on the first principal component (PC I) showed the same sign and were of similar magnitude, suggesting that this axis represents general size-related variation (Jolicoeur and Mosimann, 1960; Humphries et al., 1981; Bookstein et al., 1985). Therefore, Burnaby's method was used to correct for size effect (Burnaby 1966; Rohlf and Bookstein 1987; Doadrio et al., 2002; Domínguez-Domínguez et al., 2007a). All the subsequent analyses were conducted with the corrected matrix. A second PCA was conducted using a covariance matrix for morphometric characters and a correlation matrix for meristic characters. All analyses were made with the statistics package PAST v 1.75b (Hammer et al., 2001).

*Molecular analysis*

Six sequences of the gene Cytochrome *b* of *Xenotoca variata* from three different populations belonging to two drainages were obtained from GenBank (AF510803-AF510808), and one was obtained for the outgroup (*Goodea atripinnis* Jordan, 1880, AF510773). Fourteen sequences of the complete gene Cytochrome *b* (1140 bp) of *Xenotoca variata* from 11 populations belonging to four drainages (Table 1 and Fig. 1) were obtained using the following protocol. DNA was isolated from tissues by a standard proteinase K

and phenol/chloroform extraction method (Sambrook et al., 1989). Two overlapping fragments of the cytochrome *b* gene (total of 1140 bp) were amplified via polymerase chain reaction (PCR) for each individual DNA sample. The primers used for cytochrome *b* in all species were those discussed in Machordom and Doadrio (2001). The amplification process was conducted as follows: 94 ºC (2 min), 35 cycles at 94 ºC (45 s), 48 ºC (1 min), 72 ºC (90 s), and 72 ºC (5 min). PCR mixtures were prepared in 25 µl reactions with a final concentration of 0.4 µM of each primer, 0.2mM of each dNTP, 1.5mM MgCl2, and 1U of Taq DNA polymerase (Biotools). PCR products were checked on 1.5% agarose gels, and cloned using the pGEM-T vector (Promega) into *Escherichia coli* JM109. Positive clones were sequenced using the Big Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers. All samples were sequenced on an Applied Biosystems 3700 DNA sequencer following manufacturer's instructions. Chromatograms and alignments were visually checked. Modeltest 3.7 (Posada and Crandall, 1998) was used in order to determine the best fitting models of nucleotide substitution for the dataset. The Akaike information criterion indicated that the General Time Reversible (GTR+G) is the most appropriate for the datasets (data available from authors on request). The aligned data were analysed with the Bayesian inference (BI) method and the model selected with the program Mr. Bayes 3.1.1 (Hueselsenbeck and Ronquist, 2001). We ran one million generations of four simulating Markov chain Monte Carlo (MCMC) with trees and likelihood scores sampled each 100 generations. The burn-in period was evaluated graphically by plotting the likelihood scores against the generation times, and 100 sampled topologies for each run were burning and discarded. The remaining trees were used to build 50% majority-rule consensus trees and posterior probability values were used as support for the Bayesian topology. Based on the GTR model obtained by Modeltest and uncorrected *p* distance, pairwise genetic distances between the two groups were obtained using the program Sequencer 6.1.0 (written by B. Kessing and available at http://nmg.si.edu/).

***Xenotoca dibblenii* sp. nov.**

*(Figures 2 A-B, Table 2)*

**Holotype.** (Fig. 2A, Table 2) CPUM -1529, 55 mm SL. La Mintzita spring, Cuitzeo basin, Morelia, Michoacan, Mexico. Geographic coordinates: 101° 16' 12'' W and 19° 38' 21'' E. Coll. Ivan Dibble, Martina Medina Nava and Xavier Madrigal Guridi. 15th of may 2004.

**Paratypes.** CPUM-1525, 8 males, 10 females. MNCN-23562 2 males, 3 females; IBUNAM-P-1989, 2 males, 3 females. Same data as for the holotype

**Diagnosis.** *Xenotoca dibblenii* sp. nov. differs from *Xenotoca variata* by the following set of characters: 14 rarely 13 or 15 branched rays in the dorsal fin (*vs.* 13 rarely 12 or 14 in *X. variata*); 21 rarely 17-20 or 22-24 gill rakers in the first arch (*vs* 14 rarely 12-13 or 15-16 in *X. variata*); forty-five fixed nucleotide positions (molecular autopomorphies) in the mitochondrial cytochrome b gene (Table 3). The genetic divergences when comparing all the population of *X. dibblenii* with respect to all the populations of *X. variata* were $\bar{D}_{GTR}$ = 6.1% and $\bar{D}_{P}$ =5.5%.

**Description.** D = (13)14; A = 13-14; P = (11-12) 13-15; GR = (7) 9-12. Measurements are shown in Table 2. Body relatively deep, laterally compressed and elongated, maximum height $\bar{x}$ = 3.1 (range = 2.8-3.6) times the standard length in males and $\bar{x}$ = 3.3 (range = 2.9-3.7) times in females. Minimum body height $\bar{x}$ = 6.5 (range = 6.2-7.0) times the standard length for males and $\bar{x}$ = 7.0 (range = 6.2-7.7) in females. Head short, cephalic length $\bar{x}$ = 3.5 (range = 3.3-3.7) times standard length in males and $\bar{x}$ = 3.7 (range = 3.3-4.1) in females. Preorbital distance short, preorbital distance $\bar{x}$ = 16.6 (range = 14.6-21.8) times standard length in males and $\bar{x}$ = 20.0 (range = 12.8-27.4) in females. Anal fin inserted before the origin dorsal fin at same axis. Dorsal fin length long $\bar{x}$ = 5.2 (range = 3.4-7.0) standard length in males and $\bar{x}$ = 5.8 (range = 5.3-6.6) in females.

**Pigmentation pattern.** When alive, the coloration varies with respect to the locality, the age and sex of the organism. In general, mature females present the upper half of the body and the caudal peduncle with brown light coloration, down the eye, the opercle and down to the lateral line until the anal fin is silver-white, with some iridescence in the opercle and in some scales in the upper half of the body. The coloration in males is more variable than in females. Reproductive males have only the upper part of the body with brown light coloration, and most of the body possess a silver-white colour, with iridescence

in scales along the body and in the opercle. Males and females present dark patchy blotches in the body, the size, form, number and darkness vary among population and sex, in some specimens the dotches form a line in the middle part of the body from the opercle to the caudal fin, this line is normally more apparent in males than in females. In general, in populations occurring in sites with clear water the number and intensity of blotches are higher (e.g. La Mintzita populations) than in populations living in turbid waters (e.g. Cuizteo Lake). The fins are normally clear to dusky in adult specimens. In adult males, the caudal fins possess a yellow subterminal and dark terminal band, and the dorsal fin shows a yellowish terminal band, more apparent in males during courtship and in the localities with clear waters. Males often present an iridescent color in the pelvic and anal fins, the later with some yellow-orange coloration. The juveniles have the brown light to transparent body with the ventral area with white coloration. Fins are clear and with small black dots in the upper part of the body and caudal peduncle. Bluish or greenish hue on the lateral side of the body and some scales can produce iridescence.

**Sexual dimorphism and reproduction.** As the other members of the Goodeinae subfamily, sexual dimorphism is marked, with males showing a reduced length on the first five to seven anal-fin rays (Hubbs and Turner, 1939). The two way ANOVA revealed sexual dimorphism in some measurement. The females are larger than males SL $\bar{x}$ = 48.38 (range = 24.62-69.56) in females and $\bar{x}$ = 41.03 (range = 22.22-54.91). All the following measurements are divided by SL, the females present a smaller body dept length $\bar{x}$ = 0.239 (range = 0.237-0.333) and $\bar{x}$ = 0.308 (range = 0.284-0.347) in males. The caudal peduncle is larger in females EDUP $\bar{x}$ = 0.264 (range = 0.229-0.307) in females and $\bar{x}$ = 0.261 (range = 0.236-0.298) in males, EDLP $\bar{x}$ = 0.298 (range = 0.270-0.350) in females and $\bar{x}$ = 0.293 (range = 0.268-0.332) in males, EAUP $\bar{x}$ = 0.297 (range = 0.260-0.325) in females and $\bar{x}$ = 0.293 (range = 0.264-0.332) in males, EALP $\bar{x}$ = 0.264 (range = 0.227-0.301) in females and $\bar{x}$ = 0.260 (range = 0.239-0.286) in males. Pigmentation pattern is more lighted in males. The dark blotch are more conspicuous in males than females, some reproductive males present a black stripe along the middle part of the body. The males present a yellow terminal band in the caudal fin and evident iridescent colors in some scales in the body.

**Distribution.** The type locality of *X. dibblenii* sp. nov. is La Mintzita Spring. Following the molecular and morphometric diagnostic characters, we support the hypothesis that this species is endemic from the Cuitzeo Basin, and was previously largely recognized as *X. variata*, but should henceforth be considered as *X. dibblenii.* Thus, the distribution of *X.dibblenii* occupies all the basin of Cuitzeo Lake and collection for at least 22 localities within the basin are reported in different fish collection databases (Fig. 1), mainly at the University of Michigan Museum of Zoology and Colección de Peces de la Universidad Michoacana de San Nicolás de Hidalgo.

