# Two New Species of *Xiphophorus* (Poeciliidae) from the Isthmus of Tehuantepec, Oaxaca, Mexico, with a Discussion of the Distribution of the *X. clemenciae* Clade

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## ABSTRACT

The swordtail, *Xiphophorus clemenciae* (Poeciliidae), has been considered a species of special concern because of its apparent limited range. Although described in 1959, it is officially still known only from three locations in the Rio Coatzacoalcos basin, Mexico. Zoogeographic studies have now shown that this species is widespread and abundant but restricted to the uplands of the Rio Coatzacolacos basin where it replaces in many areas the common swordtail, *X. helleri*. Two new swordtail taxa, *X. mixei* and *X. monticolus*, are described from headwater streams of the Rio Jaltepec, a major Rio Coatzacoalcos tributary, Oaxaca, Mexico. The new forms are sympatric in part of their range and replace both *X. clemenciae* and *X. helleri*. Morphometric and molecular analyses revealed that *X. clemenciae* and the two new species constitute a monophyletic clade that exhibits a closer evolutionary affinity to the ''northern'' swordtails and the ''platyfish'' group of the genus rather than to *X. helleri* and the other ''southern'' swordtails. The evolutionary relationships of these taxa are discussed.

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## INTRODUCTION

The genus *Xiphophorus* (Poeciliidae) comprises 24 recognized species of freshwater fishes of which 20 are restricted to the Atlantic versant of the Sierra Madre of Mexico and adjacent parts of Central America. Most of the species (17) occur within 400 km to the north and south of the Trans-Mexican Volcanic Belt, suggesting that the genus evolved in this region. Only two species have a wide distribution: *X. maculatus*, which is restricted to the coastal plain, occurs from Veracruz to Guatemala (about 1000 km); and *X. helleri*, which is found in the front ranges and coastal plain, extending from central Veracruz to Honduras (1100 km).

Traditionally, the genus had been divided into swordtails, taxa with a caudal appendage or "sword", and platyfishes without such a structure, although the two terms have never been rigorously defined. As more species became known during the last 50 years, it became apparent that there exist "swordtails" without a sword and "platyfishes" with a small caudal appendage. Therefore, the vernacular and scientific usage of the two terms to describe and classify groups of *Xiphophorus* can be misleading and confusing. A group of nine species, many with swords, inhabiting the Rio Panuco and Tuxpan systems (the "northern" swordtails) were shown to be monophyletic and clearly distinct from the five species with a caudal appendage (the "southern" swordtails) south of the Trans-Mexican Volcanic Belt (Rauchenberger et al., 1990). These authors thought that one of the southern swordtails, *X. clemenciae*, could be the closest relative of the northern swordtail. The same report also suggested that the sword may have evolved more than once in the genus. Three reports (Meyer et al., 1994; Morizot and Siciliano, 1982; Meyer and Schartl, 2002) raised the possibility that the "southern" swordtails may not be monophyletic and that *X. clemenciae* is apparently unrelated to the other members of the group which we shall refer to as the *helleri* clade (*X. helleri, X. alvarezi, X. signum, X. mayae*).

*Xiphophorus clemenciae* was described in 1959 from small tributaries to the Rio Sarabia near Rancho San Carlos, Oaxaca, Mexico (Alvarez, 1959). Since then it has been re-

ported from only four additional localities in the Isthmus of Tehuantepec, Rio Coatzacoalcos basin, Oaxaca, Mexico (Rosen, 1960; Ramirez, 1999). Although we and other researchers have looked for this form in the isthmus region during the ensuing 30 years, no additional locations had been discovered and the distribution of *X. clemenciae* had remained an enigma. Because of its apparent restricted range and rarity it has been categorized as a "species of special concern" (Groombridge, 1993). Three years later *X. clemenciae* was listed as a taxon for which inadequate information was available to make an assessment of its risk of extinction (Baillie and Groombridge, 1996). We reinitiated a search for this species in the upper Rio Coatzacoalcos basin after 1993 in order to determine its true status. We report here on the results of our exploration.

## MATERIALS AND METHODS

Sample Collection, Deposition, and Zoogeography: Specimens were collected in the field with a $10 \times 3$ foot long seine with a 0.125 inch mesh. Fishes were usually preserved in 10% buffered formalin and after arrival in the laboratory transferred to 70% ethyl alcohol, except for fish used in DNA studies (see below). At a number of locations the fish were examined in the field and then returned to the stream, because permission to collect was not obtained from the local authorities.

Names of rivers at fish localities (appendix 1) were taken from Carta Topografica map sheets (1:50,000 and 1:250,000 scale; Instituto Nacional De Estadistica Geografia e Informatica; INEGI). It is noteworthy that river names and their exact spellings are not always consistent between different issues of the same map sheet or between different sheets. On the 1988 edition of Carta Topografica, Donaji E15C43, the name Rio Tolosita is used for that section of the river downstream from the confluence of the Rio Jumeapa and Rio Tortuguero; however, on Carta Topografica, Minatitlan E15–7, 1998 edition, the river is referred to as Rio Junapan along its entire length. One of the major headwater streams of the Rio Jaltepec is referred to as Rio Ahuacatengo, San Juan Ma-

zatlan sheet E15C42, 1:50,000, 1984 edition. The same river is spelled Rio Aguacatenco on the Matias Romero sheet E15C53, 1988 edition, and Rio Aguacatengo on the Minatitlan sheet E15–7, 1:250,000, 1998 edition. There are numerous other examples. We have endeavored to use the names and spellings of the most recent map issues. All distances given in the text are straight line measures. Coordinate measurements were obtained by handheld units from Garmin (GPS38, GPS50, and GPS III Plus). Collection points were imported from Excel (Microsoft) spreadsheets using ArcView GIS (version 3.2, ESRI) with a background map derived from the Digital Chart of the World series (1:1,000,000 scale; www.gisdatadepot. com). This map was extensively modified using ArcView GIS.

Specimens were deposited at Universidad Nacional Autónoma de Nuevo Leon (UNL; San Nicolas de los Garza, NL, Mexico), Universidad Nacional Autónoma de Mexico (UNAM; Mexico City, Mexico), and the American Museum of Natural History (AMNH). We thank the Government of Mexico for permission to collect specimens (Permiso de Pesca de Fomento 200597–213–03, and 150598–213–03 issued to Dr. Alexandra L. Basolo, and 200302.613.03.0147 issued to Dr. Steven Kazianis).

MORPHOMETRIC ANALYSES: Morphometric measurements were made on 105 preserved female and male *X. helleri* (5 females, 6 males, Rio Junapan; 7 females, 7 males, Rio Sarabia; 5 males Rio Romay), *X. clemenciae* (7 females, 11 males, Rio La Polvora, tributary to Rio del Sol; 2 females, 3 males, headwater of Rio Chalchijapan), *X. mixei* (6 females, 14 males, tributary to Rio del Sol at Platanillo; 6 females, 5 males, Arroyo Cuchara, tributary to Rio Aguacatengo), and *X. monticolus* (3 females, 5 males, tributary to Rio del Sol at Chahuitepec; 2 females, 3 males, Rio Aguacatengo near Loma Sta. Cruz; 4 females, 4 males, Rio San Miguel, tributary to Rio San Andres). The Rio La Polvora site of *X. clemenciae* is the one closest to the area of the Rio del Sol occupied by *X. mixei* and *X. monticolus*.

Fishes were mounted on white backing paper with pins to enable accurate measurements of fin origins and termini, and were then digitally photographed with a Nikon Coolpix 950 along with a ruler and a label depicting collection locality, individual numbers and such. All fishes were measured on both flanks. Fish images produced for figure 1 were obtained by film-based photography using live fishes and techniques described elsewhere (www.xiphophorus.org/ photography.htm). Morphometric measurements were performed digitally using TPSdig software (F. James Rohlf, State University of New York at Stony Brook). Morphometric values were computed in Excel (Microsoft) and are average measurements for both sides based on 23 (21 for females) landmark data points for each individual fish (fig. 2). Only fully mature males were photographed, as indicated by the developmental stage of the gonopodium, a modified anal fin, and secondary sex character (Kallman and Schreibman, 1973). Definitions of morphometric variables are as previously published (Miller, 1948) except for measurements of the sword, a secondary male characteristic that results in an extension of the ventral caudal fin in some members of the genus. Total sword length is defined as the linear distance between the ventral insertion of the caudal fin and the distal tip of the longest ray. Extended sword length is defined as the linear measurement between the origin of the sword as it extends from the caudal fin proper to the tip of the longest ray at the posterior end of the sword. All indexed values were computed by dividing raw measurements by standard length. Average basic measurements, indexed values, and standard deviations are presented in table 1 for females, and in table 2 for males. The averaged and indexed measurements were compared in a pairwise manner by use of the Tukey HSD (honestly significantly different) ANOVA test as well as for standard length.

GONOPODIAL STRUCTURE: Gonopodia were examined with the aid of a Zeiss Stemi 2000c microscope at 50× magnification. Individual gonopodia were excised near the insertion point in the body wall. Because gonopodia are three-dimensional structures with relatively intricate features, they were placed on glass slides and manipulated using forceps under the microscope. Particular attention was placed on the terminal region which
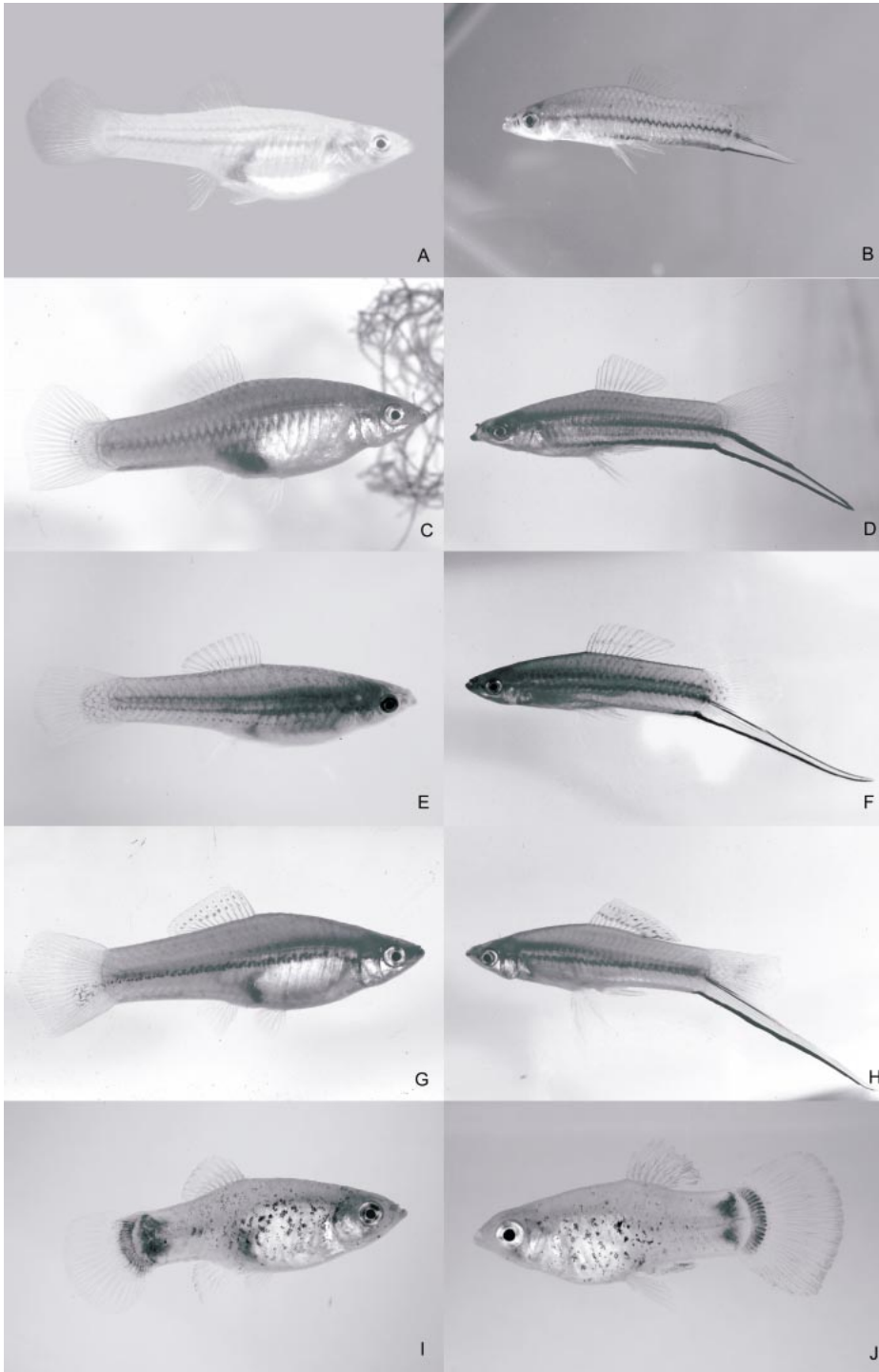
Fig. 1. *Xiphophorus* fishes of the Coatzacoalcos River System. **A, B.** *X. mixei* female and male. **C, D.** *X. monticolus* female and male. **E, F.** *X. clemenciae* female and male. **G, H.** *X. helleri* female and male. **I, J.** *X. maculatus* female and male.
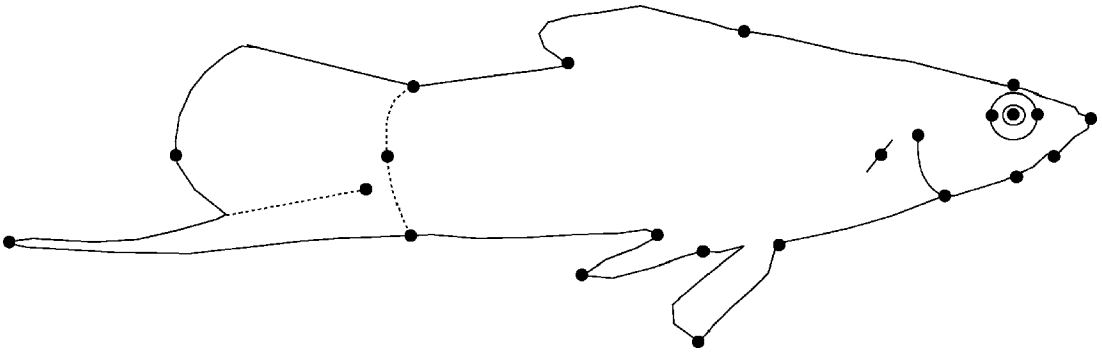
Fig. 2.   Data points recorded for morphometric measurements. Drawing of a male *X. clemenciae* with morphological landmarks indicated (see text).

includes bony elements derived from rays 3, 4a, 4p, and 5a, as these have been extensively utilized in several *Xiphophorus* taxonomic studies (e.g., Rosen, 1960; Rauchenberger et al., 1990; Meyer and Schartl, 2002). Figure 3 depicts some of the anatomical features and unique nomenclature used for this structure (Gordon and Rosen, 1951). For photographic purposes, gonopodia were first positioned and then pressed carefully using a glass block. A Zeiss Axiovert 35 microscope with Hoffman contrast filters and overhead lighting was utilized as well a C-mount Minolta Maxxum 9xi camera and Kodak TechPan film. Exposure and processing parameters were optimized for high contrast. Gonopodia were examined from eight *X. clemenciae*, seven *X. mixei*, and seven *X. monticolus* males (see table 3 and fig. 4). Individuals of *X. helleri* (fig. 3) and laboratory-raised *X. maculatus* and *X. montezumae* were also examined for comparison.

