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First record of the Conchos Shiner *Cyprinella panarcys* (Hubbs & Miller, 1978) from the mainstem of the Rio Grande along the USA–Mexico border

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Abstract

Cyprinella panarcys is considered to be endemic to the upper Río Conchos drainage of Mexico. A single individual of *C. panarcys* was collected from the Rio Grande along the USA–Mexico international border (Presidio Co., Texas) in April 2018. This is the first record of *C. panarcys* from outside of the Río Conchos and the first record from the USA. A brief description of the external morphology of the individual collected is provided along with an updated distribution map for the species.

Key words

North America; Chihuahuan Desert; Texas; Río Bravo Del Norte; Cypriniformes.

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Introduction

The genus *Cyprinella* Girard, 1856 is widely distributed throughout eastern North America, from the St Lawrence River drainage in eastern Canada to the Río Pánuco in north-eastern Mexico (Mayden 1989, Schönhuth and Mayden 2010). Though some species of *Cyprinella* are infamous for extensive ranges, in some cases spanning multiple large river drainages (e.g., *C. lutrensis* [Baird & Girard, 1853], Matthews 1987, Richardson and Gold 1995, 1999, Boschung and Mayden 2004, Osborne et al. 2016), the majority of the Mexican species of *Cyprinella* exhibit narrow ranges, with many confined to a single river drainage or cienega complex (e.g. Contreras-Balderas and Lozano 1994, Miller 2005). The Conchos Shiner *Cyprinella panarcys* (Hubbs & Miller, 1978) was described based on a large series of specimens obtained from 3 localities within the upper Río Conchos, a major tributary of the Rio Grande (Río Bravo Del Norte) in Mexico. This included 120 specimens from the Río San Pedro at Meoqui (type locality; Chihuahua), 11 specimens from the Río Florido at Estacion La Cruz (Chihuahua), and 4 specimens from the Río Florido between El Cristo and Villa Ocampo (Durango) (Hubbs and Miller 1978). Based on the specimens available to them, Hubbs and Miller (1978: 583) considered the distribution of *C. panarcys* to be "restricted" to the upper parts of the Río Conchos, extending from the Río San Pedro at Meoqui (Chihuahua) to the Río Florido (Durango). Subsequent authors working with *Cyprinella* (e.g., Mayden 1989, Schönhuth and Mayden 2010: fig. 1)

or the Mexican freshwater ichthyofauna more generally (e.g., Miller 2005) have also interpreted the range of this species to be restricted to the upper parts of the Río Conchos. The restricted range of *C. panarcys* has led some authors to consider the species at risk of extinction through habitat alteration and reduction of populations (Contreras-Balderas et al. 2003, Jelks et al. 2008).

Herein, we report on the recent collection of a single individual of *Cyprinella panarcys* from the mainstem of the Rio Grande at the confluence with Alamito Creek (Presidio Co., Texas, USA). This individual represents not only the first record of *C. panarcys* from outside of the Río Conchos drainage, but also the first record of this species from the USA. We provide a brief description of this individual, compare the cytochrome *b* mitochondrial gene sequence obtained from this individual to those available from specimens collected in the Río Conchos drainage (Schönhuth and Mayden 2010), and provide an updated distribution map for this species.

Methods

We sampled for freshwater fishes at multiple sites on the mainstem Rio Grande, downstream of the Río Conchos confluence, between Presidio (Presidio Co., TX) and Lajitas (Brewster Co., TX) over a 2-day period (16–17 April 2018). Fishes were collected at each site using a 4.6 × 1.8 m seine with a minimum of 20 5-m long seine hauls through targeted habitat considered to be homogeneous along the northern (USA) shoreline of the Rio Grande following the protocol of Heard et al. (2012). Specimens collected in each seine haul were typically released after identification but select specimens were euthanized (using MS-222), fixed in a solution of 10% formalin and preserved in 70% ethanol. Prior to fixation in formalin, tissue samples (fin clips or muscle) were obtained from select specimens and stored in 95% ETOH. Specimens were identified using keys available in Miller (2005), Hubbs et al. (2008) and Hendrickson and Cohen (2016). Identified specimens and associated tissues were deposited at the Biodiversity Research and Teaching Collections, Texas A&M University, College Station, TX, USA (TCWC).

Locality information for *Cyprinella panarcys* was obtained from the literature (Hubbs and Miller 1978, Contreras-Balderas and de Lourdes Lozano 1994, Miller 2005) or FishNet 2 (http://www.fishnet2.net/) and analyzed in ArcMap v. 10.5.1. Counts and measurements reported generally follow those of Hubbs and Lagler (1958) and Mayden (1989) except that the 2 posteriormost fin rays of the dorsal and anal fins that articulate with the same pterygiophore are counted separately and scales bearing the lateral line canal on the body and base of caudal fin are counted separately (scales on caudal-fin base are reported in parentheses). Measurements of the body are presented as a percentage of standard length (SL) and measurements of the head are presented as a percentage of head length (HL). Observations on tubercles were made using a Zeiss SteReo Discovery V20 microscope equipped with a Zeiss Axiocam MRc5 digital camera. Photographs of specimens or parts thereof were obtained either with the aforementioned microscope and digital camera or a Nikon D90. Digital images were processed using Adobe Photoshop and Illustrator CC 2018 (Adobe Systems Inc., San Jose, CA, USA).

Genomic DNA was extracted from the right pectoral fin of a single individual of *Cyprinella panarcys* using a DNeasy Tissue Extraction Kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocols. A portion of the mitochondrial cytochrome *b* (cyt *b*) gene was amplified using polymerase chain reaction (PCR) and the primer pair LA-danio and HA-danio (Mayden et al. 2007). PCR conditions followed those listed in Kim and Conway (2014) and were performed in 25.0 µl, containing 12.5 µl of GoTaq Green Master Mix (Promega, Madison, WI, USA), 10.95 µl of nuclease-free water, 300 ng of template DNA, and 10 µM each of forward and reverse primer. Amplified PCR product was sequenced using the high-throughput sequence facilities at Yale University (New Haven, CT, USA). Obtained sequences were checked for accuracy of base determination and assembled using SEQUENCHER v. 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). The final sequence has been deposited on Genbank under accession number MH796138. Two additional cyt *b* sequences for *C. panarcys* available from Schönhuth and Mayden (2010; GQ275196.1, GQ275197.1) and 3 sequences of the hypothesized sister taxon *Cyprinella proserpina* (Girard, 1856) available from multiple studies (Schönhuth et al. 2006, 2008, Schönhuth and Mayden 2010; DQ324101.1, EU082521.1, GQ275200.1) were downloaded from Genbank and aligned against the sequence generated as part of this study in BBEdit (Bare Bones Software Inc., Bedford, MA, USA). The resulting aligned data set (6 sequences, each 1,141bp) was first viewed in Mesquite v. 3.51 (Maddison and Maddison 2018) to check for spurious stop codons and analyzed for genetic distances in PAUP* v.4.0b10 (Swofford 2003).

Results

New record. *Cyprinella panarcys*, TCWC 19724.01, 36.0 mm SL (Fig. 1); USA, Texas, Presidio County, Rio Grande at the confluence with Alamito Creek (29.520640, −104.291924; Fig. 2), Kevin W. Conway, Stephanie D. George, Joshuah S. Perkin & Amanda K. Pinion (collectors), 16 April 2018.

At the time of sampling, the water of the Rio Grande was turbid (Fig. 3) and of slow to moderate flow (<0.1–1.3 m/s). The substrate ranged from sand/silt and fine gravel to cobble, with a shoreline formed predominantly by cobble (US shoreline) or riparian vegetation (Mexican shoreline). Alamito Creek was dry at the time of sampling. Fishes were sampled along the northern (US) shoreline of the Rio Grande in water ranging in depth from 0.1 to 1.07 m. The individual of *C. panacrys* was

Figure 1. A. *Cyprinella panarcys*, TCWC 19724.01, male, 36.0 mm SL; Rio Grande, Presidio Co., Texas, USA; pectoral fin split at center. **B.** *Cyprinella proserpina*, TCWC 16885.09, male 60.0 mm SL; Independence Creek, Terrell Co., Texas, USA.

collected over cobble substrate in water of moderate flow (0.61 m/s) and depth (1.07 m). Other species collected at this site together with *C. panarcys* included: *C. lutrensis, Macrhybopsis aestivalis* (Girard, 1856)*, Notropis braytoni* Jordan & Evermann, 1896, *Rhinichthys cataractae* (Valenciennes, 1842) (Cyprinidae), *Carpiodes carpio* (Rafinesque, 1820), *Cycleptus* sp. cf. *elongatus* (Lesueur, 1817) (Catostomidae), *Pylodictis olivaris* (Rafinesque, 1818) (Ictaluridae), and *Gambusia affinis* (Baird & Girard, 1853) (Poeciliidae).

Identification. We initially attempted to use the key to the Cyprinidae of Mexico available in Miller (2005: 97–103) for identification because it covers a greater number of species of *Cyprinella* than are covered in Hubbs et al. (2008) or Hendrickson and Cohen (2016). In this key, Miller (2005: 99, couplet 29) separates *C. panarcys* from *C. proserpina* based on minor differences in head and mouth width and upper jaw length, and also by location (upper Río Conchos for *C. panarcys* vs Atlantic Slope, tributaries to middle Río Bravo for *C. proserpina*). Measurements obtained from the Rio Grande specimen were intermediate between those reported for *C. panarcys* and *C. proserpina* by Miller (2005) which prevented us from arriving at a positive identification using this resource. Our final identification of the Rio Grande specimen as *C. panarcys* (vs *C. proserpina*) is based largely on features of coloration as documented in Hubbs and Miller (1978) and Lozano-Vilano et al. (2009). This includes: (1) pelvic and anal fin orange with a broad white margin in *C. panarcys* (Fig. 1A) vs pelvic and anal fin pinkish-whitish with whiter margin in *C. proserpina* (Fig. 1B); (2) caudal fin orange with a narrow iridescent pale blue margin in *C. panarcys* vs yellow with iridescent pale blue markings restricted to tip of upper and lower lobe and not forming a uniform margin around fin in *C. proserpina* (Fig. 1B); and (3) upper part of body metallic green/bronze in *C. panarcys* (Fig. 1A) vs bronze in *C. proserpina* (Fig. 1B).

The segment of the cyt *b* gene obtained from the Rio Grande specimen of *Cyprinella panarcys* (Genbank accession # MH796138) is very similar to those available on Genbank from 2 specimens collected at different locations within the upper Río Conchos in Chihuahua (Schönhuth and Mayden 2010), differing only by 1 (GQ275196.1; Río Balleza) or 5 (GQ275197.1; Río Gallos) base pair positions, respectively. The uncorrected P-distance between the sequence obtained from the Rio Grande specimen and the 2 sequences obtained from the Río Conchos specimens is minimal (0.0039075), suggesting that all 3 individuals are conspecific and further corroborating our original identification. The uncorrected P-distance between the 3 cyt *b* sequences of *C. panarcys* and 3 sequences of *C. proserpina* available on Genbank (EU082521.1, GQ275200.1 and DQ324101.1) is 0.06005111.

Discussion

Select external measurements and counts obtained from the Rio Grande specimen of *C. panarcys* (Table 1) are

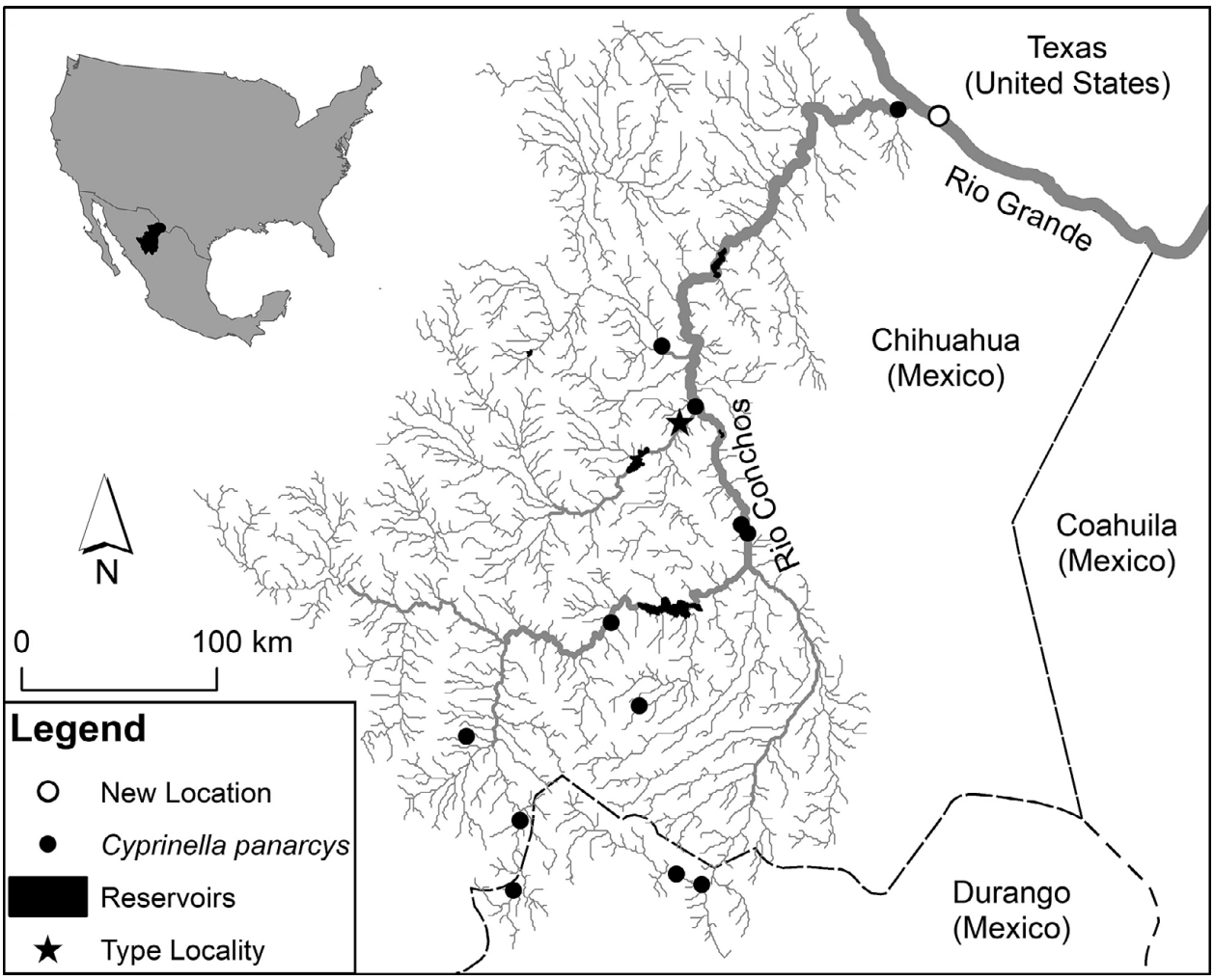

Figure 2. Known localities of *Cyprinella panarcys* based on museum vouchered material in collections located in the USA and from the literature (Contreras-Balderas and de Lourdes Lozano 1994). Type locality identified by a black star. New USA locality indicated by an open circle.

Figure 3. Rio Grande at the confluence with Alamito Creek (Presidio Co., Texas, 16 April 2018). The dry bed of Alamito Creek, representing the northern (USA) shoreline, is shown in the foreground.

Table 1. Measurements and counts obtained from the individual of *Cyprinella panarcys* collected from the Rio Grande (TCWC 19724.01). The total number of rays is provided (in parentheses) for the dorsal, anal, and paired fins.

Standard Length (SL)	**36.0**
% of SL	
Head length	27.8
Predorsal length	52.8
Pranal length	66.7
Prepelvic length	50.0
Preanus length	63.9
Body depth (at dorsal-fin origin)	27.8
Caudal peduncle length	25.0
Caudal peduncle depth	13.9
Dorsal-fin height	25.0
Anal-fin depth	19.4
% of HL	
Head depth (at orbit)	65.0
Head width (at orbit)	40.0
Interorbital width	40.0
Eye diameter	30.0
Snout length	30.0
Mouth width	30.0
Dorsal-fin rays	iii,7,i (11)
Anal-fin rays	iii,8 (11)
Pectoral-fin rays	i,9,ii (12)
Pelvic-fin rays	i,6,i (8)
Principal caudal-fin rays	9+8
Scales in lateral line scale row	34(+2)
Circumpeduncular scale rows	8
Scale rows above lateral line scale row	6
Scale rows below lateral line scale row	3

largely congruent with those provided by Hubbs and Miller (1978) and Mayden (1989). The coloration of the specimen in life (Fig. 1) also conforms to the descriptions provided by these authors. Hubbs and Miller (1978), Mayden (1989) and Contreras-Balderas and Lozano (1994) have provided detailed accounts of the tuberculation of *C. panarcys*. Tuberculation of the Rio Grande specimen of *C. panarcys* is relatively advanced and corresponds with these previous accounts except for a few minor differences. Hubbs and Miller (1978: 587) reported a triangular wedge of small tubercles to be present along the dorsal midline (presumably on the surface of the predorsal scales), extending from a point just posterior to the occiput to "mid-dorsally (rarely almost to the dorsal-fin origin [sic]". In the Rio Grande specimen, the dorsal-midline is devoid of tubercles and the predorsal scales lack the thick layer of keratin that is obvious on the scales present on the lateral body surface (which appear whitish). Hubbs and Miller (1978) and Contreras-Balderas and Lozano (1994) also reported all fins (except for the pectoral fins) to be devoid of tubercles in their material from the upper Río Conchos yet small conical tubercles (~40 μm in diameter) are present along the surface of the anteriormost 2–3 dorsal-, anal-, and pelvic-fin rays in the Rio Grande specimen. These small tubercles may have been overlooked by Hubbs and Miller (1978) and Contreras-Balderas and Lozano (1994), though we note that Hubbs and Miller (1978) observed tubercles of similar size on the lateral surface of the head and on the surface of the anterior body scales. These minor differences in tuberculation are difficult to reconcile but could be related to differences in reproductive state. At 36.0 mm SL, the Rio Grande specimen of *C. panarcys* is smaller than the smallest nuptial male (39.0 mm SL) reported by Hubbs and Miller (1978: 583) and it is possible that this specimen, though sporting well-developed tubercles on the dorsal surface of the head and dorsal and lateral surfaces of the snout (Fig. 4), was collected prior to the peak state of tuberculation.

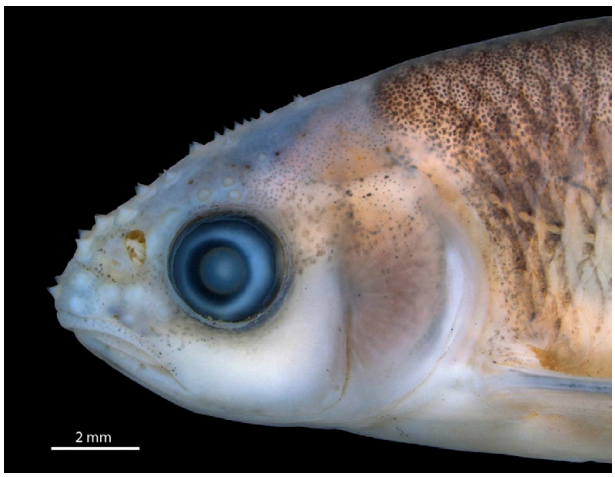

Figure 4. *Cyprinella panarcys*, TCWC 19724.01, male, 36.0 mm SL; head in lateral view.

Though *Cyprinella panarcys* has been considered by previous authors to be restricted to the upper parts of the Río Conchos (Hubbs and Miller 1978, Mayden 1989, Contreras-Balderas et al. 2003, Miller 2005, Schönhuth and Mayden 2010) our investigation of museum vouchered specimens in US collections suggests that the range of this species may extend also to the lower part of the Río Conchos (Fig. 2), as reported by Lozano-Vilano et al. (2009). A single lot of *C. panarcys* from the Kansas University Biodiversity Institute and Natural History Museum (KU 4991) was collected in 1959 from the lower Río Conchos north-west of Ojinaga, approximately 5 miles from the confluence of the Río Conchos and the Rio Grande at the international border between the USA and Mexico. These specimens were collected prior to the original description of *C. panarcys* by Hubbs and Miller (1978) and were not examined as part of that study, though were examined as part of a subsequent redescription of *C. panarcys* by Mayden (1989) who continued to interpret the range of this species as restricted to the upper parts of the Río Conchos ("Known only from the Upper Río Conchos [sic]"; Mayden 1998: 66). In combination, the overlooked record of *C. panarcys* from the lower Rio Conchos (KU 4991, 5 specimens) and the record reported herein (TCWC 19724.01, 1 specimen) from the Rio Grande extend the known range of the species beyond the currently accepted range (e.g., Miller 2005). Though

it cannot be confirmed with vouchered specimens in US collections, it is possible that *C. panarcys* persists in the middle reaches of the Río Conchos drainage and this should be further explored through examination of museum vouchered specimens in Mexican collections and/or additional field work.

Based on aspects of coloration (Fig. 1) and the presence of large tubercles (Fig. 4) on the dorsal surface of the head and dorsal and lateral surfaces of the snout, we interpret the Rio Grande specimen of *C. panarcys* to have been either mature or approaching maturity at the time of collection. We did not encounter additional specimens of *C. panarcys* in the area immediately upstream or downstream from the confluence between the Rio Grande and Alamito Creek. It is possible that we may have mistaken immature males and females of *C. panarcys* for individuals of the sympatric *Cyprinella lutrensis* (= *C.* sp. 1 of Schönhuth and Mayden 2010). Though mature "nuptial" males of *C. panarcys* and *C. lutrensis* can be easily distinguished by aspects of coloration, including body color (metallic green/bronze body in *C. panarcys* vs metallic blue/salmon pink in *C. lutrensis*) and length of throat stripe (extending to isthmus in *C. panarcys* vs not extending past lower jaw in *C. lutrensis*), and also head shape (males of *C. panarcys* exhibit a deeper head and more rounded snout than *C. lutrensis*), distinguishing between immature males or females of these 2 species is likely to be more challenging in the field and likely will require more detailed examination of preserved specimens.

In a time when many species of Rio Grande endemic fishes are declining or disappearing entirely (Hubbs 1990, Edwards et al. 2002, Contreras-Balderas et al. 2003), it is encouraging to be able to report on the range-extension, albeit a relatively minor one, of a Mexican endemic species of freshwater fish within the USA that is considered endangered in Mexico (Contreras-Balderas et al. 2003, Jelks et al. 2008). Additional collections-based work and field work in the stretch of the Rio Grande between the confluence of the Río Conchos and Alamito Creek will be needed to confirm whether *C. panarcys* is established within the mainstem Rio Grande along the USA–Mexico international border and potentially beyond. This area includes the north bank (USA) spring-fed tributaries to the Rio Grande in Texas (Presidio Co.) that are already known to be inhabited by species known otherwise only from the Río Conchos in Mexico (e.g., *Dionda* sp. 1; Schönhuth et al. 2012, Hanna et al. 2013).

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Author Contributions

All authors designed the study, participated in field work, prepared figures, and wrote the manuscript; JSP and KWC identified the specimen; KWC collected measurements and counts.

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