**Etymology.** The name "*dibblenii*" comes from the name of the English conservationist Mr. Ivan Dibble, one of the most enthusiastic and the main promoter of the international and national Goodeids conservation efforts.

**Habitat and ecology.** The type locality, La Mintzita spring, is located at the Southwest of the Cuitzeo Basin. It is a small repressed and permanent body of water fed by a large spring. The spring is used as human water supply and also for agricultural purposes. The outflow water of the spring flows to the Río de Morelia, a tributary of the endorheic Cuitzeo Lake. The bottom is composed by mud and gravel. In an annual cycle, the water temperature varies from 20 to 23°C; the dissolved oxygen ranged between 3.26 to 6.27, pH was between 7-8, conductivity between 155 to 190 µS/cm and transparency between 30 to 90 cm (Salazar-Tinoco, 2007).

The water body is surrounded by the species *Salix* sp, *Fraxinus* sp and *Taxodium* sp. The aquatic vegetation is composed by *Typha dominguensis, Nimphaea mexicana, Potamogetum pectinatum, Ceratophylum dermesum* and the introduced *Eichornia crasipes.* The fish fauna collected with the new species is represented by the goodeids *Allotoca dugesii* (Bean, 1887), *Goodea atripinnis, Alloophorus robustus* (Bean, 1892), *Zoogoneticus quitzeoensis* (Bean, 1898) and *Skiffia lermae* Meek, 1902, the cyprinids *Yuriria alta* (Jordan, 1880) and *Notorpis calientis* Jordan and Snyder, 1899, the catostomid *Moxostoma austrinum* Bean, 1880, and the poecilid *Poeciliopsis infans* (Woolman, 1894). The introduced fish fauna collected included *Cyprinus carpio* Linnaeus, 1758, *Ctenopharingodon idella* (Valenciennes, 1844), *Poecilia reticulate* Peters, 1859, *Xiphphorus hellerii* Heckel, 1848 and *Oreochromis* sp.

*Xenotoca dibblenii* sp. nov. prefer areas with flow and good cover vegetation. In La Mintzita, the first maturation size reported is 4.6 cm for females and 4.9 for males. The gestation period is reported to 55 days in captivity, giving birth between 15 to 20 fry with a maximum to 40 fry, the size at birth is 1.5 cm. In nature the mean fecundity is betwen 0.89 embryos per gram in spring to 8.63 embryos per gram in summer. The number of offspring range between 5 to 19 per female. This specie has reproductive activity during all the year, but spring and summer shown the high reproductive potential (Salazar-Tinoco, 2007). The species is considered omnivore and tolerant (Mercado-Silva et al., 2002)

**Conservation.** *Xenotoca dibblenii* sp. nov. is one of the most abundant and widespread species in the Cuitzeo basin, although, the high human impacts reported for the basin have produced an increase of alteration in the last few years (Soto-Galera et al., 1999; Domínguez-Domínguez et al., 2005; 2006a; 2007b), and this could result in a negative effect on the permanence and abundance of the populations of this species in the basin.

**Comparative data and Discussion**

As discussed above, *Xenotoca dibblenii* sp. nov. from the Cuitzeo basin shows many meristic and genetic characters differentiating it from its closets relative within the genus, *Xenotoca variata*, and therefore warrants designation as a new species.

*Morphometric and meristic comparisons*

Our ANOVA revealed significant differences between males and females ($p \leq 0.05$) in the standard length and the morphometric characters that are mainly related with the dorsal fin, anal fin and caudal peduncle measurements, indicating that *X. debblenii* sp. nov. exhibits sexual dimorphism in the caudal peduncle length, dorsal fin size and anal fin position. In meristic characters, only dorsal fin rays shows significant differences; this could explain the differences in the dorsal fin related measurements. These results justify the separation of morphometric and meristic data by sex of the fish. The interaction species/sex did not show a significant value in any of the morphometric or meristic traits (Table 4).

The exploratory PCA for morphometric variables without standardization assigned all positive values with similar weight to PC I; in males, PC I was able to explain 91.60% of the variance and in females 87.34%. Because of these results, we conducted a second PCA using a matrix corrected for the size effect according to Burnaby's method. For

morphometric variables in males, PC I was able to account for 29.81% of the variance, the highest eigenvectors were end of the anal fin- lower extreme of caudal peduncle distance (EALP) and end of the anal fin to upper extreme of caudal peduncle distance (EDUP). The cumulative variance in PC II was 45.31%, the higher eigenvectors were preorbital length (PrOL) and interorbital width (InOW). For PC III the cumulative variance was 59.22%, the high eigenvectors were dorsal fin base length (DFL) and anal fin base length (AFL), (Fig. 3-top and Table 5). In females, PC I explained 36.62% of the variance with (EALP) end of the anal fin- lower extreme of caudal peduncle distance and end of dorsal fin to upper extreme of caudal peduncle distance (EDUP) showing the highest values. PC II accounted for 51.65% of the variance with the high eigenvectors interorbital width (InOW) and anal fin base length (AFL). Finally, PC III was able to explain 62.25% of the percent cumulative variance, with body least dept as the highest eigenvector (BLD) (Fig. 3-bottom and Table 5). The variation pattern was more influenced by PCI and only males shown a somewhat segregation but not enough to be considered as differentiation pattern (Fig. 3-top). In fact no well-defined groups emerged, and no evident morphological diagnostic characters appeared among the two species (*X. variata* and *X. dibblenii*) (Fig. 3).

Even though morphology has been largely used as the basis for species discrimination among goodeids, *Xenotoca variata* exhibits a high degree of phenotypic plasticity among populations (Fitzimons, 1972; Moyaho et al., 2005), and the influence of allometry in morphometric variables has been also widely recognized (Hood and Heins, 2000; Trapani et al., 2005). We conclude that morphology alone does not provide appropriate diagnostic characters to diferentiate *X. dibblenii* from *X. variata*. Thus, other sources of information such as biogeographic, meristic and mtDNA sequences were used here for the establishment of species recognition.

The PCA conducted by population and sex for meristic characters was able to explain 48.23% of the variance in PC I for males, according to the eigenvector gill rakers (GR) and dorsal fin rays (D) shown the highest values. PC II explained 74.28% of the variance, with anal fin rays (A) as the highest value (Fig. 4-top, Table 6). For females the PCI could explain 47.99% of the variance with the gill rakers (GR) and dorsal fin rays (D) as the highest values. PC II explained 80.27% of the variance, with pectoral fin rays (P) as the highest value (Fig. 4-bottom, Table 6). For the meristic characters, the analysis

indicated a variation pattern with the formation of well-defined groups, with practically no overlap among ellipses. Inclusive the variation between the *X. dibblenii* population were recorded by the analyses. The differentiation is more evident in males that females. (Fig. 4).

*Molecular data*

In the data set, 125 characters were variable, and 86 were parsimony informative. Third codon positions were the most informative characters (67 informative characters), followed by the first codon position (14 characters), and finally the second codon position (5 characters). Saturation of transition and transversion changes was checked by plotting the absolute number of changes of each codon position against patristic distances. There was no ingroup evidence of saturation at any of the three positions (not shown). The GTR-G model was selected as the best fit to the data set. Rate matrix parameters were: -lnL = 2374.7034; K = 9; AIC = 4767.4067. The base frequencies were: freqA = 0.2563; freqC = 0.2787; freqG = 0.1450; freqT = 0.3200. Among-site rate variation was approximated with gamma distribution shape parameter $\alpha$ = 1.1379. The phylogenetic tree obtained from the Bayesian analysis (Fig. 5) clearly shows two well-differentiated groups, with a posterior probability of branch support of 100 for the *X. variata* clade (including the Basin were presumably the type locality is found) and 100 for the clade which contains the new species *X. dibblenii*. The genetic distance among groups were $\bar{D}_{GTR}$ = 6.1% (range 4.7-7.1%) and $\bar{D}_P$ =5.5% (range 4.7-6.6%). The divergences within groups were $\bar{D}_{GTR}$ = 2% (range 0.2-3.2) and $D_P$ =1.5% (0.2- 3.25) within *Xenotoca variata* populations while it was smaller among populations of *X. dibblenii* $\bar{D}_{GTR}$ = 0.3% (range 0.09-0.6%) and $\bar{D}_P$ = 0.3% (0.1-0.5%).

Both, meristic and molecular data are congruent in recognizing two well differentiated groups corresponding to the described species *X. variata* and the new taxon, *X. dibblenii*. Assuming a molecular clock of 0.9 million years per 1% pairwise differences, which is the generally accepted rate for the cytochrome *b* gene in goodeids (Doadrio and Domínguez, 2004; Domínguez-Domínguez et al., 2008a), *Xenotoca dibbleni* from the Cuitzeo basin diverged from *X. variata* in contiguous basins during the Miocene-Pliocene, between *ca*. 5 to 5.5 Ma. These results are not in accordance with findings made for other pairs of sister species or populations of freshwater fishes inhabiting the Cuitzeo basin and other contiguous drainages (e. g. Lerma river or Lake Zacapu). In those cases, divergence

events took place more recently, between *ca*. 0.5-1.8 Ma, as indicated by the populations of *Goodea atripinnis*, *Zoogoneticus quitzeoensis*, and *Skiffia lermae* from Zacapu with respect to Cuitzeo, to *ca.* 2.2 as indicated by the cladogenesis of *Allotoca zacapuensis* (Doadrio and Domínguez, 2004; Domínguez-Domínguez, 2006; 2008a,b,c). Although these differences were observed, the cladogenetic event that produced the split of the ancestor of *X. variata* and *X. dibblenii* suggests an ancient connection between Cuitzeo Lake and other regions of the Lerma River basin, with a subsequent closure of this connection that occurred probably during the late Pliocene and early Miocene. The dating for this cladogenetic event could be related with the origin and the geologic and climatic history of the Cuitzeo Lake endorheic basin whose formation is dated between *ca.* 7-8 Ma, although, the evolution of the ancient lacustrine zones in the Cuitzeo basin lasted about 5.5 Ma. During the late Pliocene and early Miocene, Cuitzeo Lake had a much larger extension and it was a deeper lake, experiencing a strong level decrease during the Pliocene as a result of the geological faults and dry climatic conditions in the region (Israde-Alcántara and Garduño-Monrroy, 1999); this pattern is in agreement with the faunal exchange and isolation of other lineages, as was previously proposed for the cladogenesis of *Neotoca bilineata*, a species suspected to be endemic from Cuitzeo Lake basin.

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FIGURE LEGENDS

**Figure 1.** Distribution range of *Xenotoca*; red circles correspond to historical occurrence points where specimens have not been collected in the last 5 years, gray circles correspond to population not included in the present study but in which specimens were collected in the last 5 years, black circles correspond to locations for *Xenotoca variata* included in the present study, and blue circles correspond to populations of *Xenotoca dibbleni* included in the present study. The numbers correspond to the localities shown in table 1

**Figure 2.** (A) Holotype, Male (CPUM-1529) and (B) Paratype, female (CPUM-1525) of *Xenotoca dibblenii* from La Mintzita Spring.

**Figure 3.** Plots of first *vs* two and two *vs* three principal components for 20 Burnaby corrected morphometric variables (males-top and females-bottom). In blue, Jesus Maria Stream; in black, Laja River; in red, La Mintzita Spring; in pink, San Cristobal Spring.

**Figure 4.** Plots of first *vs* two and two *vs* three principal components for 4 meristic variables (males-top and females-bottom). In blue, Jesus Maria Stream; in black, Laja River; in red, La Mintzita Spring; in pink, San Cristobal Spring.

**Figure 5.** Phylogenetic tree of 20 analysed specimens distributed in 16 localities of the genus *Xenotoca* recovered from cytochrome *b* sequences (1140 bp) according to the Bayesian analysis based on the best model of evolution that fit our data using the program Modeltest 3.7 (Posada and Crandall 1998). The numbers on the branches represent the Bayesian posterior probability.

Table 1. Localities and sample sizes for *Xenotoca* spp populations used for morphometric, meristic and genetic analysis. *Correspond to the area reported for the type locality for *X. variata.* ** Correspond to the type locality for *Xenoltoca dibblenii* sp. nov. +Correspond to sequences obtained from Genbank.

| Locality | Drainage | Morphometrics | | Meristics | | Genetics |
|---|---|---|---|---|---|---|
| | | Males | Females | Males | Females | |
| 1. Querendaro | Cuitzeo Lake | -- | -- | -- | -- | 1 |
| 2. La Mintzita Spring** | Cuitzeo Lake | 13 | 16 | 13 | 16 | 2 |
| 3. San Cristobal Spring | Cuitzeo Lake | 8 | 8 | 8 | 8 | 2 |
| 4. Belisario | Cuitzeo Lake | -- | -- | -- | -- | 1 |
| 5.- Zacapu | Zacapu Lake | -- | -- | -- | -- | 2 |
| 6. Yuriria | Middle Lerma River | -- | -- | -- | -- | 1 |
| 7. San Francisco del Rincón | Middle Lerma River | -- | -- | -- | -- | 1 |
| 8. Gallinero Dam | Middle Lerma River | -- | -- | -- | -- | 1 |
| 9. Allende Dam | Middle Lerma River | -- | -- | -- | -- | 1 |
| 10. Laja River at Salamanca* | Middle Lerma River | 14 | 5 | 14 | 5 | 1 |
| 11. Neutla Dam | Panuco River | -- | -- | -- | -- | 1 |
| 12. Tierra Quemada Stream | Panuco River | -- | -- | -- | -- | 2 |
| 13. Jesús María stream | Panuco River | 12 | 9 | 12 | 9 | 1 |
| 14. La Alberca lake | Chapala Lake | -- | -- | -- | -- | 1 |
| 15. Zacatecas | Aguanaval River | -- | -- | -- | -- | 1 |
| 16. Valenciana spring | Aguanaval River | -- | -- | -- | -- | 1 |

Table 2. Statistical parameters for morphometric characters in *Xenotoca dibbleni* sp. nov. Each variable is divided by standard length. Variable codes are given in the method section (SD = standard deviation). Holotype comes from San Cristobal spring population.

| | *Xenotoca dibbleni* | | | | | | | *Xenotoca variata* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 Females | | | 20 Males | | | 14 Females | | | 26 Males | | |
| Variables | Holotype | Range | Mean | SD | Range | Mean | SD | Range | Mean | SD | Range | Mean | SD |
| SL | 55 | 24.62-69.56 | 48.388 | 13.506 | 22.22-54.91 | 41.033 | 9.383 | 27.54-46.56 | 34.171 | 5.604 | 25.89-45.96 | 32.192 | 5.811 |
| HL | 0.248 | 0.231-0.307 | 0.269 | 0.020 | 0.267-0.326 | 0.285 | 0.016 | 0.259-0.320 | 0.287 | 0.019 | 0.279-0.318 | 0.297 | 0.012 |
| PrOL | 0.0691 | 0.059-0.078 | 0.071 | 0.005 | 0.054-0.080 | 0.071 | 0.007 | 0.062-0.082 | 0.072 | 0.006 | 0.053-0.091 | 0.077 | 0.008 |
| ED | 0.0828 | 0.079-0.110 | 0.084 | 0.010 | 0.85-0.100 | 0.092 | 0.005 | 0.081-0.105 | 0.096 | 0.007 | 0.089-0.114 | 0.102 | 0.005 |
| InOW | 0.0858 | 0.084-0120 | 0.101 | 0.011 | 0.90-0.126 | 0.107 | 0.011 | 0.096-0.136 | 0.116 | 0.012 | 0.102-0.135 | 0.115 | 0.009 |
| BD | 0.3111 | 0.237-0.333 | 0.293 | 0.025 | 0.284-0.347 | 0.308 | 0.019 | 0.261-0.352 | 0.308 | 0.029 | 0.273-0.329 | 0.321 | 0.026 |
| BLD | 0.1564 | 0.110-0.163 | 0.139 | 0.015 | 0.128-0.155 | 0.140 | 0.008 | 0.135-0.160 | 0.149 | 0.008 | 0.125-0.175 | 0.152 | 0.012 |
| PAD | 0.1830 | 0.148-0.215 | 0.185 | 0.017 | 0.172-0.219 | 0.191 | 0.014 | 0.151-0.213 | 0.181 | 0.018 | 0.163-0.223 | 0.186 | 0.015 |
| PDD | 0.3344 | 0.236-0.339 | 0.303 | 0.024 | 0.298-0.359 | 0.319 | 0.018 | 0.280-0.368 | 0.330 | 0.025 | 0.286-0.408 | 0.338 | 0.029 |
| PODE | 0.3678 | 0.284-0.337 | 0.346 | 0.021 | 0.340-0.390 | 0.358 | 0.015 | 0.311-0.407 | 0.361 | 0.023 | 0.332-0.428 | 0.372 | 0.023 |
| DAD | 0.3146 | 0.234-0.302 | 0.271 | 0.019 | 0.278-0.340 | 0.304 | 0.019 | 0.245-0.321 | 0.291 | 0.020 | 0.282-0.376 | 0.318 | 0.023 |
| DOAE | 0.2870 | 0.226-0.287 | 0.254 | 0.017 | 0.244-0.301 | 0.272 | 0.016 | 0.238-0.308 | 0.274 | 0.018 | 0.250-0.329 | 0.287 | 0.020 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DFL | 0.1614 | 0.132-0.158 | 0.148 | 0.006 | 0.148-0.185 | 0.163 | 0.011 | 0.137-0.170 | 0.155 | 0.012 | 0.143-0.202 | 0.167 | 0.013 |
| DEAO | 0.2598 | 0.188-0.260 | 0.230 | 0.019 | 0.237-0.275 | 0.253 | 0.010 | 0.228-0.276 | 0.250 | 0.014 | 0.230-0.299 | 0.264 | 0.017 |
| AFL | 0.1082 | 0.069-0.115 | 0.091 | 0.013 | 0.080-0.123 | 0.100 | 0.012 | 0.082-0.111 | 0.099 | 0.009 | 0.094-0.122 | 0.111 | 0.007 |
| AEDE | 0.1818 | 0.134-0.193 | 0.169 | 0.015 | 0.150-0.190 | 0.170 | 0.011 | 0.170-0.213 | 0.190 | 0.012 | 0.159-0.220 | 0.184 | 0.016 |
| EDUP | 0.2377 | 0.229-0.307 | 0.264 | 0.020 | 0.236-0.298 | 0.261 | 0.015 | 0.244-0.289 | 0.270 | 0.012 | 0.221-0.289 | 0.253 | 0.017 |
| EDLP | 0.3036 | 0.270-0.350 | 0.298 | 0.017 | 0.268-0.332 | 0.293 | 0.016 | 0.278-0.321 | 0.307 | 0.013 | 0.258-0.339 | 0.294 | 0.016 |
| EAUP | 0.2856 | 0.260-0325 | 0.297 | 0.017 | 0.264-0.333 | 0.293 | 0.018 | 0.283-0.324 | 0.301 | 0.013 | 0.274-0.309 | 0.289 | 0.011 |
| EALP | 0.2487 | 0.227-0.301 | 0.264 | 0.017 | 0.239-0.286 | 0.260 | 0.011 | 0.230-0.293 | 0.265 | 0.017 | 0.219-0.300 | 0.254 | 0.020 |
| D | | 14-15 | 14.29 | 0.46 | 13-15 | 14.04 | 0.38 | 12-14 | 13.07 | 0.61 | 12-14 | 12.92 | 0.64 |
| A | | 14-17 | 16.08 | 0.82 | 14-17 | 16.04 | 0.67 | 13-16 | 15.35 | 0.92 | 14-17 | 15.68 | 0.74 |
| P | | 12-16 | 14.75 | 1.11 | 11-16 | 14.71 | 1.42 | 13-16 | 14.57 | 1.08 | 12-16 | 14.36 | 0.95 |
| GR | | 17-24 | 20.95 | 2.07 | 17-21 | 19.42 | 1.28 | 12-16 | 14.28 | 1.13 | 12-16 | 13.96 | 1.17 |

Table 3. Molecular diagnostic characters for the cytochrome *b* gene in *Xenotoca variata* and *X. dibblenii*. Ts= transition and Tv= transversion
within the same base position.

| BP position / Species | 75 | 165 | 246 | 249 | 255 | 258 | 276 | 282 | 321 | 348 | 357 | 360 | 387 | 393 | 462 | 519 | 546 | 575 | 600 | 618 | 624 | 630 | 636 | 642 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Xenotoca variata* | T | C | G | C | C | A | T | C | T | A | A | T | A | T | T | T | C | C | G | C | T | A | C | C |
| *Xenotoca dibblenii* | C | T | A | T | T | G | C | T | C | G | T | C | T | C | C | C | T | T | A | T | C | G | T | T |
| Substitution type | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Tv | Ts | Tv | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts |

| BP position / Species | 711 | 780 | 837 | 903 | 907 | 915 | 916 | 930 | 957 | 960 | 993 | 997 | 1002 | 1041 | 1053 | 1065 | 1083 | 1095 | 1098 | 1125 | 1129 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Xenotoca variata* | T | C | A | T | G | C | A | T | T | C | C | A | C | C | C | T | A | T | T | G | C |
| *Xenotoca dibblenii* | C | T | C | C | A | T | G | A | G | T | T | G | T | T | T | C | G | C | C | A | T |
| Substitution type | Ts | Ts | Tv | Ts | Ts | Ts | Ts | Tv | Tv | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts | Ts |

Table 4. Two-way analysis of variance testing for sexual dimorphism, population variation and their interaction (Pop*Sex).

| Variable | Population | Sex | Pop*Sex |
|---|---|---|---|
| Meristics | | | |
| D | 106*** | 12.18*** | 3.49n.s |
| A | 8.53** | 0.034n.s | 1.31n.s |
| P | 1.39n.s | 0.41n.s | 0.16n.s |
| GR | 244*** | 26.75n.s | 9.87n.s |
| Morphometrics | | | |
| SL | 17.42*** | 12.75*** | 1.28n.s |
| HL | 9.93*** | 5.40* | 0.533n.s |
| PrOL | 11.17*** | 5.46* | 0.852n.s |
| ED | 9.19*** | 2.39n.s | 0.413n.s |
| InOW | 6.99*** | 3.83n.s | 0.32n.s |
| BD | 7.75*** | 3.90n.s | 0.315n.s |
| BLD | 7.87*** | 7.48*** | 0.566n.s |
| PAD | 13.17*** | 6.65* | 0.853n.s |
| PDD | 7.12*** | 4.06* | 0.464n.s |
| PODE | 9.94*** | 6.01* | 0.683n.s |
| DAD | 7.74*** | 0.761n.s | 0.23n.s |
| DOAE | 8.34*** | 3.37n.s | 0.324n.s |
| DFL | 13.16*** | 2.46n.s | 0.53n.s |

| | | | |
|---|---|---|---|
| DEAO | 8.77*** | 2.33n.s | 0.281n.s |
| AFL | 6.88*** | 1.4n.s | 0.44n.s |
| AEDE | 8.41*** | 10.3*** | 0.436n.s |
| EDUP | 23.95*** | 22.37*** | 2.02n.s |
| EDLP | 16.43*** | 14.93*** | 0.366n.s |
| EAUP | 17.65*** | 15.68*** | 1.29n.s |
| EALP | 22.67*** | 18.17*** | 1.66n.s |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$,

n.s. not significant differences

Table 5. Eigenvectors and eigenvalues for the first three principal components for 20 morphometric variables in males and females. Variable codes are given in the method section.

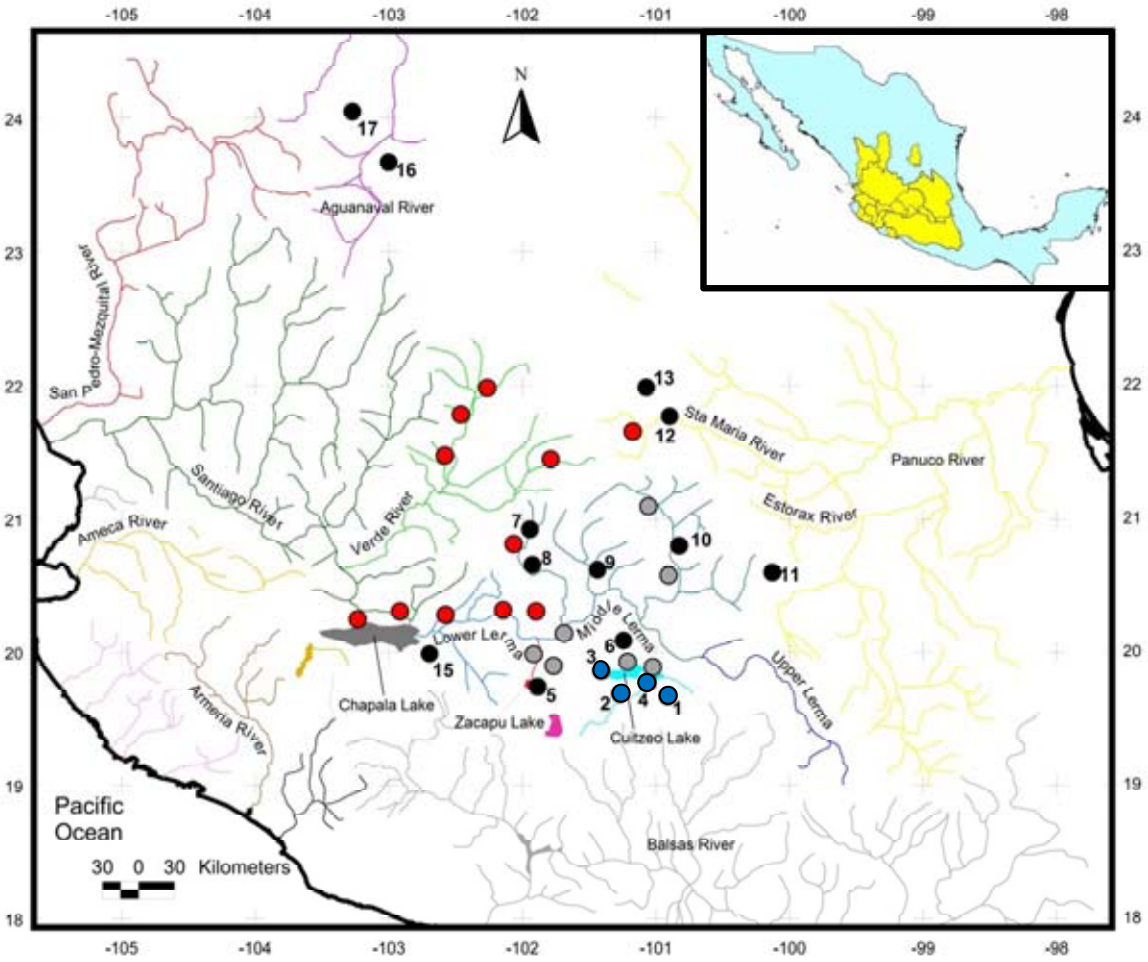
| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Eigenvectors | I | II | III | I | II | III |
| SL | 0.193 | -0.063 | 0.047 | 0.235 | -0.061 | 0.063 |
| HL | -0.002 | 0.020 | -0.002 | -0.034 | -0.063 | 0.138 |
| PrOL | -0.008 | 0.631 | 0.429 | 0.093 | -0.065 | 0.089 |
| ED | 0.104 | 0.169 | 0.205 | -0.037 | -0.032 | 0.171 |
| InOW | -0.092 | 0.513 | -0.389 | -0.178 | 0.422 | -0.775 |
| BD | -0.167 | -0.007 | -0.125 | -0.197 | 0.247 | 0.025 |
| BLD | -0.291 | -0.129 | -0.070 | -0.246 | -0.122 | 0.188 |
| PAD | 0.152 | -0.112 | -0.018 | 0.048 | 0.081 | 0.154 |
| PDD | -0.238 | 0.014 | -0.154 | -0.194 | 0.238 | 0.042 |
| PODE | -0.106 | -0.008 | -0.035 | -0.084 | 0.116 | 0.137 |
| DAD | -0.169 | -0.027 | -0.007 | -0.162 | 0.104 | 0.134 |
| DOAE | -0.200 | -0.193 | 0.037 | -0.144 | -0.090 | 0.127 |
| DFL | 0.113 | -0.048 | 0.505 | 0.162 | -0.108 | -0.028 |
| DEAO | -0.152 | -0.079 | -0.025 | -0.206 | -0.011 | 0.028 |
| AFL | -0.191 | -0.392 | 0.435 | -0.223 | -0.787 | -0.402 |
| AEDE | -0.227 | -0.074 | -0.221 | -0.271 | 0.017 | 0.021 |
| EDUP | 0.459 | -0.099 | -0.158 | 0.434 | -0.035 | -0.178 |

| | | | | | | |
|---|---|---|---|---|---|---|
| EDLP | 0.231 | -0.219 | -0.169 | 0.173 | 0.035 | 0.123 |
| EAUP | 0.244 | -0.112 | -0.161 | 0.251 | -0.028 | -0.070 |
| EALP | 0.474 | 0.020 | -0.026 | 0.486 | 0.055 | -0.097 |
| Eigenvalues | 0.021 | 0.011 | 0.009 | 0.028 | 0.011 | 0.008 |
| Percentage | 29.81 | 15.50 | 13.91 | 36.62 | 15.03 | 10.60 |
| Accumulated% | 29.81 | 45.31 | 59.22 | 36.62 | 51.65 | 62.25 |

Table 6. Eigenvectors and eigenvalues for the first three principal components for 4 meristic variables. Variable codes are given in the method section.

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Eigenvectors | I | II | III | I | II | III |
| D | -0.592 | -0.392 | -0.155 | -0.62 | 0.29 | 0.03 |
| A | -0.386 | 0.694 | 0.586 | -0.49 | -0.47 | 0.69 |
| P | -0.375 | 0.605 | -0.689 | -0.24 | -0.73 | -0.63 |
| GR | -0.600 | -0.370 | 0.137 | -0.56 | 0.41 | -0.36 |
| Eigenvalues | 1.93 | 1.04 | 0.718 | 1.92 | 1.29 | 0.49 |
| Percentage | 48.22 | 26.05 | 17.95 | 47.99 | 32.28 | 12.33 |
| Accumulated% | 48.23 | 74.28 | 92.24 | 47.99 | 80.27 | 92.6 |

Figure 1.


-105
-104
-103
-102
-101
-100
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-98
24
23
22
21
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18
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17
16
Aguanaval River
San Pedro-Mezquital River
Santiago River
Ameca River
Verde River
13
12
Sta Maria River
Panuco River
Estorax River
7
8
9
10
11
Lower Lerma
Middle Lerma
Upper Lerma
15
5
3
6
2
4
1
Chapala Lake
Zacapu Lake
Cuitzeo Lake
Armeria River
Balsas River
Pacific
Ocean
30 0 30 Kilometers

Figure 2

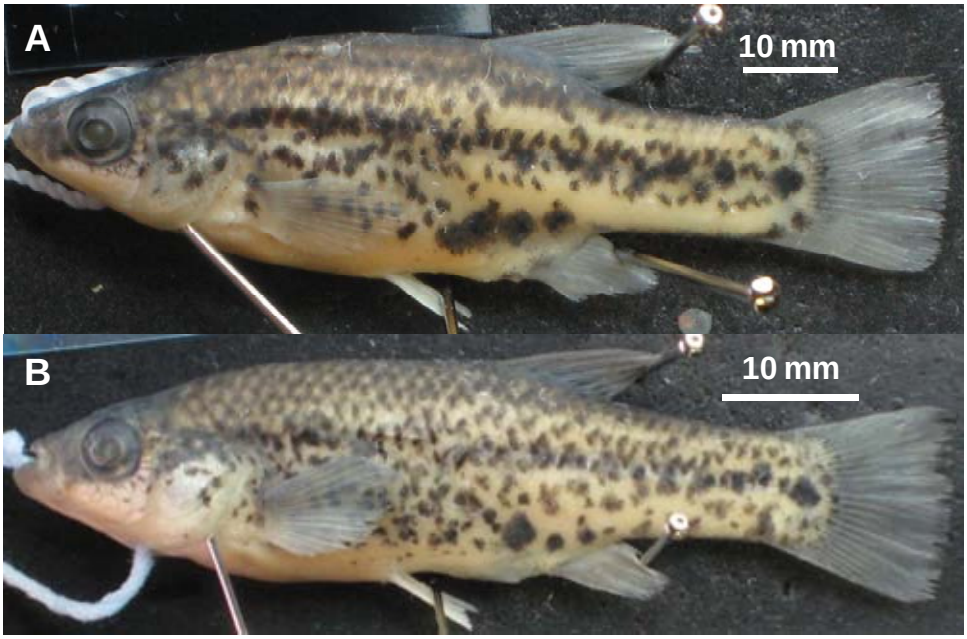


Figure 3.

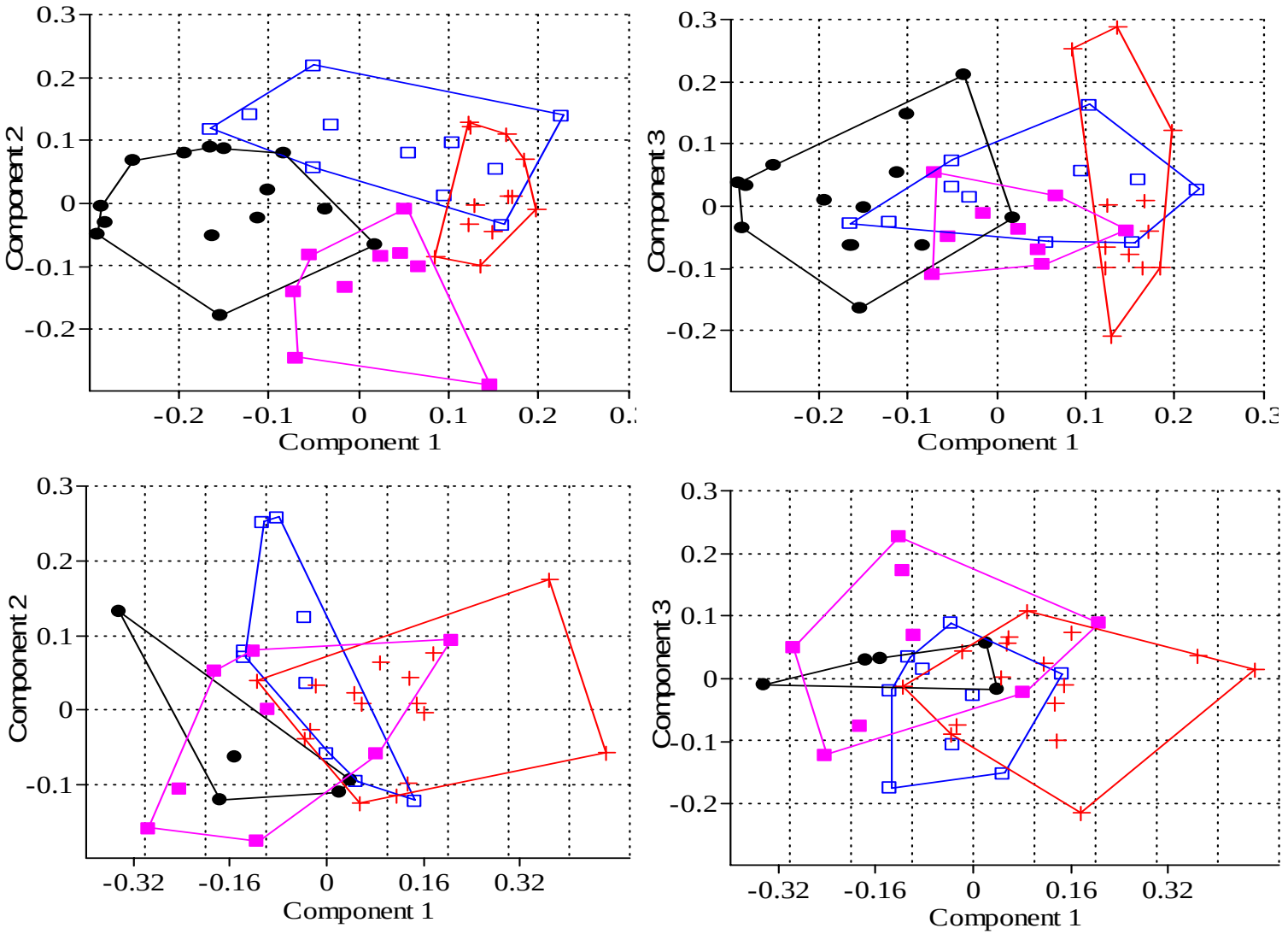

0.3
0.2
0.1
0
-0.1
-0.2
Component 2
-0.2 -0.1 0 0.1 0.2
Component 1
Component 3
-0.32 -0.16 0 0.16 0.32

Figure 4.

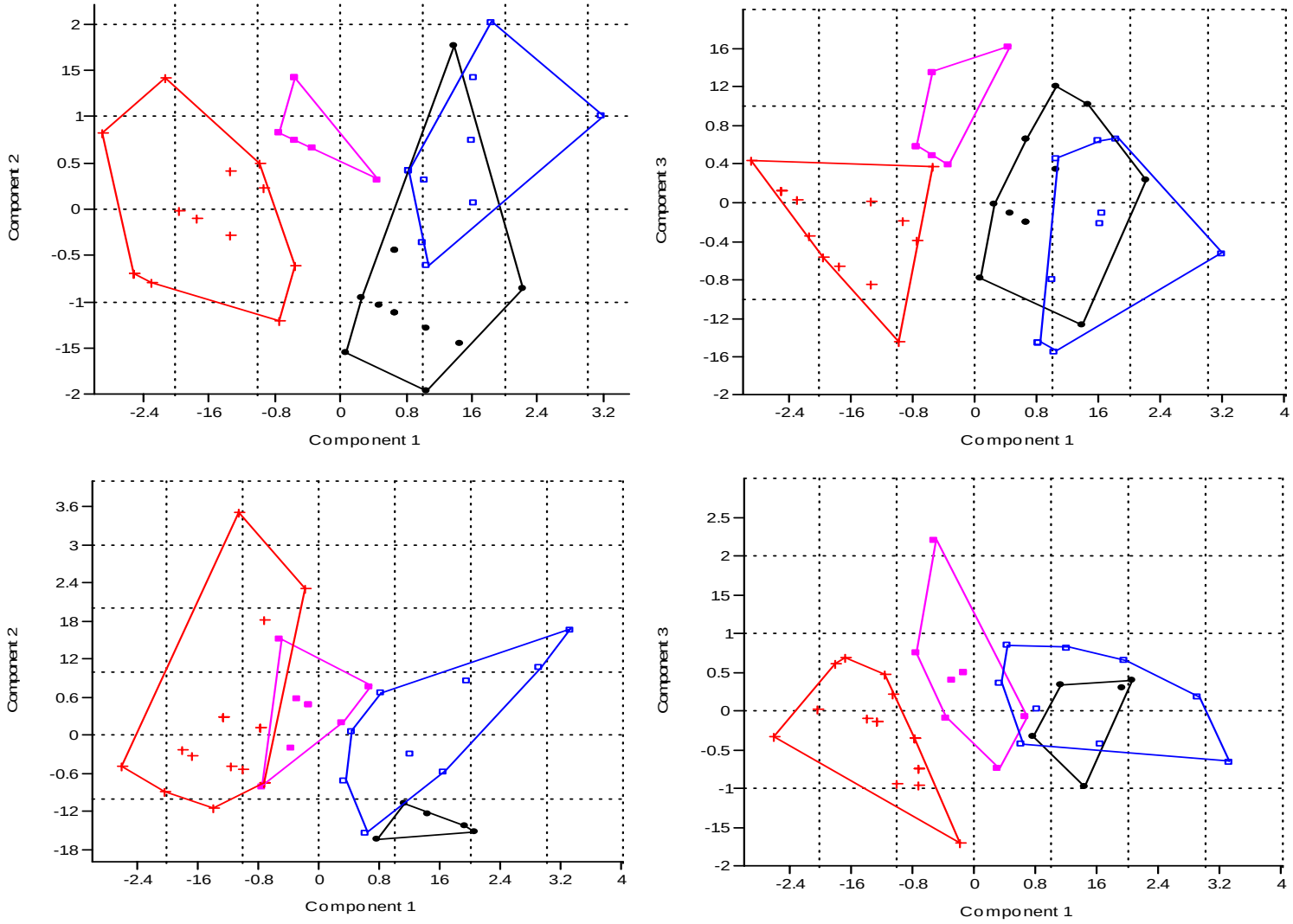
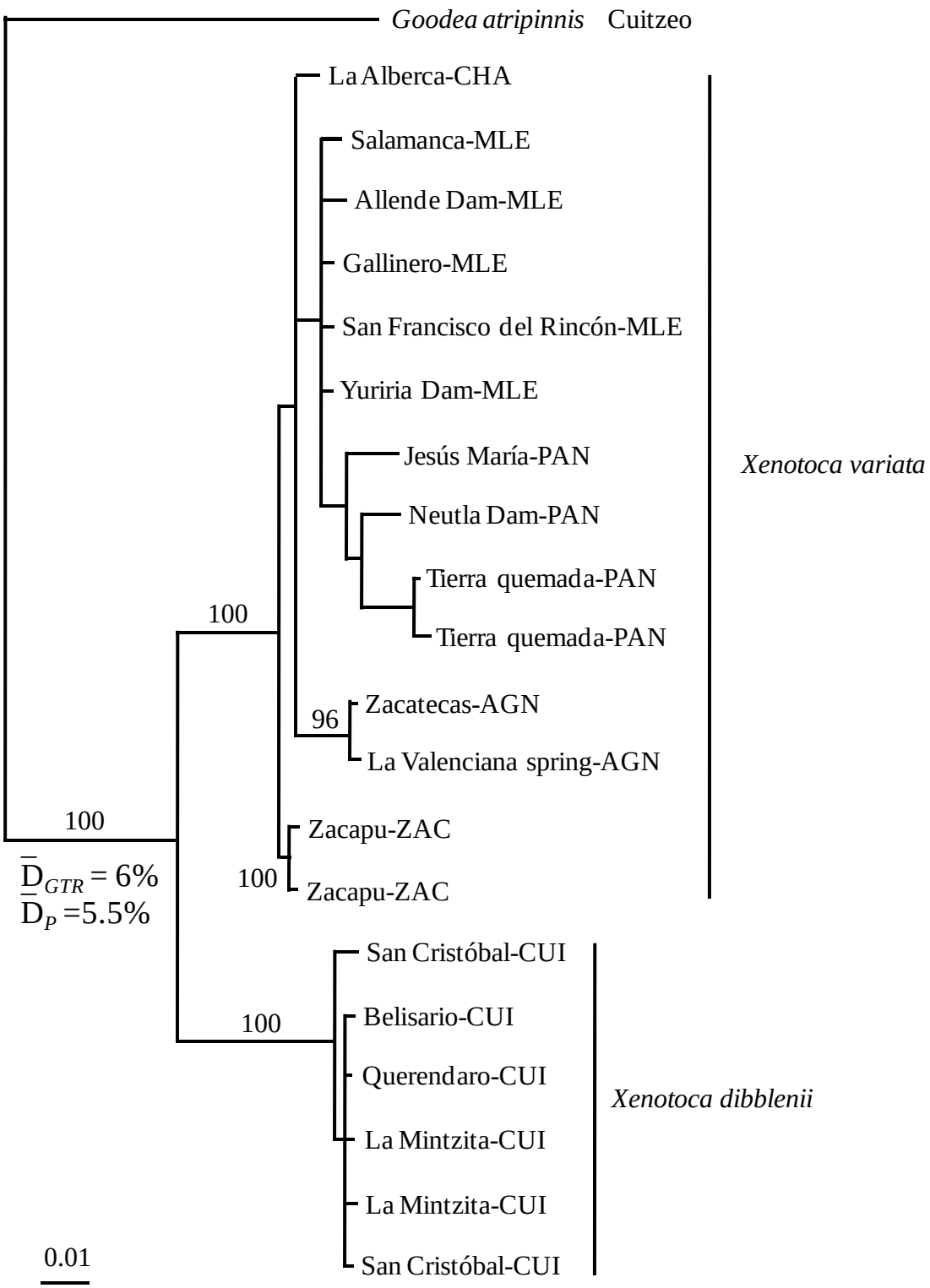

Component 2
Component 1
Component 3
Component 1
Component 2
Component 1
Component 3
Component 1

Figure 5


*Goodea atripinnis* Cuitzeo
La Alberca-CHA
Salamanca-MLE
Allende Dam-MLE
Gallinero-MLE
San Francisco del Rincón-MLE
Yuriria Dam-MLE
Jesús María-PAN
Neutla Dam-PAN
Tierra quemada-PAN
Tierra quemada-PAN
*Xenotoca variata*
100
96
Zacatecas-AGN
La Valenciana spring-AGN
100
Zacapu-ZAC
100
Zacapu-ZAC
$\overline{D}_{GTR}$ = 6%
$\overline{D}_{P}$ =5.5%
San Cristóbal-CUI
100
Belisario-CUI
Querendaro-CUI
*Xenotoca dibblenii*
La Mintzita-CUI
La Mintzita-CUI
San Cristóbal-CUI
0.01

# DISCUSION GENERAL E INTEGRACION DE RESULTADOS

## DISCUSION GENERAL E INTEGRACION DE RESULTADOS

A continuación se presenta un análisis general de los resultados que se obtuvieron en el presente estudio, donde se indica con numeros romanos (I-V) el trabajo donde una idea en particular es expresada y discutida y que corresponde con los capitulos que conforman la presente tesis.

La Mesa Central de México comprende una de las extensiones más amplias de tierras alta tropicales del mundo. Esta región ha experimentado una intensa actividad tecto-volcánica que se inicio en el Mioceno y que continua hasta nuestros días, siendo considerada una de las zonas más activas desde el punto de vista geológico. A su vez ha sido afectada por fuertes variaciones climáticas a nivel mundial, continental y regional. Toda esta dinámica ha generado un fuerte impacto en la geomorfología de la zona, lo que ha influenciado eventos de formación, destrucción y compartimentalización de cuerpos de agua a diferentes escalas de espacio y tiempo. Aunado a la fuerte actividad tecto-volcánica y climática de la región, la privilegiada posición y extensión latitudinal de México, lo que lo sitúa en una zona de transición entre la fauna Neártica y Neotropical, y a la inclusión de grupos de peces de origen marino dentro de los cuerpos de agua dulce de la Mesa Central, han influenciado procesos biogeográficos y evolutivos complejos dentro de la fauna acuática de la región, dichos eventos de vicarianza, dispersión, pulso de taxones y presiones selectivas, han hecho de la Mesa Central una de las regiones más importante en la diversidad de peces de agua dulce en el mundo, considerada por el World Conservation Monitoring Centre como una región de especial interés para la conservación de los peces de agua dulce. De manera particular, la fauna íctica está representada por aproximadamente 100 especies nativas, de las cuales el 70% son endémicas de la región, algunas de ellas con una distribución muy restringida, más aun, todas las especies dentro de la subfamilia Goodeinae son endémicas de esta región y áreas adyacentes.

Los diferentes resultados obtenidos en los diferentes capitulos que conforman el presente trabajo de tesis confirman que la dinámica de formación, destrucción, compartimentalización y captura de cuerpos de agua, inducida por la compleja historia geológica y climática de la Mesa Central de México, ha generado una compleja historia evolutiva y biogeográfica en los componentes ictiofaunísticos de esta región (ver artículos I y IV). Los datos filogeográfícos y demográficos de las tres especies de Goodeidos

estudiadas, *Xenotoca variata, Alloophorus robustus* (IV) y *Zoogoneticus quitzeoensis* (I), ponen de manifiesto que la biogeografía histórica de la Mesa Central de México, y la historia evolutiva de las especies de peces que habitan en cuencas hidrológicas en esta región del país, no puede ser explicada tan solo por eventos geológicos o climáticos únicos que afectaron de la misma forma a todas las especies o poblaciones codistribuidas. Por el contrario, los resultados del presente trabajo, habiendo analizado a tres especies de goodeidos que representan uno de los grupos de peces dulceacuícolas mas característicos de la región, demostraron que, a pesar de que las diferentes especies compartían áreas de distribución en el pasado, y que estuvieron sujetas a los mismos eventos que determinaron la fisiografía y evolución de las cuencas donde éstos habitaban, las tres especies exhiben patrones filogeográficos únicos, existiendo escasa o nula concordancia entre algunos de ellos. Las diferencias filogeográficas, demográficas y biogeográficas encontradas pueden deberse no solo a la naturaleza de los eventos geológicos y climáticos que debieron afectar a las especies, sino que parecen estar también determinadas por las características intrínsecas de las especies, como lo son los aspectos biológicos, ecológicos y etológicos. Estas diferencias intrínsecas de las especies decididamente influyeron de manera importante en la historia evolutiva y demográfica de algunas de ellas (IV), permitiendo que cada especie alcanzara diferentes capacidades de adaptarse para sobrevivir en dichas condiciones cambiantes o bien, a través de los corredores existentes y por lo tanto a la posibilidad de dispersarse por estas nuevas rutas. De igual forma, estas diferencias intrínsecas pueden inducir un aislamiento reproductivo y la segregación ecológica en algunos grupos durante un contacto secundario posterior al aislamiento. Por ello, los resultados de la presente investigación sugieren que la historia evolutiva y demográfica de los Goodeinae este influenciada tanto por eventos de pulso de taxones, vicarianza, extinciones, como de eventos de selección sexual y segregación ecológica ocurridos en diversas escalas de tiempo y espacio.

De manera particular, *Xenotoca variata* (IV) y *Zoogoneticus quitzeoensis* (I) surgen como dos especies cuyas poblaciones distribuidas en distintas cuencas hidrológicas poseen una fuerte estructura genética y las poblaciones de ambos están divididas en dos linajes evolutivos independientes y altamente divergentes. Las poblaciones dentro de los linajes de ambas especies, con excepción del linaje II para *X. variata*, también presentan una

importante estructuración, la cual, de manera general, sigue un arreglo a nivel de cuencas. Ambas especies presentan una elevada diversidad genética en la mayoría de sus poblaciones. Por otra parte, la especie *Alloophorus robustus* presenta poca diferenciación entre los grupos genéticos identificados, más aun, dichos grupos genéticos (linajes y clados), carecen de estructura interna, compartiendo haplotipos entre diferentes cuencas, y presentando una diversidad genética muy reducida en la mayoría de sus poblaciones.

La evidencia presentada también demuestra que la historia demográfica de las tres especies de goodeidos, en especial la de *A. robustus* y *X. variata* (IV), está vinculada con la historia de oscilación climática de la zona, así como con eventos de deriva génica inducida por la actividad humana (I), lo que refuerza la idea que no solo los eventos geológicos, o aquellos de conexión y aislamiento de cuerpos de agua pueden producir cambios en la estructura genética de las poblaciones, sino que eventos como la reducción del tamaño efectivo de la población, endogamia o cuellos de botella pueden afectar de manera importante la estructura genética de las poblaciones y con ello las interpretaciones filogeográficas.

El presente trabajo también demuestra que los datos moleculares y los análisis filogeográficos pueden ser usados como una herramienta para la identificación y delimitación de especies. En este sentido, el uso de marcadores moleculares permitió la identificación de linages altamente divergentes dentro de las poblaciones estudiadas de las especies *Z. quitzeoensis* y *X. variata*. Estos datos fueron posteriormente corroborados por datos morfológicos y biogeográficos, llevando a la descripción de dos nuevos taxones, *Z. purepechus* habitante de las cuencas de los ríos Ameca, Armería, Santiago y bajo Lerma, así como en el lago de Chapala, en los estados de Jalisco y Michoacán (III) y *X. dibblenii* (V) endémico de la cuenca del Lago de Cuitzeo, en los estados de Michoacán y Jalisco. De esta manera, el uso combinado de datos moleculares y morfológicos demostraron ser una poderosa herramienta para la obtención de hipótesis más robustas en el ámbito de la taxonomía y delimitación de especies, las cuales no habían sido desenmascaradas mediante métodos tradicionales en taxonomía.

Los datos moleculares y la filogeografía también demostraron ser una herramienta eficaz en el reconocimiento de unidades de conservación. Las políticas de conservación en la mayoría de los países, por lo general, se llevan a cabo tomando en cuenta a la especie

como unidad operativa en las políticas de conservación, asumiendo que las poblaciones dentro de una especie son una parte abstracta y no dinámica dentro de dicha especie, negando de antemano la importancia que tienen los procesos microevolutivos o adaptativos en la sobrevivencia a largo plazo de la especie en cuestión, y más aun, eliminando de manera automática la posibilidad de conservar los procesos evolutivos que han llevado o bien, que están llevando a la formación de nuevas especies. La fuerte perturbación humana que ha existido en cuerpos de agua del Centro de México ha dejado su huella en la diversidad y variabilidad genética de las poblaciones que los habitan. Estos eventos de perturbación humana han generado no solo la erosión genética de las poblaciones de peces, sino que han promovido su diferenciación genética (II). En este sentido, el presente trabajo arrojó información importante para la planeación de las estrategias de conservación de la fauna acuática en ecosistemas del centro de México. Por un lado, existen poblaciones ecológicamente viables, pero que, desde el punto de vista genético, su viabilidad no está del todo asegurada (II), mientras que por el otro lado existen poblaciones genéticamente viables, pero que desde el punto de vista ecológico dicha viabilidad no está asegurada. Asimismo, mientras que para las especies *Zoogoneticus quitzeoensis* (I y II) y *Xenotoca variata* (IV) la conservación de los linajes o clados independientes encontrados, de manera general, puede realizarse a nivel de cuenca, manteniendo la diversidad interna de las poblaciones y promviendo el intercambio de organismos entre poblaciones dentro de las cuencas, para la especie *Alloophorus robustus* esto no es suficiente. En el caso de esta especie es necesario considerar la posibilidad de migración y el intercambio de organismos entre cuencas, por lo que en este caso los corredores que conectan las localidades que esta especie habita, deben ser conservados con la finalidad de asegurar una calidad ambiental tal que permita el movimiento de estas especies a través de ellos (v. gr. Río Lerma).

Para finalizar, deseamos tan solo enfatizar que trabajos como el presente aportan una cantidad importante de información para describir los patrones y procesos que han determinado la historia evolutiva y biogeográfica de un grupo de organismos en particular, en este caso, de la sufamilia Goodeinae que, como se describio anteriormente, representa el grupo mas representativo de la fauna ictiológica dulceacuícola de la Mesa Central de México. Adicionalmente, los datos generados aquí aportan elementos importantes para describir los complejos patrones de biogeografía histórica de la fauna dulceacuícola de esta

región geográfica, misma que es de gran utilidad para analizarse en el conjunto de datos que se han aportado y se están aportando con otros componentes bióticos, como otros grupos de peces, particularmente de las familias Cyprinidae e Ictaluridae, pero también con otros organismos como helmintos, crustáceos, anfibios y reptiles, etc.

# CONCLUSIONES

Como resultado del trabajo presentado en esta tesis se llego a las siguientes conclusiones generales para los distintos rubros que fueron abordados:

**Filogeografía**

- Las tres especies estudiadas, de manera general, no presentan patrones filogeográficos concordantes en las áreas donde se co-distribuyen.
- La historia evolutiva de las tres especies ha estado parcialmente ligada a la fuerte actividad tectónica, volcánica y climática de la zona.
- Las características intrínsecas de las especies y las fuertes perturbaciones humanas en los cuerpos de agua de la zona, también han influido de manera importante la historia evolutiva y demográfica de las tres especies estudiadas, y por ende en la reconstrucción filogeográfica.
- Los cambios climáticos del Pleistoceno han influenciado de manera importante la historia demográfica de las poblaciones estudiadas dentro de al menos dos especies estudiadas, *Xenotoca variata* y *Alloophorus robustus*.
- Las especies *Zoogoneticus quitzeoensis* y *Xenotoca variata* presentan una fuerte estructura genética entre sus poblaciones. Dicha estructura, de manera general, se presenta a nivel de cuencas, con poco o nulo intercambio genético entre los clados identificados. De igual forma, estas dos especies tienen una alta diversidad y variabilidad genética.
- La especie *Alloophorus robustus* al contrario, presenta poca estructura entre las poblaciones estudiadas. La poca estructura encontrada no sigue a su vez un patrón a nivel de cuencas, presentando un elevado flujo genético entre poblaciones. Esta especie presenta una baja diversidad y variabilidad genética.

**Biogeografía**

- La historia biogeográfica de las tres especie ha estado influenciada de manera importante por eventos geológicos y climáticos ocurridos en la zona en los últimos ocho millones de años, sin embargo, características intrínsecas de las especies, como lo son la selección sexual o segregación ecológica, pudieron influir de manera decisiva en dicha historia.
- A pesar que se obtuvieron algunos eventos biogeográficos que aparentemente influyeron de la misma forma la historia biogeográfica temprana de a al menos dos de las especies

estudiadas (como formación de barreras o intercambio de fauna entre cuencas actualmente separadas), no se obtuvieron patrones biogeográficos concordantes bien definidos entre las tres especies. Por el contrario, de forma general se obtuvo que cada especie sigue patrones biogeográficos independientes, lo que puede deberse a que otros factores, como las características intrínsecas de las especies, pudieron afectar dicha historia antes o después de un evento de aislamiento o migración.

- El único patrón que se repite en las tres especies es la segregación de linages o clados entre los lagos distribuidos en el oeste de Michoacán (v.gr. Cuitzeo, Zacapu, Patzcuaro y Zirahuen), con respecto a las demás zonas estudiadas en el presente trabajo (v.gr. Lerma, Chapala, Ameca, etc), donde siempre se encontró una separación entre estas regiones, aunque sus relaciones internas no son concordantes.
- Eventos geológicos como la formación de fallas y grabens (v.gr. Fallas de Ameca, San Marcos, Penjamillo), tuvieron una influencia importante en la historia biogeográfica de las especies, principalmente influyendo en proceso de vicarianza entre diferentes grupos.
- Eventos de actividad volcánica (v.gr. actividad en el Corredor Tarasco o en el volcán Las Ventanas), también pudieron tener una influencia importante como generadores de procesos de vicarianza entre las poblaciones de las especies estudiadas.
- La captura de cabeceras de Ríos por cuencas aledañas parece ser una de las principales vías de dispersión dentro de las poblaciones que habitan cuencas contiguas.
- Los cambios climáticos ocurridos en la zona, como el evento húmedo y posteriormente seco ocurrido entre *ca.* 1.8 y 0.9 millones de años, parece haber influido en la historia biogeográfica de las especies, promoviendo eventos de dispersión y vicarianza mediante la conexión y posterior aislamiento de cuencas contiguas.

**Conservación**

- La fuertes alteraciones ambientales ocurridas en los últimos 200 años (v.gr. desecación de cuerpos de agua, introducción de especies exóticas) han tenido, y siguen teniendo, un fuerte impacto en las poblaciones de las especies de peces estudiadas, lo cual repercute de manera negativa en la "salud" genética de las especies y sus poblaciones, lo que puede llevar a una perdida de la variabilidad genética de la especie como tal, y con ello reducir la posibilidad de adaptación de dicha especie a las condiciones cambiantes de su medio.

- Las zonas de manantiales o cuerpos de agua alimentados por manantiales, aparentemente están sirviendo como zonas de refugio para las especies nativas, ya que las condiciones de los Ríos u otros cuerpos de agua son muy pobres. Sin embargo, de forma general, estas zonas, por su reducido tamaño o su desecación intencional con fines agrícolas, son las que tienen la menor variabilidad genética, repercutiendo en las posibilidades de sobrevivencia de dichas poblaciones a largo plazo.
- Dentro de las áreas estudiadas se observó que prácticamente cada cuenca o región debe ser considerada como una Unidad de Conservación independiente, sin embargo, el intercambio genético entre las poblaciones dentro de cada Unidad de Conservación (OCU por sus siglas en inglés) es muy importante para poder mantener la variabilidad genética de cada OCU. Más aun, para la conservación de *Alloophorus robustus* es imprescindible mantener la conectividad e intercambio genético entre OCU's, tratando de mantener el índice de migración de esta especie.
- Las políticas de conservación deben enfocarse en la identificación de áreas estratégicas, cuyo manejo y cuidado sean factibles, para conservar de mejor manera a cada una de las especies y su variabilidad genética intraespecífica. De igual forma deben plantearse programas de restauración ecológica que lleven no solo a la recuperación de áreas especificas, sino a la recuperación de corredores biológicos entre dichas áreas, de manera que se pueda mantener el intercambio genético y así mantener la diversidad genética de especies como *A. robustus*.

**Sistemática**

- Se describe una nueva especie, *Zoogoneticus purepechus* con base en caracteres meristicos, morfológicos y genéticos. La nueva especie presenta distancias genéticas de DHKY = 3.4% (3-3.8%) con respecto a *Z. quitzeoensis*, su especie hermana. En los análisis morfológicos se identificaron dos morfotipos, uno perteneciente a la nueva especie y otra a *Z. quitzeoensis*. Las nueva especie difiere de la ya descrita por tener una distancia preorbital más corta (PrOL/SL = 0.05 -0.06), la base de la aleta dorsal más larga (DFL/SL = 0.17-0.20) y presentar entre 13 y 14 radios en la aleta dorsal. La nueva especie difiere de las dos especies descritas en el género (*Zoogoneticus tequila* y *Z. quitzeoensis*) en 10 posiciones nucleotídicas fijadas para el gen citocromo b. *Zoogoneticus purhepechus* se distribuye en

las cuencas de los ríos Ameca, Armería, Santiago y Bajo Lerma, así como en el lago de Chapala.

- Se describió una nueva especie, *Xenotoca diblenii*, con base en caracteres morfológicos, meristicos y genéticos. Esta especie es endémica de la cuenca del Lago de Cuitzeo. La nueva especie es morfológicamente muy similar a *X. variata* pero difiere en presentar 14 y raramente 13 o 15 radios en la aleta dorsal y por presentar 21 y raramente 17-20 o 22-24 branquiespinas en el primer arco branquial. La distancia genética entre *X. variata* y *X. diblenii* es de $\bar{D}$GTR = 6.1% and $\bar{D}$P =5.5%. La nueva especie presenta 45 posiciones nucleotidicas fijas para el gen citocromo b.