DNA Isolation, Amplification, and Sequencing: Field-collected fishes were first over-anesthetized in 0.06% MS-222 (tricane methane sulfate) and then fixed in 95% ethanol within 15 ml plastic tubes (Falcon; Becton Dickinson, Franklin Lakes, NJ). This solution was discarded and replenished after 8–12 hours to ensure proper preservation of DNA. High-molecular-weight DNA was later extracted from these samples using the Puregene isolation kit (Gentra systems, Minneapolis, MN). Samples were resuspended in $1\times$ Tris-EDTA (TE; pH = 8.0) and then quantitated using a FLx800 (Bio-Tek, Winooski, VT) fluorometer and PicoGreen (Mo-

lecular Probes, Eugene, OR). Working DNA stocks were diluted to a concentration of 50 ng/µl.

Two distinct nuclear genes were partially amplified by polymerase chain reaction (PCR) and sequenced: *RAB27* and *CCND1* (also known as *Cyclin D1*). For *RAB27,* we amplified an 851-bp genomic region (as determined in *X. maculatus* strain Jp 163 A; data not shown) including part of the second (primer RAB-EX2F; gctcctggcgctcgggcactc) and fourth exons (primer RAB-EX4R.1; gctgattggtcaagtcgaaca). Twenty-nine individuals were utilized in the analysis resulting in 29,679-bp of sequence. *CCND1* was amplified across the first two exons and was represented by a 1554-bp amplicon (as determined in *X. maculatus*). Forward primer (primer D1F9; atggaggagcagctgctgtgc) was coupled with the reverse primer (D1R5; cctggtggcctccactga). A total of 42,254 bp representing *CCND1* were sequenced from the same 29 fishes amplified for *RAB27*. This overall strategy produced genomic sequences that could be unambiguously considered homologous among taxa since conservation in exonic regions was high. Simultaneously, these sequences also yielded the needed divergence in intronic regions. PCR was performed using an Expand Long Template PCR kit (Roche, Basel, Switzerland), which also provided additional proofreading (calculated by the manufacturer at ~1 misincorporation per $4.8 \times 10^6$ bases). PCR was performed using Hybaid PCR-Express thermal cyclers (Thermo-Hybaid, Middlesex, UK) and 50 ng of genomic DNA. Identical cy-

TABLE 1
Raw (mm) and Indexed Morphometric Measurements for Female
Individuals of Four *Xiphophorus* Species

| | *X. helleri* (N = 12) | | *X. clemenciae* (N = 9) | | *X. monticolus* (N = 9) | | *X. mixei* (N = 12) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Anal fin length | 7.3 | 1.4 | 7.5 | 1.0 | 8.3 | 1.7 | 7.1 | 1.4 |
| Anal fin length index | **0.186** d,e | 0.014 | **0.178** e | 0.014 | **0.168** b | 0.013 | **0.162** b,c | 0.015 |
| Body depth | 12.7 | 2.6 | 13.2 | 1.6 | 13.3 | 3.5 | 12.5 | 2.1 |
| Body depth index | **0.323** d,e | 0.019 | **0.314** d,e | 0.020 | **0.267** a | 0.022 | **0.289** a | 0.013 |
| Caudal peduncle depth | 7.3 | 1.6 | 8.0 | 1.1 | 7.9 | 1.7 | 7.5 | 1.1 |
| Caudal peduncle depth index | **0.185** d,e | 0.010 | **0.189** d,e | 0.009 | **0.161** a | 0.007 | **0.173** a | 0.007 |
| Caudal peduncle length | 12.3 | 2.2 | 13.6 | 1.8 | 16.0 | 2.9 | 13.7 | 1.9 |
| Caudal peduncle length index | 0.314 | 0.014 | 0.323 | 0.014 | 0.325 | 0.015 | 0.316 | 0.014 |
| Dorsal fin base | 8.3 | 1.7 | 8.4 | 1.5 | 8.1 | 1.8 | 7.5 | 1.3 |
| Dorsal fin base index | **0.212** d,e | 0.013 | **0.198** d,e | 0.020 | **0.165** b,c | 0.016 | **0.172** b,c | 0.015 |
| Eye to dorsal | 2.2 | 0.3 | 2.3 | 0.4 | 2.5 | 0.5 | 2.4 | 0.3 |
| Eye to dorsal index | 0.056 | 0.006 | 0.054 | 0.006 | 0.052 | 0.008 | 0.056 | 0.008 |
| Eye to snout | 4.2 | 0.7 | 4.4 | 0.8 | 4.7 | 1.0 | 4.3 | 0.6 |
| Eye to snout index | **0.107** d | 0.009 | 0.104 | 0.010 | **0.096** b | 0.010 | 0.100 | 0.012 |
| Eye to ventral | 3.4 | 0.7 | 3.3 | 0.5 | 3.7 | 0.7 | 3.4 | 0.7 |
| Eye to ventral index | **0.0867** d,e | 0.007 | 0.080 | 0.008 | **0.076** b | 0.009 | **0.077** b | 0.010 |
| Head length | 9.9 | 1.4 | 10.2 | 1.4 | 11.5 | 2.0 | 10.2 | 1.2 |
| Head length index | **0.255** d,e | 0.016 | 0.244 | 0.013 | **0.235** b | 0.012 | **0.237** b | 0.017 |
| Mandible length | 2.2 | 0.4 | 2.2 | 0.4 | 2.6 | 0.4 | 2.2 | 0.5 |
| Mandible length index | 0.056 | 0.011 | 0.053 | 0.008 | 0.053 | 0.009 | 0.051 | 0.008 |
| Orbital diameter | 3.4 | 0.4 | 3.4 | 0.5 | 3.8 | 0.7 | 3.4 | 0.4 |
| Orbital diameter index | **0.087** d,e | 0.010 | 0.081 | 0.006 | **0.078** b | 0.004 | **0.079** b | 0.007 |
| Predorsal length | 22.0 | 3.9 | 23.4 | 2.8 | 27.8 | 5.6 | 24.7 | 3.8 |
| Predorsal length index | 0.561 | 0.008 | **0.557** e | 0.014 | 0.564 | 0.012 | **0.57** c | 0.015 |
| Snout length | 2.6 | 0.6 | 2.8 | 0.6 | 2.9 | 0.6 | 2.7 | 0.4 |
| Snout length index | 0.066 | 0.009 | 0.066 | 0.008 | 0.060 | 0.008 | 0.063 | 0.008 |
| Standard length | **39.3** d | 6.9 | 42.0 | 5.0 | **49.2** b | 9.6 | 43.3 | 6.3 |
| Total length | 48.3 | 8.9 | 51.6 | 6.2 | 60.7 | 11.3 | 53.0 | 7.6 |

Bold values followed by letters differ significantly ($P < 0.05$, Tukey HSD test) from values for other taxa as follows: a, all other taxa; b, *X. helleri*; c, *X. clemenciae*; d, *X. monticolus*; e, *X. mixei*.

cling parameters were used for both studied loci as follows: $92°/2'$ followed by 10 cycles of $92°/10''$, $60°/30''$, $68°/2'$, and 20 cycles of $92°/10''$, $60°/30''$, $68°/2'$ (with a $20''$ increment added per cycle). Samples were incubated at $68°$ for an additional $7'$ and then kept at $4°$ until further utilized. PCR amplicons were excised from 1.0% ethidium bromide-stained agarose gels (*CCND1*) or directly purified (*RAB27*) using Geneclean-Spin (Q-Biogene, Carlsbad, CA) and quantitated using the fluorometric approach detailed above. These samples were sent for commercial (Davis Se-

quencing) fluorescent thermal-cycle sequencing utilizing internal oligonucleotides (RAB-INT2R.3 [cgttcctccaggctctaactt] and RAB-INT2FA [ttaccatctacaactctttaa] for *RAB27*, and D1F14 [cgtgccagaagcctattac] and D1R12 [gatttagttcgtagtcagttc] for *CCND1*) to generate contiguous sequences with high accuracy. Alignments for each taxon were performed using Sequencher software (version 4.1, Gene Codes, Ann Arbor, MI) which enables visualization and overlay of electropherograms for more accurate base calling. All sequences have been deposited into Genbank

TABLE 2
**Ray (mm) and Indexed Morphometric Measurements for Male
Individuals of Four *Xiphophorus* Species**

| | *X. helleri* (N = 18) | | *X. clemenciae* (N = 14) | | *X. monticolus* (N = 12) | | *X. mixei* (N = 19) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Body depth | 13.1 | 2.4 | 13.1 | 2.3 | 11.5 | 2.2 | 11.5 | 1.7 |
| Body depth index | **0.298** d,e | 0.012 | **0.293** d,e | 0.022 | **0.258** a | 0.013 | **0.275** a | 0.010 |
| Caudal peduncle depth | 8.4 | 1.5 | 9.8 | 1.7 | 8.1 | 1.6 | 8.0 | 1.0 |
| Caudal peduncle depth index | **0.192** c,d | 0.009 | **0.220** a | 0.014 | **0.182** a | 0.009 | **0.193** c,d | 0.008 |
| Caudal peduncle length | 17.6 | 3.2 | 18.2 | 3.2 | 18.0 | 2.9 | 16.6 | 2.6 |
| Caudal peduncle length index | 0.401 | 0.014 | 0.408 | 0.019 | 0.407 | 0.012 | 0.398 | 0.015 |
| Dorsal fin base | 9.9 | 1.8 | 9.7 | 1.7 | 8.2 | 1.6 | 7.6 | 1.3 |
| Dorsal fin base index | **0.225** d,e | 0.013 | **0.218** d,e | 0.021 | **0.185** b,c | 0.017 | **0.181** b,c | 0.017 |
| Extended sword length | 14.9 | 8.5 | 11.2 | 2.8 | 10.3 | 3.1 | 2.5 | 2.0 |
| Extended sword length index | **0.326** e | 0.156 | **0.254** e | 0.063 | **0.234** e | 0.065 | **0.061** a | 0.049 |
| Eye to dorsal | 2.9 | 0.4 | 2.2 | 0.4 | 2.4 | 0.4 | 2.3 | 0.3 |
| Eye to dorsal index | 0.051 | 0.007 | **0.049** e | 0.007 | 0.054 | 0.006 | **0.054** c | 0.006 |
| Eye to snout | 4.5 | 0.7 | 4.7 | 0.8 | 4.8 | 0.8 | 4.2 | 0.6 |
| Eye to snout index | 0.103 | 0.009 | 0.107 | 0.013 | 0.108 | 0.013 | 0.101 | 0.012 |
| Eye to ventral | 3.4 | 0.5 | 3.3 | 0.7 | 3.0 | 0.5 | 3.0 | 0.4 |
| Eye to ventral index | **0.079** a | 0.007 | **0.074** b,d | 0.010 | **0.069** b,c | 0.006 | **0.071** b | 0.007 |
| Gonopodial length | 8.8 | 1.4 | 8.7 | 1.2 | 8.5 | 1.7 | 8.0 | 1.0 |
| Gonopodial length index | 0.203 | 0.021 | 0.196 | 0.014 | 0.191 | 0.025 | 0.195 | 0.022 |
| Head length | 10.5 | 1.5 | 10.4 | 1.3 | 10.6 | 1.6 | 9.8 | 1.0 |
| Head length index | 0.242 | 0.014 | 0.236 | 0.020 | 0.240 | 0.022 | 0.235 | 0.018 |
| Mandible length | 1.7 | 0.4 | 1.9 | 0.3 | 2.0 | 0.3 | 1.9 | 0.4 |
| Mandible length index | **0.039** d,e | 0.008 | 0.042 | 0.006 | **0.045** b | 0.006 | **0.045** b | 0.007 |
| Maximum sword depth | 3.8 | 0.9 | 4.1 | 0.8 | 3.6 | 0.9 | 3.3 | 0.5 |
| Maximum sword depth index | **0.085** c | 0.010 | **0.093** a | 0.009 | **0.08** c | 0.010 | **0.08** c | 0.007 |
| Orbital diameter | 3.5 | 0.4 | 3.5 | 0.4 | 3.4 | 0.5 | 3.2 | 0.3 |
| Orbital diameter index | 0.081 | 0.007 | 0.079 | 0.008 | 0.077 | 0.005 | 0.077 | 0.006 |
| Predorsal length | 23.2 | 3.7 | 23.1 | 3.3 | 23.4 | 3.6 | 22.6 | 2.9 |
| Predorsal length index | **0.531** e | 0.010 | **0.521** e | 0.013 | **0.529** e | 0.014 | **0.541** a | 0.013 |
| Snout length | 2.9 | 0.5 | 3.1 | 0.6 | 3.1 | 0.6 | 2.8 | 0.4 |
| Snout length index | 0.068 | 0.008 | 0.071 | 0.011 | 0.071 | 0.013 | 0.066 | 0.009 |
| Standard length | 43.8 | 7.2 | 44.4 | 6.5 | 44.3 | 6.8 | 41.7 | 5.7 |
| Total length | 56.2 | 8.9 | 57.0 | 8.2 | 55.8 | 8.3 | 52.3 | 6.8 |
| Total sword length | 28.6 | 10.3 | 24.9 | 4.2 | 22.9 | 4.4 | 14.0 | 3.1 |
| Total sword length index | **0.641** d,e | 0.161 | **0.564** e | 0.071 | **0.517** b,e | 0.070 | **0.336** a | 0.067 |

Bold values followed by letters differ significantly ($P < 0.05$, Tukey HSD test) from values for other taxa as follows: a, all other taxa; b, *X. helleri*; c, *X. clemenciae*; d, *X. monticolus*; e, *X. mixei*.

under the following accession number series: AY211356–AY211384 (*RAB27*) and AY211327–AY211355 (*CCND1*).

SAMPLE SELECTION AND PHYLOGENETIC RECONSTRUCTION: Nuclear DNA samples were extracted, amplified, and analyzed from seven *X. clemenciae* collected at seven locations

spanning its entire experimentally determined east–west range, six *X. monticolus* from three locations and five *X. mixei* individuals from three sites. To ascertain the relationships of *X. clemenciae* and the two newly described taxa to other *Xiphophorus*, especially to the other "southern" sword-
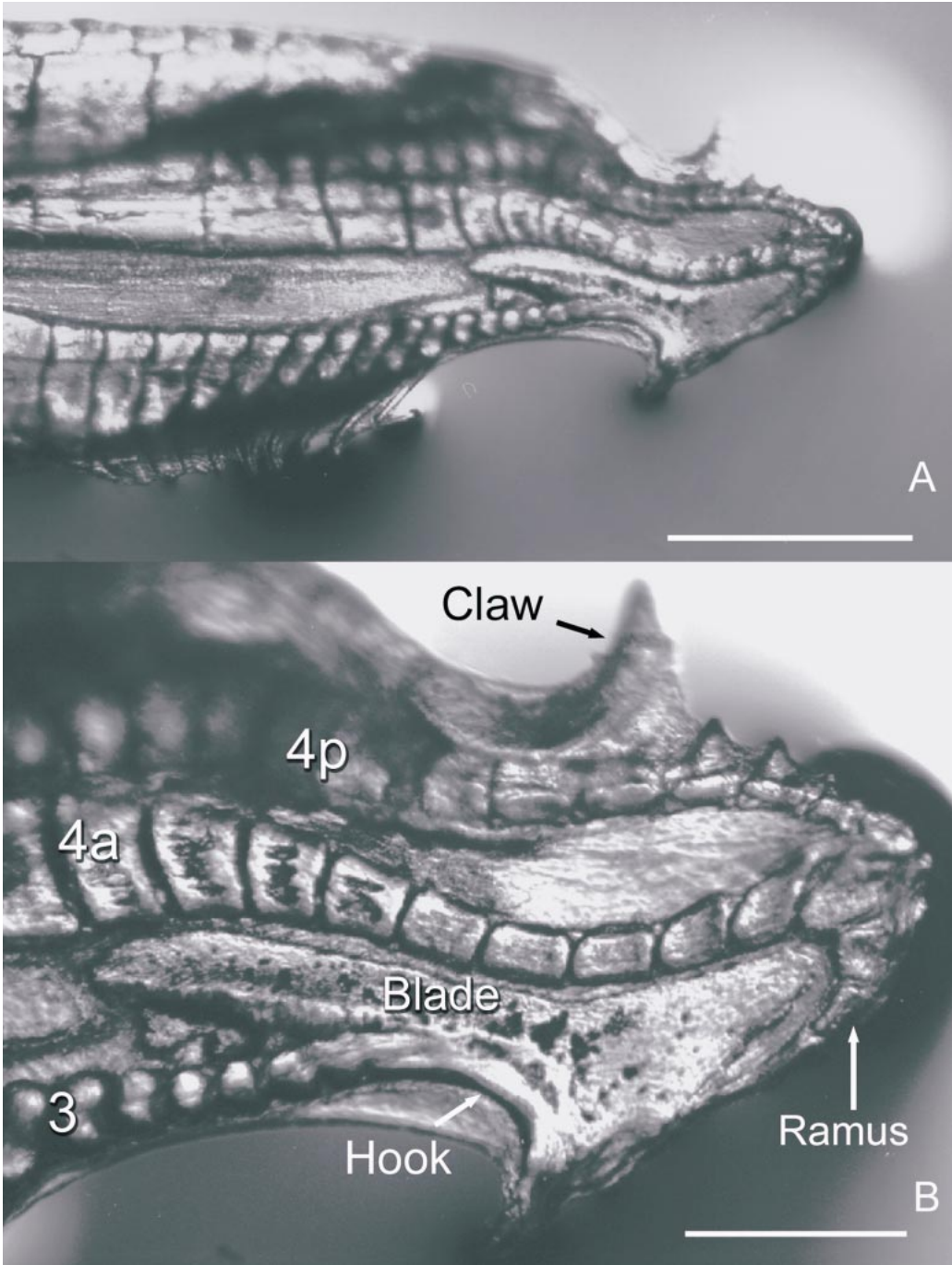
Fig. 3. **A.** Photomicrograph of the distal part of a gonopodium of a *X. helleri* (Rio Robalo). Scale bar is 0.5 mm. **B.** Close-up of gonopodium shown in panel A, showing anatomical nomenclature used in gonopodial descriptions (after Gordon and Rosen, 1951). Scale bar is 0.16 mm.

TABLE 3
**Gonopodial Characteristics of the *clemenciae* Clade**

| | X. clemenciae (N = 8) | X. mixei (N = 7) | X. monticolus (N = 7) |
|---|---|---|---|
| **RAY 3** | | | |
| Hook | Crescent to sickle shaped | Crescent to sickle shaped | Crescent to sickle shaped |
| Subterminal segments | 6–9 | 7–10 | 6–9 |
| Spines | 7–9 | 8–10 | 7–9 |
| Distal spine | Angled proximally then curves, ends anteriorly | Angled proximally, ends at angle | Angled proximally, ends at angle |
| Blade | Pointed proximally, blunt distally | Pointed proximally, blunt distally | Pointed proximally, blunt distally |
| **RAY 4a** | | | |
| Tip of ramus | Slightly angled anteriorly[a] | Slightly angled anteriorly[a] | Slightly angled anteriorly[a] |
| Subterminal segments | 6–12 | 7–10 | 7–10 |
| Ray curved or straight | Straight | Straight | Straight |
| **RAY 4p** | | | |
| Distal serrae | 5–6 | 4–6 | 4–5 |
| Subterminal segments | 3–6 | 3–5 | 3–5 |
| Proximal serrae | 6–8 | 6–9 | 7–11 |
| **RAY 5a** | | | |
| Claw | Yes | Yes | Yes |
| Size | Smaller than distal serrae of 4p | Smaller or equal to distal serrae of 4p | Smaller to slightly larger than distal serrae of 4p |

[a] Exceptional animals show single elements that form a 90° angle with those more proximal.

tails, DNA samples were also analyzed from four *X. helleri* (four locations) and from one specimen each of *X. signum, X. alvarezi, X. andersi, X. couchianus, X. maculatus,* and *X. montezumae* (stocks derived from the *Xiphophorus* Genetic Stock Center, San Marcos, TX). *Priapella intermedia* was included as an outgroup and has been utilized as such in several other studies (Marcus and McCune, 1999; Meyer et al., 1994; Rosen, 1979). Detailed information as to the geographical origin of these fish is included in the figure legend accompanying the provided phenogram (fig. 5).

Derived DNA sequences were aligned using the ClustalW program (Thompson et al., 1994) built into the MacVector software suite (version 7.0, Accelrys, San Diego, CA) and exported into PAUP (version 4.0b10, Apple Macintosh edition; Swofford, 2002). The datasets derived from both studied loci were examined before and after pooling, by boot-strapped (1000 replicates) neighbor-joining and parsimony methods as well as likelihood (100 replicates) techniques. Similar topologic outcomes were obtained in all cases (as discussed below). Results derived from the pooled dataset are provided in figure 5.

RESULTS

***Xiphophorus mixei,*** new species
(Mixe swordtail)
Figure 1A, 1B

DIAGNOSIS: A small to medium-sized swordtail with a short sword (sword index 0.06), one or two orange lateral stripes and black spots in the base of the caudal fin, closely allied to the two species below but differing from them by a shorter sword.

TYPE SPECIMENS: The holotype, UANL15253, is a mature adult male, 39.0 mm SL, 6 mm sword, collected on the 16th of March, 2002, by Kazianis and Kallman in
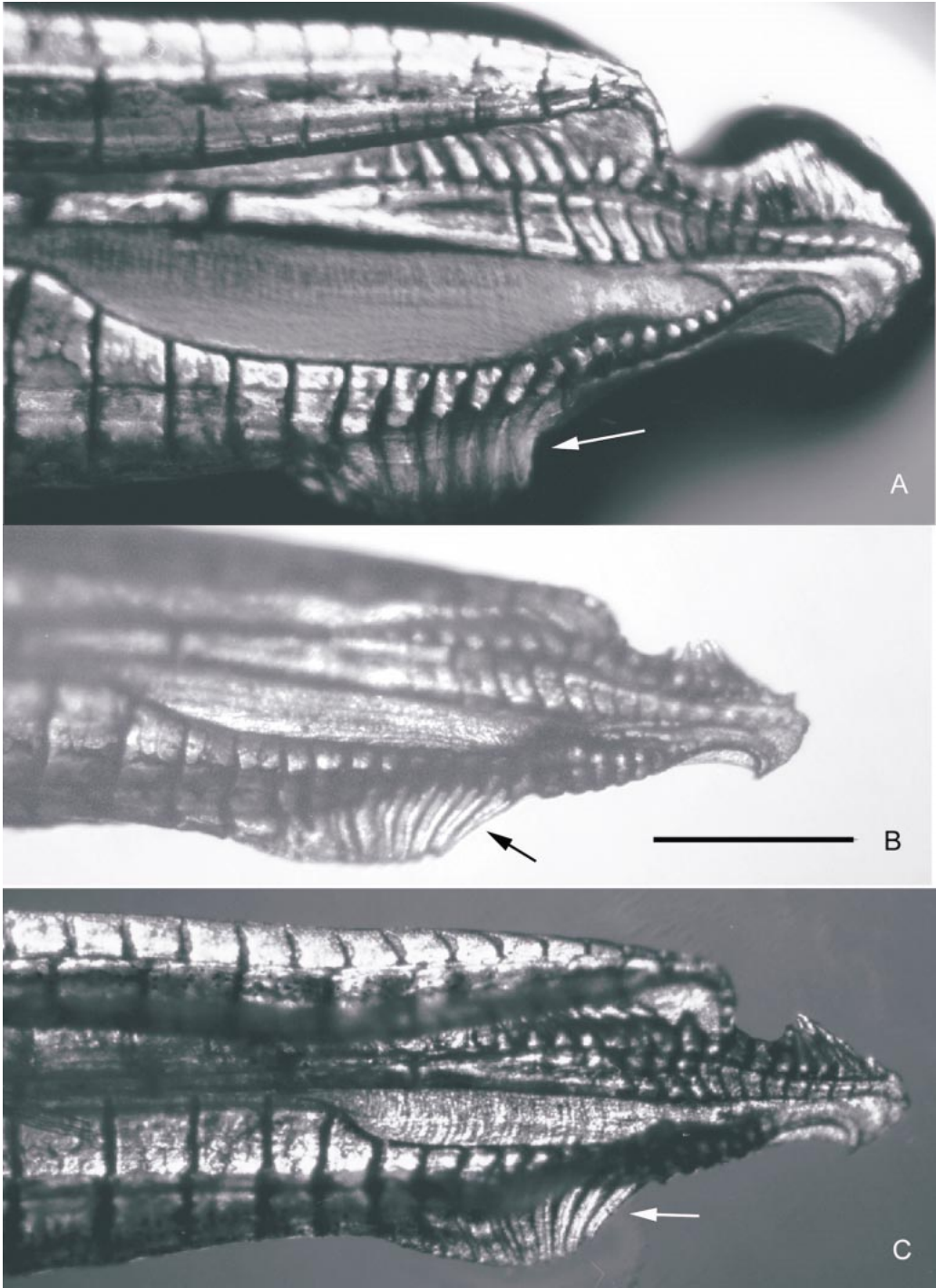
Fig. 4. Photomicrographs of the distal part of gonopodia of *clemenciae*-clade fishes. **A.** Gonopodium of a *X. clemenciae*. **B.** Gonopodium derived from a *X. mixei*. **C.** Gonopodium excised from a *X. monticolus*. Scale bar for *X. mixei* is 0.5 mm and applies to all three images. Arrowheads depict the most distal spine of ray 3, which differs among taxa (see text).

a tributary to the Rio Cuchara (16°58.42′N, 95°17.31′W), Rio Aguacatengo drainage, Oaxaca Mexico. The allotype, UANL15254, is a mature female, taken together with the holotype. The paratypes (IBUNAM 13100, three females, seven males; UANL15255, one female) were collected on the 16th of March 2002, by Kallman and Kazianis in a small stream at Platanillo (16°57.29′N, 95°15.35′W), Rio del Sol drainage, Oaxaca, Mexico.

DESCRIPTION: Males with rather short swords projecting from 1 to 6 mm beyond the posterior margin of the caudal fin. The short stubby sword tapers rapidly and is up-turned. Ventral margin of the sword edged with black pigment cells. The central portion or field of the sword has an orange coloration. The short free distal, dorsal margin of the sword is usually unpigmented. The Grave spot (Rosen and Kallman, 1969) is always well developed, but does not obscure the fin rays. Males always exhibit a well-developed primary axillary stripe composed of melanophores that runs from the base of the pectoral fin past the gonopodium into the caudal peduncle. Some of the males exhibit a fainter second stripe that runs one scale row above the primary one. One male has been seen that had a third stripe below the primary one that ended in front of the gonopodium. Four to nine black spots develop in the caudal fin base.

The terminal segment of ray 3 of the gonopodium is differentiated into a crescent- or sickle-shaped hook and the blade is blunt distally. The more distal serrae of ray 3 are angled proximally (fig. 4B). The ramus (ray 4a) of the gonopodium ends bluntly in most cases; only rarely is the last segment of ray 4a recurved over the blade. Proximal serrae on ray 4p are rather thick near their base. Terminal segment of ray 5a produced into a claw which is about the same height as the distal serrae on the terminal segments of ray 4p. Counts for gonopodial features in rays 3, 4, and 5 are provided in table 3.

The males always have from one to two subdued orange- to salmon-colored stripes along the flank, sometimes up to four. The one along the midlateral line is best developed and runs from the eye to the base of the caudal fin, where it connects with the Grave spot. The next one above begins slightly more posteriorly and may not extend as far posteriorly as the caudal fin. A third still more dorsal stripe is still shorter and fainter in coloration. A fourth stripe below the midlateral one is only weakly developed. In females the orange pigmentation is even more subdued and the caudal fin spots are absent.

ETYMOLOGY: Named after the native inhabitants, the Mixe, which live in villages along the Rio del Sol and the Rio Jaltepec headwaters (Lipp, 1991). The Mixe previously occupied large sections of southern Mexico, speaking a language of the Zoquean linguistic group, but by the mid-18th century have become restricted on the Isthmus of Tehuantepec within areas close to and including the town of Guichicovi (Thomas and Swanton, 1911).

REMARKS: A strain of *X. mixei* derived from the upper Rio del Sol drainage at Sta. Cruz Chahuitepec has been maintained in the *Xiphophorus* stock center for several generations. The pigmentary and morphological traits that distinguish this species from the other two forms have remained constant. Males (34.1 mean SL, range 24–42, n = 70) of the laboratory stock were maintained for up to a year after attaining sexual maturation. Their swords (3.6 mm, range 1–8 mm) were just as short as those of wild-caught males.

DISTRIBUTION: Headwaters of Rio del Sol, Rio Sarabia system and tributaries to Rio Aguacatengo (Rio Jaltepec drainage) west of Platanillo, Oaxaca, Mexico, Rio Coatzacoalcos basin (fig. 6B; appendix 1).

MATERIAL: From Oaxaca: tributaries to Rio Aguacatengo UANL14341 (4), UANL14342 (4), UANL15255 (1), AMNH233383 (8), AMNH211352 (2); from Rio del Sol IBUNAM13100 (10) paratypes, AMNH211353 (4).

### *Xiphophorus monticolus*, new species
### (Southern Mountain Swordtail)
#### Figure 1C, D

DIAGNOSIS: This species is closely allied to the form above and can be distinguished from it by a longer caudal appendage (sword index 0.25), fainter orange stripes that fade in older individuals, and by the pronounced melanophore pigmentation that edges the dorsal and ventral sword margins (in males).
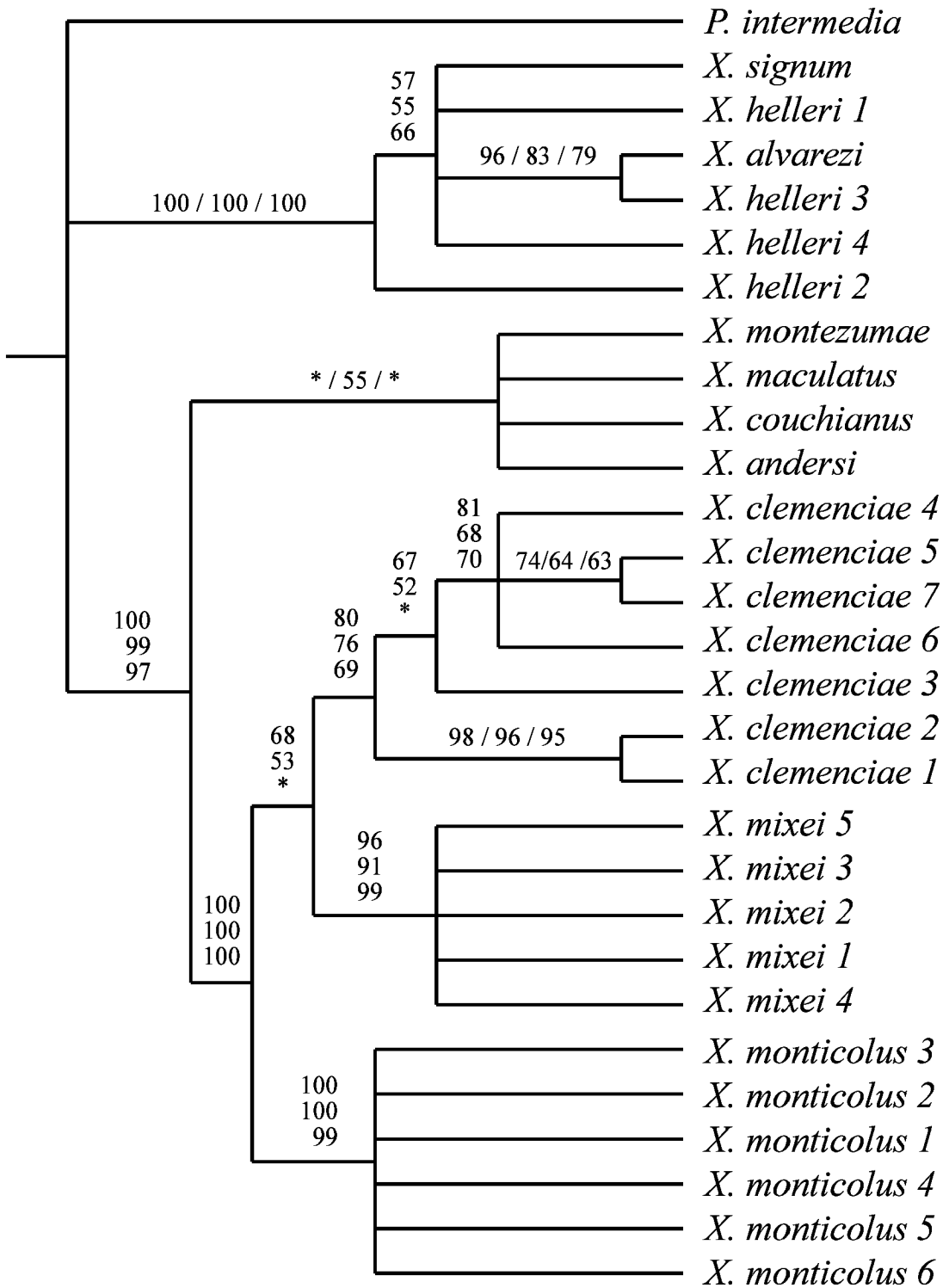
TYPES: The holotype, UANL15256, is a mature male, 50.0 mm SL, taken on the 16th of March 2002 by Kallman and Kazianis in the Rio Cahuitepec (upper Rio del Sol). The allotype, UANL15257, is a mature female, 64.0 mm SL, taken with the male at the same location.

DESCRIPTION: A medium to large-sized swordtail with heavy black pigmentation. Usually two axillary stripes are present; the more ventral one is always more strongly expressed. Beyond the gonopodium the stripe is not continuous, but may be produced in a series of spots. The midventral line is pigmented by melanophores and this pigmentation is especially heavy near the base of the caudal fin. From four to nine black spots are present in the base of the caudal fin. The sword is usually well developed and thickly edged with black pigment cells along ventral and dorsal margins. It is also straight and faintly orange in color. The Grave spot is very dark and obscures fin rays. One to two faint orange lateral stripes may be present along the flank but are absent in older individuals, resulting in an overall steel, blue-gray appearance. The structure of the gonopodium is similar to that of the preceding species, including the most distal spines of ray 3, which also tend to be angled proximally (fig. 4C). Counts for gonopodial features in rays 3, 4, and 5 are provided in table 3.

ETYMOLOGY: The name is derived from the Latin for a dweller in the mountains which in reference to the restricted range of the species to headwater streams inside the Sierra Madre.

REMARKS: This species occurs sympatrically with *X. mixei* in the Rio del Sol. Anecdotal evidence from the Rio del Sol suggests that it prefers deeper pools. We have witnessed a phalanx of nine native women, each holding each other's outer apron (specially designed for seining), and then diving to scour the bottom of the 2–3m deep river and coming up against the steep-sided bank. The fish in their ''apronseine'' were mostly *X. monticolus*. In the Rio Aguacatengo near Loma Santa Cruz, which is a rapidly flowing river with boulders and riffles, we have taken this species only. Only *X. monticolus* was found in the Rio San Miguel drainage.

A strain of this species derived from the upper Rio del Sol has been maintained in the *Xiphophorus* Genetic Stock Center (Southwest Texas State University, San Marcos, TX) for many generations. The differences that separate this species from the other two forms have remained constant. Sword length of the laboratory strain (36.0 mm mean SL,

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←

Fig. 5. Phylogenetic tree of the *clemenciae* clade. Bootstrap values (%) are provided on the illustrated tree, which was derived from the parsimony approach (50% majority-rule consensus tree; consistency index 0.91, retention index 0.95, rescaled consistency index 0.87). Neighbor-joining bootsrap values are included first at each node, followed by parsimony- and maximum likelihood-derived values. Unresolved nodes showing possible polytomies (bootstrap values under 50%) are shown with an asterisk. Source of specimens: *P. intermedia*: tributary of the Rio Polvora, Oaxaca, Mexico; *X. signum*: Rio Chajmaic, Alta Verapaz, Guatemala; *X. alvarezi*: Rio Dolores, Alta Verapaz, Guatemala (XGSC stock); *X. helleri*: no 1, Rio Sarabia stock, Oaxaca, Mexico (XGSC); no. 2, Jalapa stock, Rio Chachalaca system, Veracruz (XGSC); no. 3, Lancetilla stock, Honduras (XGSC); no. 4, tributary to Rio Aguacatengo at Santiago Tutla Nuevo, Oaxaca, Mexico; *X. montezumae*: Rascon, Rio Gallinas, Rio Panuco system, San Luis Potosi, Mexico (XGSC); *X. maculatus*: Jp 163 A strain, Rio Jamapa, Veracruz, Mexico (XGSC); *X. andersi*: Rio Atoyac, Veracruz, Mexico (XGSC); *X. couchianus*: Xc stock, Huasteca Canyon, Nuevo Leon, Mexico (XGSC); *X. clemenciae*: no. 1, Rio Verde, tributary to Rio Uxpanapa; no. 2, tributary to Rio Uxpanapa, 4 km NW of Los Amarillos; no. 3, Rio Romay, Rio Coachapa system; no. 4, tributary to Rio Sarabia at Rancho San Carlos; no. 5, tributary to Rio La Polvora, 2.5 km NW of San Juanita, Rio Sarabia system; no. 6, Arroyo El Boqueron, Rio Jaltepec system; no. 7, tributary to Rio Aguacatengo near Santiago Tutla Nuevo, Rio Jaltepec system; *X. mixei*: nos. 1–3 tributary at Platanillo, Rio Sarabia system; no. 4, Arroyo Cuchara, tributary to Rio Aguacatengo, Rio Jaltepec; no. 5, Rio Cuautepec at Santa Cruz Chahutepec, Rio Sarabia system; *X. monticolus*: nos. 1–3, three locations from the Rio San Miguel, Rio Jaltepec system; nos. 4,5, Rio Cuautepec at Santa Cruz Chahutepec, Rio Sarabia system; no. 6, Rio Aguacatengo at Loma Santa Cruz, Rio Jaltepec system.
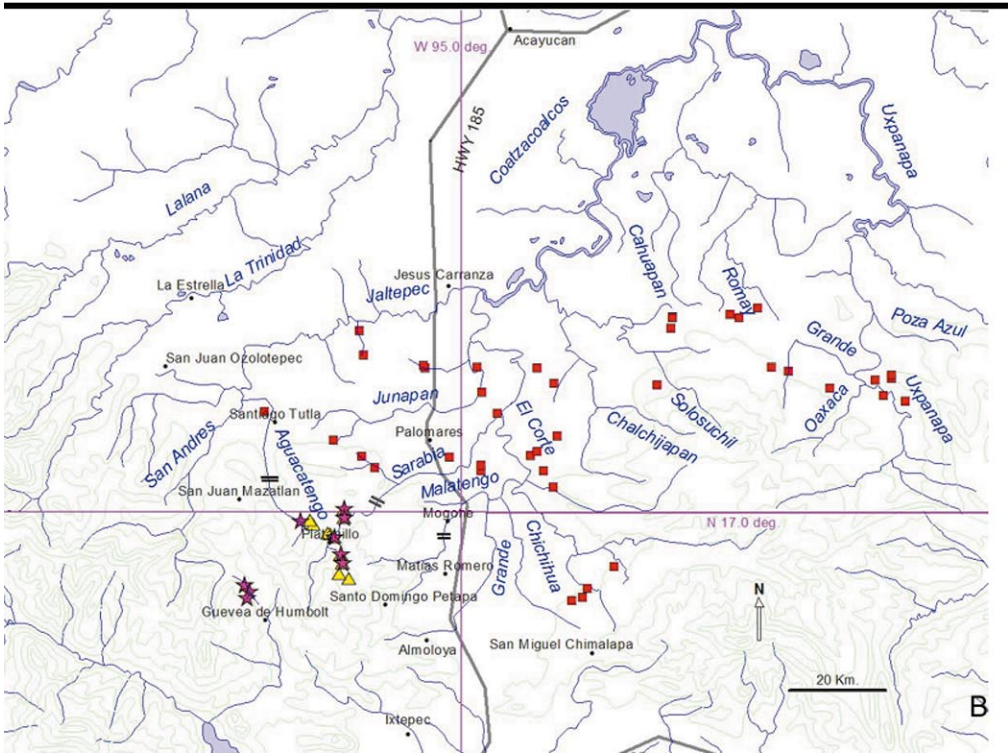
n = 40) was 10.5 mm (range 5–18 mm), significantly larger than that of *X. mixei*.

DISTRIBUTION: Upper Rio del Sol (Rio Sarabia drainage); upper Rio Aguacatengo near Loma Santa Cruz and Rio San Miguel (Rio Jaltepec drainage); all in Rio Coatzacoalcos basin, Oaxaca, Mexico (fig. 6B; appendix 1).

MATERIAL: From Oaxaca: Rio San Miguel, AMNH211358 (8); Rio Aguacatengo, UANL15258 (6); Rio del Sol, IBUN-AM13101 (5 paratypes).

### *Xiphophorus clemenciae* Alvarez, 1959
### (Yellow swordtail)
#### Figure 1E, F

DIAGNOSIS: This species has been previously described (Alvarez, 1959) and has been utilized in several evolutionary studies (Morizot and Siciliano, 1982; Borowsky et al., 1995; Rosen, 1960; Meyer et al., 1994; Meyer and Schartl, 2002).

DESCRIPTION: Males display two to four intense orange to red lateral stripes along the flank. In males, six to nine deep orange spots are always present in the base of the caudal fin. These spots develop at the time of sexual maturation. Coloration of the sword is either green or orange and edged with black pigment. The sword of mature males is well developed (sword index 0.25) and slightly upturned. Dorsal and ventral margins of the sword are edged with black. The gonopodium is similar to those of the preceding species except the more distal spines on ray 3 point anteriorly, as opposed to being angled proximally (fig. 4A). Counts for gonopodial features in rays 3, 4, and 5 are provided in table 3.

REMARKS: We cannot confirm Alvarez's description of these fish as having a deep yellow coloration in addition to the brilliant orange-red stripes. Among the hundreds of adult males sampled by us over the entire range of this species, including the type lo-cation near Rancho San Carlos, we have not seen a single individual with this coloration. Nor have any fish with this coloration appeared in the *X. clemenciae* maintained in the laboratory for more than 25 years. The vernacular name is inappropriate, in our opinion. The distinguishing traits of this species have remained unchanged during this period.

The species is especially common in small streams and brooks with light current and rockpools in areas with extensive limestone outcroppings, and it is relatively uncommon in fast-flowing streams. Thousands of these fish were present in the small streams at La Guadalupe, Arroyo El Boqueron, Arroyo San Antonio, Arroyo Pita, and an arroyo tributary to the Rio Negro. Isolated pools with hundreds of *X. clemenciae* were encountered along the Rio Uxpanapa, Rio Grande, and Rio Sarabia and in the rock pool near San Jose Morelos. The fish were uncommon in the Rio Paquital, Rio Grande, and Rio Escolapa. We do not think that these differences reflect degrees of difficulty in seining the fish, because many *X. monticolus* and *X. mixei* were collected in fast flowing rivers.

DISTRIBUTION: Rio Uxpanapa drainage, south of 17°17′N latitude; tributaries to Rio Coachapa at Vidal Diaz Munoz and Hidalgo Amayac, Veracruz; Rio Las Cuevas and Rio Paquital and tributaries near La Guadalupe, Veracruz, Rio Chalchijapan drainage; Arroyo El Boqueron, south of La Victoria, Oaxaca, rill on west bank of Rio Aguacatengo just north of a bridge, near Santiago Tutla Nuevo, Oaxaca, Rio Jaltepec drainage; tributaries to lower Rio Junapan, from Santa Maria Morelos, Veracruz, north, and headwater streams descending C. Pico de Aguila; tributaries to Rio Sarabia from confluence with Rio El Corte to Rio Polvora and upstream along Rio Polvora, Oaxaca; Rio Verde, Rio Escolapa and tributaries, Oaxaca; tributaries to Rio

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Fig. 6.    Drainage map of the Rio Coatzacoalcos basin in the Isthmus of Tehuantepec, Oaxaca, Mexico. **A.** Overview of the study area within Mexico. **B.** Distribution of the *clemenciae* clade. Squares: *X. clemenciae*; triangles: *X. mixei*; stars: *X. monticolus*. The clade ranges from the continental divide as far north as the mountain front bordering the coastal plain. Note its absence from the southcentral part of the isthmus. Solid bars indicate the observed existence of waterfalls.

Negro, Oaxaca, all above stations in Rio Coatzacoalcos basin (fig. 6B; appendix 1).

MATERIAL: From Oaxaca: Rio Escolapa drainage, UANL14333 (4), UANL14334 (4); Rio Negro drainage, UANL14335 (4); Rio Sarabia drainage, UANL14337 (4), UANL15250 (10) AMNH211355 (8); from Veracruz: Rio Junapan drainage UANL14336 (4), UANL-14339 (4); from Rio Chalchijapan drainage, UANL14338 (4), AMNH211354 (5).

### *Xiphophorus helleri* Heckel
### (1848; Green Swordtail)
#### Figure 1G, H

DIAGNOSIS: A medium to large swordtail with a long straight caudal appendage. Midlateral stripe may be dusky or brownish (northern populations) or red. Two additional reddish stripes may be present above midlateral line and one beneath. Terminal segment of gonopodial ray 3 produced into a crescent-shaped hook and blade pointed distally (fig. 3). Ray 4a curves strongly backward over the blade at an angle greater than 90°. Distal serrae of ray 4p reduced in size and number and proximal serrae rather slender. Terminal segment of ray 5a produced into a claw, several times larger than the distal serrae of ray 4p (fig. 3).

DISTRIBUTION: In coastal plain and front ranges. From Rio Nautla, Veracruz, Mexico, to northern Honduras (Rosen, [1960: fig. 7A, appendix 1] provided data for the upper Rio Coatzacolacos drainage).

MATERIAL EXAMINED: From Oaxaca: Rio del Sol, IBUNAM 13102 (2); Rio Junapan, IBUNAM 13103 (3), IBUNAM 31104 (8) UANL15251 (3), AMNH 211356 (14); from Veracruz: Rio Coachapa, UANL15252.

### COMMENTS

Within both sexes of the four species, significant pairwise differences were discovered in indexed measures of body and caudal peduncle depth, dorsal fin base, predorsal length, eye to snout and eye to ventral distance, head length, orbital diameter, and anal fin length (tables 1, 2). Several of these characters exclusively distinguished the three taxa of the *clemenciae* clade. Body depth and caudal peduncle depth indices are significantly different for all three forms. *X. mon-ticolus* possesses a rather narrow body and caudal peduncle; *X. clemenciae* exhibits the deepest body shapes while *X. mixei* is intermediate. Dorsal fin base length is significantly shorter in *X. mixei* and *X. monticolus* than in *X. clemenciae*. Predorsal length is longest in *X. mixei* and differs significantly from the two other species in males (only from *X. clemenciae* in females). The two newly described species differ significantly from *X. helleri* in body depth, length of dorsal fin base, and eye to ventral distance. Additional significant differences exist in caudal peduncle depth and mandibular length indices in males and head length and orbital diameter indices in females.

The sword of *X. mixei* is significantly shorter than those of the other taxa. The sword develops at the time of sexual maturation and continues to grow for several more months thereafter. The age of the mature males caught in the field is not known. It could be argued that all the mature *X. mixei* males had just attained maturity and that, therefore, the swords had not yet attained their final size. This is unlikely because many additional *X. mixei* males that were caught in the field and released all had short swords. Moreover, a stock of *X. mixei* has been bred in the *Xiphophoru*s Genetic Stock Center for several generations and males were maintained for at least one year past maturity. All males developed short swords, adding confidence to our conclusion from data derived from field collections. The sword of *X. clemenciae* is also significantly broader at its base than that of the other species, recalling its greater body and caudal peduncle depth.

The two newly described taxa and *X. clemenciae* possess gonopodia that are similar in structure. These similarities are present in studied structures within rays 4a, 4p, and 5a (table 3). There are, however, consistent differences that concern the spines on ray 3. The more distal spines in *X. clemenciae* are facing anteriorly, whereas in all 14 specimens of *X. mixei* and *X. monticolus* the distal spines are angled toward the more proximal. Because the most proximal spines of ray 3 are thick and angled toward the medial spines, in the two newly described taxa the spines are produced into a somewhat trian-

gular structure whereas in *X. clemenciae* they form a broader, more comblike feature. Ray 4a (also called the ramus) usually ends bluntly (11 out of 14 gonopodia), but in three gonopodia (one from each taxon) the small terminal segment angled sharply anteriorly, a condition previously illustrated for *X. clemenciae* (Rosen, 1960). The last segment of ray 5a of all three species is differentiated into a small claw that is approximately of the same size as the distal serrae on ray 4p.

## PHYLOGENETIC RELATIONSHIPS

Several traits unite *X. clemenciae, X. monticolus,* and *X. mixei* and set them apart from the other swordtails. The orange lateral stripes of the three species are produced by carotenoid pigment, whereas the pigment responsible for the red stripes of the *helleri* clade is drosopterin (Kallman and Bao, 1987).

A second diagnostic trait for the three species concerns the spots which develop in the proximal portion of the caudal fin. Every adult male exhibits red (*X. clemenciae*) or black (*X. mixei, X. monticolus*) spots, a trait not seen in any other *Xiphophorus*. The black spots in the proximal portion of the caudal fin of *X. mixei* and *X. monticolus* develop from the reticulum, bands of melanophores that border each scale pocket. When the males are exposed to agents that cause melanosomal (pigmentary) aggregation, orange spots are revealed. Thus with respect to this character the difference between *X. mixei* and *X. monticolus* on the one hand and *X. clemenciae* on the other is that in the two former species the melanosomes are invariably in the dispersed state and obscure the orange pigment cells, whereas in *X. clemenciae* the situation is reversed.

A third pigmentary trait is more difficult to evaluate. It concerns the axillary stripe, a band of melanophores extending from below the base of the pectoral fin to beyond the base of the gonopodium and into the caudal peduncle, one scale row above the midventral line. It is present in all adult males of the clade and expressed as a continuous zigzag line (at least in front of the gonopodium) following closely the reticulum. The only other *Xiphophorus* in which a similar pattern is present are adult males of the northern *X. helleri* populations (Rio Nautla, Rio Chachalaca, Rio Antigua, Rio Jampa systems; Rosen, 1960; Meek, 1904). In the *Xiphophorus* Genetic Stock Center, a strain derived from the Rio Chachalaca shows an axillary stripe that is produced as a row of spots rather than a continuous band, and it does not extend into the caudal peduncle. The same condition is exhibited in a male from the Rio Jamapa as illustrated in figure 21A by Rosen (1960), and figure 22 by Zander (1967). Because none of our other data reveals a close phylogenetic relationship between the *clemenciae* clade and *X. helleri*, we consider the trait(s) as being independently derived or, alternatively, the resemblance may be superficial. According to our observations on the Rio Chachalaca stock, in which two kinds of males occur, some with and some without this pattern (Zander, 1967), presence or absence of this trait appears to be controlled by a recessive sex-linked allele. Because no variation is present within the *clemenciae* clade, no genetic analysis can be easily performed. $F_1$ hybrids between *X. clemenciae* and *X. helleri* (Sarabia stock) do not exhibit an axillary stripe. In many backcross hybrids to *clemenciae* the axillary stripe is poorly expressed (Kallman, unpubl. ms.), suggesting that the genetic control of the axillary stripe in the two species may not be the same. Such a situation would be analogous to the presence of the Sc (spotted caudal) pattern in *X. variatus*, a platyfish, and *X. cortezi*, a northern swordtail (Rauchenberger et al., 1990).

## MOLECULAR PHYLOGENY

A total of 71,933 total bases of genomic DNA sequences were derived from two nuclear genes (*RAB27* and *CCND1*) in 29 fishes. This approach yielded 2359 nucleotide positions/characters that were conserved between all taxa. In addition, 153 were variable characters and, of these, 57 were informative for parsimony analyses. Data analysis revealed that *X. clemenciae* as well as the newly described species *X. mixei* and *X. monticolus* are unambiguously monophyletic. This conclusion is based on examination of phenograms/dendrograms derived from phenetic (neighbor-joining), parsimony, and maxi-

TABLE 4
**Jukes and Cantor Corrected Distance Matrix Between Exemplative Individuals**

|                 | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11 |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| P. intermedia   | —     |       |       |       |       |       |       |       |       |       |    |
| X. signum       | 0.029 | —     |       |       |       |       |       |       |       |       |    |
| X. montezumae   | 0.029 | 0.014 | —     |       |       |       |       |       |       |       |    |
| X. maculatus    | 0.027 | 0.012 | 0.005 | —     |       |       |       |       |       |       |    |
| X. helleri 1    | 0.030 | 0.005 | 0.015 | 0.014 | —     |       |       |       |       |       |    |
| X. couchianus   | 0.028 | 0.013 | 0.006 | 0.005 | 0.015 | —     |       |       |       |       |    |
| X. andersi      | 0.029 | 0.014 | 0.008 | 0.006 | 0.016 | 0.007 | —     |       |       |       |    |
| X. alvarezi     | 0.029 | 0.002 | 0.015 | 0.012 | 0.006 | 0.013 | 0.015 | —     |       |       |    |
| X. monticolus 1 | 0.030 | 0.016 | 0.010 | 0.009 | 0.018 | 0.008 | 0.011 | 0.016 | —     |       |    |
| X. clemenciae 1 | 0.031 | 0.016 | 0.011 | 0.010 | 0.018 | 0.009 | 0.012 | 0.017 | 0.005 | —     |    |
| X. mixei 1      | 0.032 | 0.016 | 0.010 | 0.009 | 0.018 | 0.009 | 0.010 | 0.016 | 0.005 | 0.006 | —  |

mum likelihood methods of phylogenetic reconstruction. A phylogenetic tree is provided (fig. 5) which shows bootstrap values derived from each of the three algorithmic methods. Using the parsimony method, strict consensus of 1000 reconstructed trees revealed absolute support for monophyly between *X. clemenciae* and the newly described taxa (data not shown). *X. clemenciae* individuals utilized in the analysis were specifically chosen to provide coverage of its entire geographic range. It is noteworthy that *X. clemenciae* individuals show more divergence from each other than do those of *X. mixei* and *X. monticolus*. This phenomenon is probably associated with the geographic ranges of these two newly identified species, which are relatively restricted compared to that of *X. clemenciae*, although this assumption is not proven by the data.

The *clemenciae* clade is also clearly more related to those included platyfish (*X. maculatus, X. couchianus,* and *X. andersi*) species and the included northern swordtail (*X. montezumae*) taxon than to *X. helleri, X. alvarezi,* and *X. signum*, as evidenced by bootstrap values (100%, 99%, and 97%, depending on the method of reconstruction; see fig. 5). Evidence for *X. mixei* and *X. monticolus* being distinct from other *Xiphophorus* taxa is supported by the phylogenetic reconstruction depicted in figure 5, which shows strong support for monophyly between all included individuals, regardless of geographic position. The genetic distance between species in the *clemenciae* clade, which includes the

newly described taxa, are similar to distances between *X. montezumae* and *X. maculatus* for example, taxa of "northern" swordtail and platyfish clades, respectively (table 4).

The position of *X. clemenciae* itself within phylogenetic trees involving members of the genus has been the topic of debate in the literature (Morizot and Siciliano, 1982; Rosen, 1979; Meyer et al., 1994; Borowsky et al., 1995; Meyer and Schartl, 2002). Although this study does not completely address the exact taxonomic relationship of *X. clemenciae* and the two newly described species within the genus *Xiphophorus*, it clearly shows with statistical confidence that these species are not closely related to the *helleri* clade. Undoubtedly, further inclusion of other *Xiphophorus* taxa and further data generation will address this research topic.

Support for monophyly of *X. helleri, X. alvarezi,* and *X. signum* is strongly supported by the data. The recently described *X. mayae* was not available and not included in the analysis, but presumably would also show close phylogenetic relationship to the "*helleri* clade" taxa (Meyer and Schartl, 2002). The common green swordtail, *X. helleri*, has the widest distribution of any *Xiphophorus*. The three other members of the *helleri* clade are found in Guatemala, suggesting that some populations in the eastern part of its range diverged into new forms. The similarity of the DNA sequences from both gene loci of *X. alvarezi* and the Lancetilla stock of *X. helleri* argues for a recent divergence from a common *X. alvarezi/X. helleri*-like

ancestor. Moreover, the apparent closer phylogenetic relationship of *X. alvarezi*, and perhaps also *X. signum*, to some (eastern) *X. helleri* populations than to others (Jalapa) has interesting taxonomic implications. If a similar relationship should be revealed by analyses of additional characters, then what is now known as *X. helleri* is really a paraphyletic assemblage of populations. This recalls an earlier description of the Jalapa population of swordtails as a separate species (Meek, 1904). In addition, extremely high levels of allozyme diversity within and among the *helleri* clade was previously reported (Morizot and Siciliano, 1982), providing additional evidence for genetic differentiation within this group of fishes.

Undoubtedly, further examination of the *clemenciae* and *helleri* clades and all *Xiphophorus* taxa will be required to fully address their exact evolutionary positioning. This study, however, does provide robust support that *X. clemenciae* and the two closely related species, *X. monticolus* and *X. mixei*, do not belong to the evolutionary grouping that includes *X. helleri*, which supports the conclusions of other studies (Morizot and Siciliano, 1982; Rosen, 1979; Meyer et al., 1994; Borowsky et al., 1995; Meyer and Schartl, 2002).

## DISCUSSION

*Xiphophorus mixei* and *X. monticolus* are more similar to each other in gonopodial structure than to *X. clemenciae*, but the overall similarly in the shape of the gonopodia of the three species is striking and sets them apart from all other *Xiphophorus*. Such overall similarities in gonopodial structure between closely related *Xiphophorus* species have been previously reported (Rosen, 1960; Rauchenberger et al., 1990). As an example, the nine species comprising the monophyletic northern swordtail group predominantly differ only in the size of the claw derived from ray 5a and the size of the distal serrae of ray 4p (Rauchenberger et al., 1990). The distal segment of ray 3 in the swordtails of the *helleri* clade is produced into a crescent-shaped hook (fig. 3), while in the three species of the *clemenciae* group it is a more elongated structure resembling the northern

swordtails (fig. 4). In the *clemenciae* group the ramus (ray 4a), found just posterior to the hook and in direct contact to it, is usually blunt at the distal tip or barely curved backward, a condition closely resembling that in the northern swordtails. The distal section of ray 4a of all three studied species is comprised of segments that usually show little or no taper anteriorly. In contrast, the ramus in the *helleri* clade is always strongly curved backward distally at an angle of greater than 90° and this portion consists of from three to six robust segments (fig. 3 shows one such gonopodium). In addition, ray 4a distally shows a wavy convex taper in the *helleri* clade that is not observed elsewhere in the genus (fig. 3). In all examined individuals of *X. clemenciae, X. mixei,* and *X. monticolus*, the last segment of ray 5a is produced into a claw, but there was never a pronounced size difference between it and the distal serrae of ray 4p. In most instances, the claw failed to protrude above the distal serrae altogether. This condition again resembles that in some members of the northern swordtail group. By contrast, the claw in the *helleri* clade is robust (fig. 3) and several times larger than the distal serrae of ray 4p which are also reduced in number (Rosen, 1960). This condition is not present in any other *Xiphophorus* taxon. Of the nine species of *Xiphophorus* conventionally called platyfish, only one exhibits a small claw and a second species, *X. maculatus*, is polymorphic for presence or absence of the claw in one population (Rosen, 1960).

### Topographic Setting

The Isthmus of Tehuantepec is the narrow waist of Mexico where the distance from the Gulf of Mexico in the north to the Pacific Coast to the south is a mere 190 km. The continental divide is located 35 km north of the Pacific Ocean. The very center of the isthmus, roughly followed by Mexican Route 185 (trans-isthmian highway) and by the Rio Coatzacoalcos and Rio El Corte, is also an area of low elevation throughout. Altitude immediately north of the divide is 220 m (plain of Chivela). There are rather few places in the center of the isthmus that reach an elevation of 300 m above sea level. The barrier ranges at the low section of the conti-

nental divide extend approximately 30 km east to west and 10 km north to south and they vary from 300 to 500 m in altitude, but there are several low passes through them. Towards the east and west-northwest the divide gains rapidly in altitude with some peaks reaching 2000 m and above. About 100 km to the east, the Sierra Espinazo del Diablo, a steep mountain range exceeding 1000 m in elevation and composed of cretaceous limestone, runs north-south and separates the Rio Coatzacoalcos basin from the Rio Grijalva drainage.

On the west the low section of the isthmus is bordered by the eastern limb of the Sierra Madre del Sur which rises steeply approximately 7 km west of the trans-isthmian highway and extends in a north-northwesterly direction for about 75 km as far as the Rio Jaltepec. There are several high points well north of the divide. Cerro el Borrego, 10 km southwest of Matias Romero, peaks at 1150 m and the Cerro Pico Del Aguila-Cerro Verde massif, 30 km northwest of Matias Romero, ranges from 800 to 1120 m. The mountains gradually descend to the 100–150 m level, roughly along a line westward from Palomares near the Rio Sarabia to the Rio Jaltepec, but then the land rises again to 300 m before dropping off sharply to the Rio Jaltepec, about 25 km southwest of Matias Romero. This northernmost rise may be more orogenically related to the ridge composed of cretaceous slate between the Rio Junapan and Rio Coatzacoalcos toward the east. It is lined up with the mountain front bordering the coastal plain to the east, but not with the front of the Sierra Madre del Sur to the west. Its maximum height of slightly above 300 m corresponds well with similar maximum elevations to the east (see below).

The Sierra Madre del Sur is being dissected by the Rio Malatengo, Rio Sarabia, Rio Aguacatengo and Rio San Andres and their tributaries. The rivers flow from the continental divide in a north-northeasterly direction in steepsided valleys, 100–400 m above their base level, except for the Rio Malatengo, which hugs the eastern front of the sierra. The Rio Junapan originates from streams that descend from Cerro Pico Del Aguila to the east and from the plateaulike mountain front along the Rio Jaltepec to the south and east. Shortly before these rivers reach their base level and assume an easterly course toward the Rio Coatzacoalcos and Rio El Corte, they descend over major waterfalls and cascades (Rio Malatengo just south of Mogone; Rio del Sol downstream from Guasamann; Rio Junapan downstream from Monte Aguila).

There are a few areas of karst outcroppings west of the Rio Coatzacoalcos. They occur along the base of the high ground that separates the Rio El Corte and Rio Junapan, on the north side of the mountain front that faces the Rio Jaltepec (there is also a row of karst towers on top of this plateau) and near the confluence of the Rio San Andres and Rio Aguacatengo to form the Rio Jaltepec and in the intramontane valley of the Rio San Miguel, just north of the continental divide at Guevea de Humboldt. This last river meanders in a northwestern direction for 17 km until, according to the map, it disappears into the ground at El Boqueron (elev. 360 m). From there a narrow gorge at 400 m elevation between Cerro El Centinela (elev. 1300 m) and Cerro El Otate (elev. 750 m) leads to the Rio San Andres (elev. 350 m) about 1.5 km west of El Boqueron. Although we have not visited this site, most likely there was a direct connection between the two rivers in the past.

The eastern part of the isthmus (east of the Rio El Corte) from the divide north for about 75 km consists of a series of folded ridges and valleys that trend northeasterly, then easterly and ultimately southwesterly. A prominent massive Paleozoic granite intrusion, the jagged Sierra Tres Picos, exceeds 1000 m, but most ridges are considerably lower. North of the Sierra Tres Picos the ridges are formed of Oligocene and Eocene sandstone and are in general higher toward the south and east. A trellislike network of small rivers dissects and erodes the ridges since the Pliocene south of the Sierra Tres Picos and since Quaternary times to the north (Geissert Kientz, 1999). Note that the Rio Uxpanapa, Rio Verde, and Rio El Corte and several smaller streams flow west or east for considerable distances before turning north. The Sierra Espinazo Del Diablo, 3–7 km northeast and east of the Rio Uxpanapa, forms the eastern limit of the Rio Coatzacoalcos basin.

The mountain front meets the Atlantic coastal plain approximately 80 km south of the town of Coatzacoalcos or roughly between 17°22′N and 17°29′N. Along most of the front the difference in elevation between coastal plain and upland is not very pronounced, a mere 100–150 m. Only few places near the front exceed 300 m in altitude which then gradually slope down toward the south. Such high points are found just south of the Rio Jaltepec and also south of the junction of the Rio Junapan and Rio El Corte (a north–south ridge of cretaceous slate surrounded by Miocene sandstone), near the source of the Rio Cahuapan and just west of the Rio Uxpanapa (Cerros La Numeracion).

The elevated terrain along the mountain front descends gently toward the south for about 20 km as far as the Valle de Uxpanapa, contrary to the overall prevailing dip in the isthmus, which is from south to north (Durham et al., 1955). Several small streams descend from the high ground along the mountain front in a southerly direction until they join the major rivers. The term ''Valle de Uxpanapa'' does not refer to the valley of the Rio Uxpanapa. This term is applied to the 5–10-km-wide level valley that extends at an elevation of 100 m from the Rio El Corte at Cuauhtemoc for 60 km eastward to the Rio Grande. It then continues southwesterly for 30 km along the Rio Uxpanapa at an elevation of 50 m. The Valle de Uxpanapa shows the same trend as the ridges: northeasterly then southwesterly.

Wherever the sandstone ridges have been eroded away between the mountain front and the Sierra Tres Picos, cretaceous limestone is exposed, often present as karst towers and sinkholes (Geissert Kientz, 1999). The Valle de Uxpanapa has a solid floor of limestone covered by a thin layer of soil. The major rivers in this region have cut down close to the base level. Elevation of the Rio Uxpanapa at its junction with the Rio Grande is 40 m, and further upstream near 17°03′N it runs at 100 m. Along latitude 17°10′N—roughly along the Valle de Uxpanapa—elevation of the Rio El Corte is 40 m, the Rio Chalchijapan is 60 m, and the Rio Solosuchil is 70 m.

The coastal plain just north of the mountain front consists of uncountable low hillocks between 60 and 80 m in elevation above sea level. All the major streams run at an elevation of 40 m. The coastal plain gently slopes toward the Gulf. As far as 45 km inland from the Gulf coast there are extensive swamps, lagoons, and oxbows along the Rios Coatzacoalcos and Uxpanapa.

Since Cretaceous times there was never a sea passage across the Isthmus, although the emergent land mass must have been exceedingly narrow until the Pliocene (Durham et al., 1955). Neocene marine deposits have been found south along route 185 as far as Mogone along the Rio Malatengo (Durham et al., 1955). Marine ostracods of mid-Pliocene age have been reported from a locality 15 km north of Jesus Carranza (Michain-Castillo, 1985), marine foraminifera of lower Pliocene age near Jesus Carranza (Akers, 1984), and late Miocene marine fossils from a site 3 km south of the Rio Jaltepec (Durham et al., 1955). It appears that until the Pliocene the Gulf of Mexico extended as far at the present day mountain front.

## DISTRIBUTION

*Xiphophorus clemenciae* is the most widespread and common *Xiphophorus* in the uplands of the Rio Coatzacoalcos basin (fig. 6). It is absent from the coastal plain. Like virtually all other *Xiphophorus*, it is a species restricted to the Sierra Madre. Between the Rio El Corte and the Rio Uxpanapa, the northern limit of its distribution coincides closely with the mountain front facing the Gulf coastal plain. This species was not taken in any of the small streams that descend the mountain front to the north, but it was abundantly present in every stream sampled south of it. Conversely, *X. helleri* ranges over the coastal plain (see locality records listed by Rosen, 1960) as far as the mountain front but not beyond (fig. 7A). Only in the Rio Romay and Rio Cuahapan, streams that arise a short distance south of the front and flow north, did the two species occur sympatrically. This area has been severely eroded and can be considered as a southward extension of the coastal plain.

West of the Rio Coatzacoalcos the distribution of *X. clemenciae* is rather spotty. In the very center of the isthmus, *X. clemenciae*
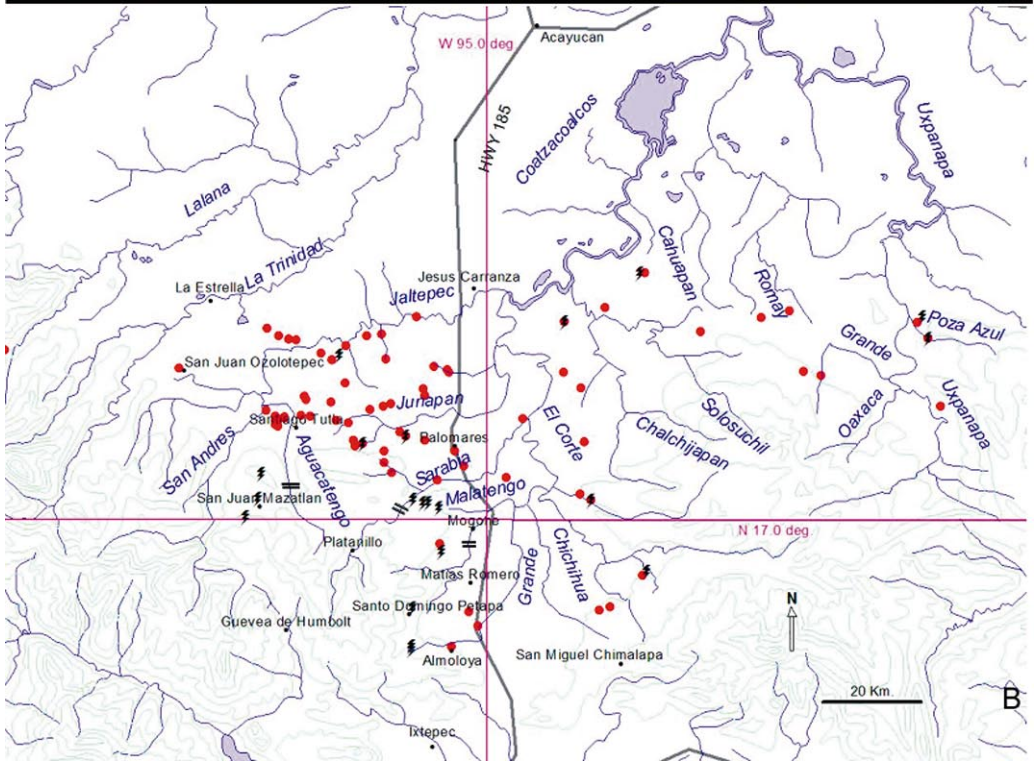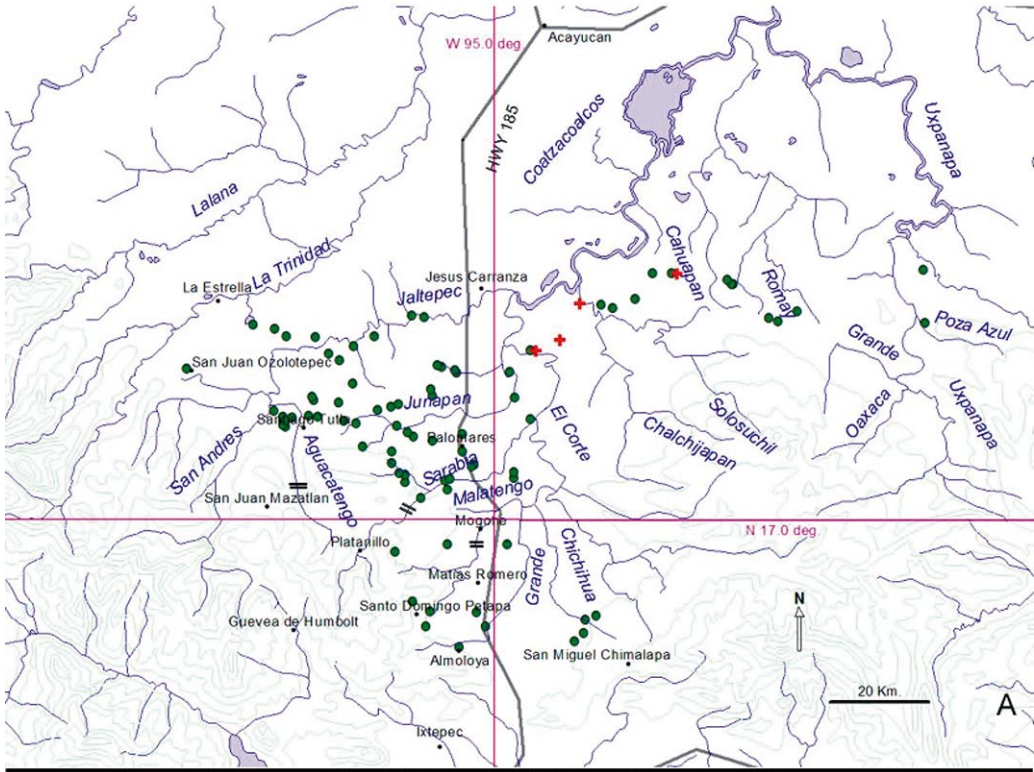
is found from near the mountain front to about 25 km south of it along some of the tributaries to the Rio Sarabia and Rio Junapan. At each station it occurs sympatrically with *X. helleri*. Yet farther south in the low center of the isthmus—the area drained by the Rio Malatengo, Rio Grande, and Rio Chichihua—*X. clemenciae* is absent (19 sites sampled), but *X. helleri* is ubiquitous. *X. clemenciae* is found in small tributaries of the Rio Sarabia as far upstream as the Rio del Sol and Rio La Polvora, but it does not reach above the waterfall and cascades downstream from Guasamann. At all but one location, it occurred together with *X. helleri*, but there were many stations where only *X. helleri* was found.

Along the lower Rio Junapan we found *X. clemenciae* present in three tributary streams (fig. 6) and Ramirez (Ramirez, 1999) listed an additional station in the Rio Tortuguero, where we caught only *X. helleri*. From then on *X. clemenciae* was not encountered in this drainage until the headwaters (Rio El Robalo) that descend from Cerro Pico del Aguila. One station is approximately 2.5 km NW of the *X. clemenciae* site in the Polvora drainage across a narrow ridge. A member of the *clemenciae* clade also occurs in the Rio Junapan at Monte Aguila, but because only small immature fish were collected, we could not identify the species. Both streams were also sampled repeatedly further downstream near route 147 over a period of nearly 10 years, but only *X. helleri* was found.

Only 2 of 27 sites in the Rio Jaltepec drainage yielded *X. clemenciae*. It was abundant throughout the Arroyo El Boqueron (not to be confused with El Boqueron in the Rio San Miguel drainage near the continental divide) which descends the mountain front to the Rio Jaltepec, and in a small rill (elev. 100 m) near Santiago Tutla Nuevo, not far from where the Rio San Andres and Rio Aguacatengo join. These two *clemenciae* sites are 25 km apart. A straight line drawn along the Rio Sarabia where it enters the Sierra, and continued to the Tutla Nuevo site, will pass through all *clemenciae* locations in the upper Rio Sarabia and Rio Junapan.

*Xiphophorus monticolus* and *X. mixei* replace *X. clemenciae* at higher elevation in the interior section of the Sierra Madre del Sur. They occupy headwater streams of the Rio Jaltepec (Rio Aguacatengo, Rio San Miguel) and Rio Sarabia (Rio del Sol) drainages. *X. mixei* is abundant in the Rio del Sol, which is a fast-flowing river with riffles and boulders, and in all tributaries that flow into it from the mountain spur that separates the Rio del Sol from the Rio Malatengo. The new forms of *Xiphophorus* are also present in small tributaries on both sides of the low Sol–Aguacatengo divide near Platanillo and one Rio del Sol tributary east of the divide. Elevation of collecting sites ranged from 500 to 380 m.

We have not found the two species in a tributary near Guasamann, just before the Rio del Sol spills over a waterfall and descends 160 m over a distance of 5 km to join the Rio Polvora. This cascade appears to be the barrier between *X. monticolus* and *X. mixei* in the uplands and *X. helleri* and *X. clemenciae* in the downstream section of the Rio del Sol/Rio Sarabia drainage. Note also that three additional genera of poeciliid fishes (*Priapella, Poecilia,* and *Poeciliopsis*) do not occur above the waterfall, although they are common below (fig. 7B; see appendix 1). There appears to be a narrow belt in the region between the upper and lower Rio del Sol where no *Xiphophorus* is present. In addition to the tributary at Guasamann, no

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Fig. 7.   Drainage map of the Rio Coatzacoalcos basin in the Isthmus of Tehuantepec, Oaxaca., Mexico. **A.** Collection sites of *X. helleri* (circle) and *X. maculatus* (cross). *X. helleri* occurs in the coastal plain (distribution not shown) as far south as the mountain front, except in the central part of the isthmus, where its range extends to the continental divide. *X. maculatus* occurs in the coastal plain as far as the mountain front. Note that the distributions of *X. helleri* and the *clemenciae* clade (fig. 5B) are complementary to each other. **B.** Collecting sites of *P. intermedia* (circles). The ''lightning'' symbol represents sites where no *Xiphophorus* was collected. The distribution of *P. intermedia* coincides with that of *X. helleri* (fig. 6A) and *X. clemenciae* (fig. 5B). Solid bars indicate the observed existence of waterfalls.

*Xiphophorus* is present in the Rio San Juan Viejo, which drops 400 m over a distance of 5 km and joins the Rio del Sol just below the waterfall. The Rio Guajalote (Rio Malatengo tributary), which flows in the opposite direction 3 km east of San Juan Viejo also lacks *Xiphophorus*. A similar situation may exist in the Rio Aguacatengo and Rio San Andres area, but here our knowledge is less complete because of the difficulty of access to the region. In the foothills of the Sierra Madre del Sur near the area where the Rio Aguacatengo and Rio San Andres join, *X. helleri* is common. However, a short distance inside the Sierra two tributaries to the Rio San Andres, Rio Coyol (sampled at 450 m and 320 m) and Rio Ovo (380 m) near San Juan Mazatlan, lacked *Xiphophorus*. These streams descend the divide that separates the Rio Aguacatengo to the east and the Rio San Andres to the west. As in the Rio Sarabia–Rio del Sol drainage, *Priapella, Poeciliopsis,* and *Poecilia* are not present in the Rio Aguacatengo and Rio San Andres headwaters.

In the uplands of the Rio Coatzacoalcos basin, *X. helleri* is replaced by members of the *X. clemenciae* clade. This recalls the relationship of *X. helleri* with *X. signum* and *X. alvarezi* (and perhaps also *X. mayae*) in Guatemala where *X. helleri* is found in the coastal plain and along the front ranges, whereas the other two forms inhabit interior drainages, many of which are isolated (Rosen, 1979). East of the Rio El Corte the mountain front separates *X. helleri* from *X. clemencia*e. *X. helleri* occupies the streams along the northern edge of the front (fig. 7A), and according to the locality records listed by Rosen (1960) this is the only swordtail species that occurs in the coastal plain of the Rio Coatzacoalcos basin.

In the western range of the Rio Coatzacoalcos basin, the distribution of the two species is more complex. *X. helleri* is common in the tributaries of the Rio Jaltepec, Rio Junapan, and Rio Sarabia that descend the foothills, but it is absent in the interior valleys. *X. clemenciae*, which shows a spotty distribution along the three rivers, is always sympatric with *X. helleri* except in Arroyo El Boqueron. In the low southern central part of the isthmus, which is drained by the Rio Malatengo and its two major tributaries, *X.*

*helleri* is common, but members of the *clemenciae* clade are absent. The only stream where *X. helleri* occurs at a higher elevation is a tributary (elev. 500 m) of the Rio Malatengo at Zacatal that descends the steep eastern limb of the Sierra Madre.

In our interpretation, the *clemenciae*-clade ancestor occupied the old uplands of the Coatzacoalcos basin and was the only *Xiphophorus* in this region. Since then there appears to have been renewed uplift of the Sierra Madre, as indicated by the steep valleys and major waterfalls which isolated the fish in the headwaters from those farther downstream. At present the *X. clemenciae* population of the Rio Polvora near its junction with the Rio del Sol is the closest one to *X. mixei* and *X. monticolus*. The two species very likely evolved in different upland rivers which subsequently were joined by stream capture. Northeast of Gueva, headwater streams of the Rio del Sol, Rio San Miguel and Rio Aguacatengo come in close proximity on the same mountain spur. The Rio del Sol could have been a tributary to the Rio Aguacatengo west of Platanillo where the ridge between the two rivers is rather low. Subsequently, the Rio del Sol could have been captured by a major Rio del Sol tributary (called Rio Cuchara, but not to be confused with the stream of the same name that joins the Rio Aguacatengo) which descends Cerro de Pico Aguila to the south and joins the river upstream from Guasamann. *X. helleri* arrived at a later time after the emergence of the coastal plain, and its dispersal was along the mountain front and coastal plain. Because the mountain front east of the Rio El Corte rises abruptly from the coastal plain and dips south and few rivers pass through it, it was a barrier to the southward dispersal of the green swordtail. It penetrated farther south in the low part of the isthmus along the major rivers Rio Junapan, Rio Sarabia, Rio Malatengo, and Rio El Corte. This geologic scenario leaves unanswered the questions as to why *X. clemenciae* is absent from the central southern section of the isthmus and why *X. helleri* did not penetrate farther south in the Rio Uxpanapa. During Pliocene and Quaternary times stream systems in this highly tectonically active region may have been considerably different.

In view of the distribution of *X. clemenciae*, the failure of earlier attempts to determine the true status of this form now becomes apparent. Ichthyologists including ourselves traveled with vehicles along the Trans-isthmian Highway and collected fishes in streams close by. With the exception of three locations, however, the highway passes through the only region of the isthmus where the species is absent. The collectors failed to go into the backcountry to the east and west.

*Xiphophorus clemenciae* is widespread and abundant and can be removed from the list of endangered and threatened *Xiphophorus*. However, *X. monticolus* and *X. mixei* occupy limited ranges; as long as the headwaters of the Rio Sarabia and Rio Jaltepec north of the continental divide remain intact, the two species will not be threatened. Other species of *Xiphophorus* that are in danger of becoming extinct are the three northernmost species (*X. meyeri, X. gordoni, X. couchianus*) in the Chihuahuan desert. These are forms that are restricted to a few small isolated spring pools. *X. couchianus* is currently considered extinct in nature, but this determination is pending further exploration of proximal localities to the type collections. A fourth species that we think is in danger is *X. andersi*, which is restricted to the small watershed of the Rio Atoyac above the cataracts at Atoyac, Veracruz. Although located in a region of high annual rainfall, diversion of water for agricultural purposes and industrial pollution from sugar mills seriously degrades the habitat. Similarly, *X. continens*, a species of very narrow distribution could also be considered threatened, requiring further study.

## ECOLOGY

In many places *X. helleri* and members of the *clemenciae* clade exist in close proximity, but in different watersheds across rather minor barriers. A 10–km wide section of limestone country separates *X. helleri* in the Rio Poza Azul from *X. clemenciae* in the Rio Verde (Rio Uxpanapa system). No swordtail is present in the headwaters of the Rio Zacatal, but 6.5 km distant in a small stream that flows south, *X. clemenciae* occurs by the thousands (Chalchijapan drainage). On the divide (300 m) between the Rio Chichihua and a tributary to the Rio Escolapa, *X. helleri* is present in a swampy area, whereas 2.5 km away in the Arroyo San Antonio (250 m) *X. clemenciae* is common. Tributaries of the Rio Malatengo that drain the eastern limb of the Sierra Madre southwest (Arroyo La Canada, 300 m) and northwest (Rio Los Nanches at Zacatal, 500 m) of Matias Romero are occupied by *X. helleri*, but in the streams that drain 5–7 km to the west into the Rio del Sol only *X. mixei* and *X. monticolus* are found. The sharp separation of *X. helleri* from members of the *clemenciae* clade over a wide area suggests that this distributional pattern is of long standing.

The complementary distribution of *X. helleri* north and of *X. clemenciae* south of the mountain front east of the Rio El Corte might also suggest that the two species occupy similar niches and cannot exist together. We reject this idea, because in the Rio Sarabia and Rio Tolosita drainages the two species are broadly sympatric. It is also possible that the two forms prefer different habitats. We found *X. clemenciae* usually associated with limestone habitats. This may be merely a reflection of the geological condition of this species' distribution and not its cause, but on a very local level we found a few sites where *X. clemenciae* seemed to prefer limestone habitats and *X. helleri* did not. The streams (16 sites sampled) that drain the high ground 20 km southwest of Matias Romero along the Rio Jaltepec are occupied by *X. helleri* with the single exception of the Arroyo El Boqueron. This is the only stream in this region with massive limestone outcroppings, and here *X. helleri* was absent and *X. clemenciae* abundant. A second site where we found such a separation was in two small streams that flow into the Rio Sarabia southeast of Palomares. One stream with muddy bottom and decaying leaves sampled repeatedly since 1982 contains only *X. helleri* whereas the other stream, one km distant and 20 m lower in a ravine with limestone bottom and decaying leaves, is occupied only by *X. clemenciae*. A third such site is the ravine southeast of Jose Maria Morelos. In the small stream at the mouth of the ravine we took only *X. helleri*, but farther inside where the

large rockpools were located, both species were common.

## SUMMARY

Our data clearly show that *X. clemenciae* is an abundant and widespread inhabitant of the uplands of the Rio Coatzacoalcos system, Veracruz and Oaxaca, Mexico. The species can be removed from the list of endangered or threatened species. Based on morphological and molecular evidence derived from two distinct loci, two new taxa, *X. monticolus* and *X. mixei*, have been described that are closely allied to *X. clemenciae*. These new forms are sympatric in parts of their range that comprises headwater streams of the Rio Jaltepec and Rio Sarabia drainages inside the Sierra Madre del Sur, Oaxaca, Mexico. According to our dataset and methods of phylogenetic reconstruction, the three species of the *clemenciae* clade are monophyletic and are more closely related to the platyfishes and the northern swordtail group of the Panuco river basin, rather than to the *helleri* swordtail clade in the south. The distribution of the swordtails is closely correlated with geological features in the Rio Coatzacoalcos basin. East of the Rio El Corte, *X. clemenciae* is the only swordtail between the mountain front bordering the Gulf coastal plain and the continental divide. It is absent from the coastal plain. *X. helleri* occurs in the coastal plain but not south of the mountain front. West of the Rio El Corte, *X. clemenciae* and *X. helleri* are sympatric in tributaries of major rivers—Rio Jaltepec, Rio Junapan, and Rio Sarabia—, that drain the foothills. Inside the Sierra Madre above waterfalls and cataracts, *X. mixei* and *X. monticolus* replace *X. clemenciae* and *X. helleri*. These complex zoogeographic patterns suggest even more complex relationships derived from the combination of historic geologic events, populational isolations, and subsequent divergence. These same events theoretically contributed to probable differences in microhabitat preference, morphometric change/fixation, and possible behavioral divergence.

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APPENDIX 1

ICHTHYO-FAUNAL SAMPLING OF THE RIO COATZACOALCOS DRAINAGE SYSTEM

| Location | Samplings | Latitude N, longitude W | X. helleri | X. clemenciae | X. mixei | X. monticolus | X. maculatus | Priapella sp. | Poecilia sp. | Poeciliopsis sp. | Heterandria sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RIO UXPANAPA** | | | | | | | | | | | |
| Small tributary to Rio Nanchital, ~3 km SW of Cerro Nanchital, Ver. | 1 | 17°28.55′, 94°10.75′ | Yes | No | No | No | No | No | Yes | No | Yes |
| Small tributary to Rio Nanchital, Ver. | 1 | 17°23.09′, 94°10.00′ | No | No | No | No | No | No | No | No | Yes |
| Rio Poza Azul at Poza Azul, tributary to Rio Uxpanapa, Ver. | 1 | 17°22.41′, 94°10.55′ | Yes | No | No | No | No | Yes | No | No | Yes |
| Rio Poza Azul enters cave, Ver. | 1 | 17°20.67′, 94°09.34′ | No | No | No | No | No | Yes | No | No | No |
| Stagnant pool in dry arroyo, tributary to Rio Verde, 1 km W of El Trece or Gustavo Diaz Ordaz, Ver. | 1 | 17°15.93′, 94°09.50′ | No | Yes | No | No | No | No | No | No | Yes |
| Stagnant pool in dry arroyo, tributary to Rio Verde, Ver. | 1 | 17°15.53′, 94°09.50′ | No | Yes | No | No | No | No | No | No | No |
| Arroyo El Hule, tributary to Rio Uxpanapa, ~8 km NW of Los Amarillos, Ver. | 1 | 17°15.30′, 94°11.40′ | No | Yes | No | No | No | No | No | No | No |
| Arroyo, tributary to Rio Uxpanapa, ~4 km NW of Los Amarillos, Ver. | 1 | 17°13.55′, 94°10.35′ | No | Yes | No | No | No | No | No | No | Yes |
| Small stream, tributary to Rio Uxpanapa, 1.5 km NE of Los Amarillos, Ver. | 1 | 17°12.79′, 94°07.87′ | No | Yes | No | No | No | Yes | Yes | No | No |
| Rio Grande, 10 km E of Chinantla, Ver. | 1 | 17°16.38′, 94°21.63′ | No | Yes | No | No | No | No | No | No | No |
| Rill, next to Rio Grande, Ver. | 1 | 17°16.38′, 94°21.63′ | No | Yes | No | No | No | Yes | No | No | No |
| Arroyo, tributary to Rio Grande, Ver. | 1 | 17°16.86′, 94°23.60′ | No | Yes | No | No | No | Yes | No | No | No |
| Rio La Garganta, Ver. | 1 | 17°12.68′, 94°15.00′ | No | Yes | No | No | No | No | No | No | No |
| **RIO COACHAPA** | | | | | | | | | | | |
| Tributary to Rio Los Juanes, 2 km SSE of Vidal Diaz Munoz, Ver. | 1 | 17°22.64′, 94°27.42′ | Yes | Yes | No | No | No | No | No | No | Yes |
| Tributary to Rio Los Juanes at Vidal Diaz Munoz, Ver. | 1 | 17°22.99′, 94°28.48′ | Yes | Yes | No | No | No | Yes | No | No | Yes |
| Rio Romay, 2.5 km S of Hidalgo Amayac, Ver. | 1 | 17°23.74′, 94°25.22′ | Yes | Yes | No | No | No | Yes | No | No | Yes |
| Rio Cahuapan at Cahuapan, Ver. | 1 | 17°27.36′, 94°33.21′ | Yes | No | No | No | No | No | Yes | No | No |
| Small creek flowing N, Ver. | 1 | 17°26.90′, 94°32.61′ | Yes | No | No | No | No | No | No | No | Yes |
| Stagnant pool in pasture, Ver. | 1 | 17°26.93′, 94°32.80′ | Yes | No | No | No | No | No | Yes | No | No |
| **RIO CHALCHIJAPAN** | | | | | | | | | | | |
| Stagnant pool in pasture, Ver. | 1 | 17°28.14′, 94°42.35′ | No | No | No | No | No | No | Yes | No | Yes |
| Small brook flowing N, Ver. | 1 | 17°28.14′, 94°41.78′ | Yes | No | No | No | No | Yes | Yes | No | No |
| Small brook flowing N, Ver. | 1 | 17°28.12′, 94°38.93′ | Yes | No | No | No | Yes | No | No | No | No |
| Arroyo, 4 km SE of village of Chalchijapan, Ver. | 1 | 17°24.59′, 94°47.74′ | Yes | No | No | No | No | No | No | No | Yes |
| Small stream 1 km NW of Las Flores, Ver. | 1 | 17°24.19′, 94°46.37′ | Yes | No | No | No | No | Yes | Yes | No | No |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | X. helleri | X. clemenciae | X. mixei | X. monticolus | X. maculatus | Priapella sp. | Poecilia sp. | Poeciliopsis sp. | Heterandria sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RIO CHALCHIJAPAN *(continued)* | | | | | | | | | | | |
| Dos Hermanos, Ver. | 1 | 17°22.68′, 94°35.24′ | No | Yes | No | No | No | No | Yes | No | Yes |
| Arroyo, tributary to Rio Las Cuevas, 3.5 km N of Benito Juarez Uno, Ver. | 1 | 17°21.39′, 94°35.45′ | No | Yes | No | No | No | Yes | Yes | No | Yes |
| Arroyo, tributary to Rio Solosuchil near Poblado Dos, Ver. | 1 | 17°14.70′, 94°37.00′ | No | Yes | No | No | No | No | No | No | No |
| Arroyo, tributary to Rio Las Cuevas, 6 km N of Benito Juarez Uno, Ver. | 1 | 17°22.75′, 94°35.24′ | No | Yes | No | No | No | No | No | No | No |
| Arroyo, 1 km W of village of Chalchijapan, Ver. | 1 | 17°24.73′, 94°50.06′ | No | No | No | No | Yes | No | No | No | No |
| Rio Zacatal at Adolfo Lopes Mateos, Ver. | 1 | 17°22.50′, 94°51.00′ | No | No | No | No | No | Yes | Yes | Yes | No |
| Rill, Rio Zacatal drainage, Ver. | 1 | 17°20.50′, 94°52.34′ | No | No | No | No | Yes | No | No | No | No |
| Small stream at La Guadelupe, tributary to Rio Paquital, Ver. | 2 | 17°16.78′, 94°51.09′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Rio Paquital at Veinticuatro de Febrero, Ver. | 1 | 17°14.89′, 94°49.11′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Arroyo at Luna Media, tributary to Rio Paquital, 9 km NE of Cuauhtemoc, Oax. | 1 | 17°08.78′, 94°48.71′ | No | Yes | No | No | No | Yes | No | No | No |
| Arroyo Azul, tributary to Rio Chalchijapan, Oax. | 1 | 17°04.65′, 94°46.37′ | No | Yes | No | No | No | No | No | No | Yes |
| RIO JALTEPEC | | | | | | | | | | | |
| Small tributary to Rio Jaltepec, 3 km ESE of Los Angeles, Oax. | 1 | 17°23.16′, 95°08.00′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Small tributary to Rio Jaltepec, 1 km SE of Los Angeles, Oax. | 1 | 17°23.33′, 95°09.43′ | Yes | No | No | No | No | No | No | No | Yes |
| Arroyo El Boqueron, 0.5 km S of La Victoria, Oax. | 1 | 17°21.09′, 95°11.97′ | No | Yes | No | No | No | Yes | Yes | No | Yes |
| Headwater pool of El Boqueron, 6 km SSE of La Victoria, Oax. | 1 | 17°18.30′, 95°11.51′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Rio Chayotal, Nuevo Madero, Oax. | 1 | 17°20.89′, 95°13.68′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Rio La Cal at Tortuguero, Oax. | 1 | 17°19.76′, 95°16.07′ | Yes | No | No | No | No | Yes | No | Yes | No |
| Small tributary to Rio La Cal, Oax. | 1 | 17°18.76′, 95°16.83′ | No | No | No | No | No | No | No | Yes | Yes |
| Rio La Cal near San Juan del Rio, Oax. | 1 | 17°15.53′, 95°16.12′ | Yes | No | No | No | No | Yes | Yes | Yes | No |
| Rio Huasmole at Jaltepec de Candayoc, Oax. | 1 | 17°21.75′, 95°25.12′ | Yes | No | No | No | No | Yes | Yes | No | Yes |
| Tributary to Rio Cabeza at San Juan Otzolotepec, Oax. | 1 | 17°17.26′, 95°35.16′ | Yes | No | No | No | No | Yes | No | No | Yes |
| Arroyo Toposito at San Jose de las Flores, Oax. | 1 | 17°20.89′, 95°23.82′ | Yes | No | No | No | No | Yes | No | Yes | No |
| Rio Puxmecatan at Constitution Mexicana, Oax. | 1 | 17°20.51′, 95°22.67′ | No | No | No | No | No | Yes | No | Yes | No |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | X. helleri | X. clemenciae | X. mixei | X. monticolus | X. maculatus | Priapella sp. | Poecilia sp. | Poeciliopsis sp. | Heterandria sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RIO JALTEPEC** (*continued*) | | | | | | | | | | | |
| Small stream E of Constitution Mexicana, Oax. | 1 | 17°20.49′, 95°21.73′ | No | No | No | No | No | Yes | No | Yes | Yes |
| Arroyo E of Constitution Mexicana, Oax. | 1 | 17°20.79′, 95°20.40′ | Yes | No | No | No | No | No | No | Yes | Yes |
| Tributary to Rio Juan at Agua Fria, Oax. | 1 | 17°18.97′, 95°18.95′ | No | No | No | No | No | Yes | No | Yes | No |
| Damned spring pool at Agua Fria, Oax. | 1 | 17°18.90′, 95°18.95′ | Yes | No | No | No | No | No | No | No | Yes |
| