

Report of *Xiphophorus hellerii* in the arid Metztitlán Canyon Biosphere Reserve in Mexico

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Abstract Biosphere reserves have been established around the world as a means to protect sensitive or threatened ecoregions and the biodiversity within them. As such, the organisms occurring within these reserves are important from a conservation perspective as they often represent endemic species or remnant populations. Here we provide genetic evidence of widespread occurrence of *Xiphophorus hellerii* within the Metztitlán Canyon Biosphere Reserve in Hidalgo, Mexico. One nuclear and two mitochondrial genes were sequenced from four populations within the canyon and confirmed that the specimens collected were *X. hellerii*. When compared to published sequences of *X. hellerii* from multiple localities within the documented natural range of the species, the specimens from Metztitlán were found to exhibit between 0 and 1.6 % sequence divergence. Possible scenarios for colonization of the canyon and conservation implications are discussed.

Keywords Conservation · Non-native species · Poeciliidae

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Introduction

Natural protected areas such as Biosphere Reserves are intended to conserve the biological integrity of an area and promote the sustainable use of natural resources with particular emphasis on local community development and involvement. One such protected area is the Reserva de la Biosfera Barranca de Metztitlán or Metztitlán Canyon Biosphere Reserve, in Hidalgo, Mexico. The reserve covers an area of approximately 96 000 ha and was designated as a biosphere reserve in 2000. The reserve is situated in the rain shadow of the Sierra Madre Oriental, producing an arid climate and receiving just one quarter of the rainfall of nearby areas located at higher elevations within the Sierra Madre Oriental (CONANP 2003). However, the canyon is innervated by a number of small streams coming down from the mountains that converge on the Metztitlán River in the valley floor and that form a part of the larger Rio Tulancingo watershed within the Rio Panuco drainage. Several streams run dry during parts of the year and only flow freely during rainy periods or during isolated, extreme rainfall events although others flow year-round.

Within Metztitlán Canyon is the Laguna de Metztitlán (Metztitlán Lake), which is an important fishery for many in the local communities. However, prior to its designation as a Biosphere Reserve, regular introductions of non-native species including tilapia, carp, and catfish were conducted by the government and, because native species are no longer available in sufficient numbers, these species are now cultivated in the lake in floating cages. This was done in order to supplement low numbers of native species, due in large part to

significant fluctuations of the water level that occasionally even leave the lake dry (Ibáñez-Aguirre et al. 2002; CONANP 2003). The effects of these introductions both direct and indirect are not entirely known.

Here we provide one of the first reports of occurrence of the live bearing fish, *Xiphophorus helleri* (Haekel), in the Metztitlán Canyon Biosphere Reserve. *Xiphophorus helleri* is one of the most recognized species of *Xiphophorus* largely due to its presence in the aquarium hobby around the world, but also within the scientific community particularly in the literature on sexual selection (Basolo 1990; Rosenthal and Evans 1998; Royle et al. 2008; Tatarenkov et al. 2008; Walling et al. 2008). It is currently recognized natural range - one of the broadest of any *Xiphophorus* - is throughout the eastern part of Mexico, bounded on the west by the Sierra Madre Oriental mountains, to the north by the Trans-Volcanic Belt, and extending as far south as Honduras and Guatemala (Kallman and Kazianis 2006). However, due to its popularity in the pet trade, feral populations of *X. hellerii* have been reported well outside of its range around the world from Hawaii to Australia (Poort et al. 2006; Simmons et al. 2008). Additionally, records of *X. hellerii* in other parts of Mexico that lie outside of the described range, such as Distrito Federal, Ixmiquilpan (Hidalgo), among others, have been reported (<http://www.gbif.org/>). These instances are almost certainly due to accidental or intentional introduction for ornamental use or as pet fish are discarded. Only recently have specimens morphologically resembling *X. hellerii* been reported from sixteen localities within the Metztitlán Canyon (Miranda et al. 2012).

We used DNA sequencing for comparison with published sequences of *X. hellerii* to confirm the identity of specimens collected within the Metztitlán Canyon. Specimens were found to be widespread in streams throughout the reserve from headwaters to the valley floor. Given the unexpected discovery of *X. hellerii* within this arid environment beyond the Sierra Madre Oriental, the origin and consequences of its widespread distribution inside the Metztitlán Canyon merit consideration.

Methods

Specimen collection, DNA extraction and sequencing

Adult and juvenile fish were collected in 2008 by electrofishing, dip nets, and throw nets (attaraya) in multiple

sites throughout the Metztitlán Canyon (see Miranda et al. 2012 for details). Specimens were then euthanized and preserved in 95 % ethanol. Preliminary identification was made using information on morphology from Wischnath (1993). For DNA extractions a small portion of the caudal fin was removed and whole genomic DNA was extracted using proteinase K digestion followed by isopropanol extraction and washing with 70 % ethyl alcohol. Specimens were sequenced for partial regions of the same three genes used in Meyer et al. (1994): mitochondrial control region (CR), cytochrome B oxidase (CYTB) and tyrosine kinase (XSRC). Primer sequences for CR and XSRC were as in Meyer et al. (1994 and references therein). Primers Glu31 (5'-TGRCTTGAAAAACCACCGTTGT-3') and Poec15931 (5'-CCCTCGACTTTCGGTTTACAAG-3') were used to amplify CYTB (Jones and Johnson 2009). We sequenced two individuals from each of four populations representing four separate stream reaches within the canyon (Table 1).

All genes were amplified in 25 uL total reaction volume with the following PCR conditions: 2.5 mM MgCl₂, 0.25 mM each forward and reverse primer, 0.1 uM each dNTP, 0.625 U Taq polymerase and 20–30 ng genomic DNA. All PCRs were done on a MyCycler thermocycler (BioRad) with initial denaturation at 94°C for 6 min followed by 35 cycles of 94 °C for 45 s, 56 °C for 60 s, 72 °C for 90 s with a final elongation step at 72 °C for 6 min. Following all reactions, PCR products were verified on a 2 % agarose gel and then sequenced with the forward and reverse primer by the Nevada Genomics Center at the University of Nevada-Reno.

Sequence comparisons

In order to confirm that the specimens collected were *X. hellerii*, sequences obtained from specimens from Metztitlán were aligned with published sequences of *X. hellerii* that were available on Genbank. The following Genbank sequences were used: Control Region U06607–U06608, Cytochrome B AF404291–AF404295, and Tyrosine Kinase U06508, U06509, and AF404297–AF404301. Alignment was done by eye using Sequencher v4.2 (Gene Codes). In a few cases the sequences obtained in this study were slightly longer than published sequences. For these cases, non-overlapping portions were trimmed, but the full sequences were used for Genbank submissions. None of the non-overlapping portions of the sequences from

Table 1 List of localities within the Metztlán Canyon Biosphere Reserve used for sequencing of mitochondrial and nuclear genes

Locality	River	<i>N</i>	<i>W</i>	Elevation (m)
Jihuico	San Agustín	20.541722	98.727306	1,282
Jilotla	Metztlán	20.551972	98.741583	1,279
Macuila	Viejo	20.647722	98.810833	1,261
Milpillas	Mezquitlán	20.520611	98.661722	1,321

Metztlán contained polymorphisms that differentiated populations within this study. Two CR sequences – AF404291 and AF404292 – were excluded from alignments as they differed from other published *X. hellerii* sequences by approximately 40 base pairs. This is a three times greater divergence than was observed when comparing published sequences of *X. hellerii* and a published sequence from the more distantly related platyfish, *X. variatus* (DQ235821). Thus, these two sequences were assumed to be labeled erroneously or to contain errors and were excluded.

Results

DNA sequencing

All sequences from this study have been deposited on Genbank and accession numbers are provided (Table 2). Sequencing produced 1379 base pairs (bp) total for specimens from Metztlán and 1,218 bp of overlap for comparison with published sequences (CR: 390 bp, CYTB: 307 bp, XSRC: 521 bp). Alignment with sequences from Genbank confirmed that the specimens collected in Metztlán were *X. hellerii*. Among the three genes sequenced, haplotypes from Metztlán either matched published sequences or differed by only a few

base pairs. There was a single haplotype recovered for CR in Metztlán specimens that was distinct from all other sequences. This haplotype differed from the Honduras Rio Lancetilla (AF404294) and Tabasco Rio Oxolotan (AF404295) sequences by only one base pair each and from the Veracruz Rio San Juan sequence just by two base pairs. The only CYTB haplotype recovered from Metztlán was identical to that found in four of the published sequences of *X. hellerii*, but differed from all three sequences from Veracruz by 1–5 base pairs.

The nuclear XSRC gene revealed the greatest polymorphism of any of the three sequenced genes. There were four haplotypes observed among the four populations in Metztlán. All four of these haplotypes were distinct from the three published sequences available for comparison. One of the haplotypes recovered, XhXSRC1, was observed in all four populations included in the analysis (Table 2). Interestingly, this haplotype closely resembled the sequence from the Rio Lancetilla in Honduras differing by only a single base pair and shared indel polymorphism with the fish from Rio Lancetilla. This haplotype was noticeably more divergent from fish from Rio Coatzacoalcos in Tabasco and Rio Sarabia in Oaxaca, which differed by 4 bp. However, the remaining three haplotypes were equally divergent from all published sequences (4 bp) but contained a cytosine at the indel site matching the

Table 2 Haplotypes sequenced from Metztlán populations and matches to known *X. hellerii* sequences from Genbank

Gene	Haplotype	Population*	Genbank Accession No.	Matches Haplotype†
CR	XhCR1	JL (1), JH (2), MA (1)	KC155334	None
CYTB	XhCYTB1	JL (2), JH (2), MA (2), MI (2)	KC155333	U06508
				U06509
				AF404300 AF404301
XSRC	XhXSRC1	JL (2), JH (1), MA (1), MI (1)	KC155329 KC155330 KC155331 KC155332	None
				None
				None
				None

**JL* Jilotla, *JH* Jihuico, *MA* Macuila, *MI* Milpillas. Numbers in parentheses indicate the number of individuals with that haplotype

†Genbank accession numbers for matching haplotypes

fish from Coatzacoalcos and Sarabia. Two of these three haplotypes, XhXSRC2 and XhXSRC3, were observed in two populations and the XhXSRC4 haplotype was observed only in Jihuico (Table 2).

Discussion

This is the first report to confirm the presence of *X. hellerii* in the Metztitlán Canyon Biosphere Reserve based on DNA sequence data. Sequencing of nuclear and mitochondrial genes confirmed that the specimens collected within the reserve were *X. hellerii* and, in general, sequences were distinct from known *X. hellerii* gene sequences but exhibited low divergence. The data were inconclusive regarding a definitive geographic origin for the specimens that were collected in the reserve.

There are two primary, not mutually exclusive, explanations for the occurrence of *X. hellerii* in the Metztitlán Canyon. The first possibility is that these fish represent a relict population of *X. hellerii* that was isolated during orogeny from the eastern coastal plain where *X. hellerii* is common. The other possible scenario - that does not preclude historical isolation - is that *X. hellerii* colonized the canyon as a result of anthropogenic introduction. Such introduction could stem from at least one of three sources: the pet trade, use of baitfish, or being introduced accidentally during introductions of species that are commercially exploited. *Xiphophorus hellerii* has been introduced accidentally into non-native habitats around the world due to its popularity in the pet trade. These introductions have produced stable populations that even have been the focus of scientific investigation (Poort et al. 2006; Simmons et al. 2008). A second potential source of introduction is through accidental by-catch of *X. hellerii* during the collection of baitfish that were used subsequently in the Laguna de Metztitlán - a important local fishery. Since the time that the Laguna de Metztitlán was depleted of native species, the lake has been routinely stocked for years with non-native omnivores like tilapia and catfish (CONANP 2003), representing an opportunity for accidental translocation of *X. hellerii*.

The sequencing of portions of three genes was successful in confirming that the specimens were *X. hellerii*, but they are somewhat limited in what they can tell us about how these fish came to colonize this area. The two mitochondrial genes, CYTB and CR, showed generally low divergence but an overall trend towards similarity to

sequences from southern *X. hellerii* (Tabasco and Honduras). The nuclear gene, XSRC, exhibited notably greater polymorphism, including those from Metztitlán, than the mitochondrial genes. The most common haplotype recovered in all four populations closely resembled that from the Rio Lancetilla in Honduras. All three other haplotypes were equally distinct from all sequences to which they were compared. One possible explanation is that the three XSRC haplotypes recovered from Metztitlán are more closely related to *X. hellerii* from a locality that was not available for comparison (e.g. Rio Oxolotan Tabasco, Veracruz, etc.). This seems a likely scenario given that XSRC is typically more conservative and exhibits less polymorphism than the other genes (Meyer et al. 1994), yet it exhibited considerably more variation than every mtDNA gene in the present study. Given the close similarity of *X. hellerii* in Metztitlán to fish from Honduras, introduction to the reserve seems plausible. It is much less likely that a relict population would either converge on the same XSRC sequence as that observed in Río Lancetilla or exhibit such shallow differentiation from the Río Lancetilla sequence after diverging in isolation since the orogeny of the Sierra Madre Oriental thousands of years ago.

While we like to think of biosphere reserves and protected areas as natural, endemic biological communities representing pristine conditions, this is often not the case. It is now well known that the Metztitlán Canyon Biosphere Reserve itself does not represent a natural community. This is particularly true and documented with respect to the fish community found in the Laguna de Metztitlán and their associated parasites. The introduction of non-native species to the fishery simultaneously introduced non-native parasites that generally parasitize native species more than the non-natives with which they were introduced (Monks et al. 2005).

If *X. hellerii* colonized the Barranca de Metztitlán Biosphere Reserve as a result of introduction, it is unlikely that they pose a conservation concern to other species. Species of the genus *Xiphophorus* often occur in sympatry with multiple other poeciliids including congeners and we know not of documented cases where competitive exclusion has replaced a native species in this group (Kallman and Kazianis 2006). For example, *Poeciliopsis gracilis* is non-native to the eastern side of the Sierra Madre Oriental but can be found in abundance at many lowland sites with native poeciliids such as *X. birchmanni*, *X. variatus*, *Heterandria jonesii* and others (ZWC pers. obs.; Obregón-Barboza et al.

1994). Hybridization within *Xiphophorus* is somewhat common (Rosenthal et al. 2003; Kallman and Kazianis 2006; Meyer et al. 2006; Culumber et al. 2011) and but unlikely a threat given that there are no other species of *Xiphophorus* present in the canyon.

Based on the present genetic data we can affirm that the specimens found broadly throughout the Metztitlán Canyon Biosphere Reserve are *X. hellerii*. What remains less clear is exactly how this species colonized this canyon located on the backside of the front ranges of the Sierra Madre Oriental. A more in-depth investigation of molecular variation including specimens from more localities throughout the natural range is necessary in order to determine the history surrounding the colonization of *X. hellerii* in this reserve as well as that of its natural range. Until the origin of these populations can be determined, the species deserves the same conservation consideration as other native species given that it occurs within a natural protected area designed to conserve the natural biodiversity of the region.

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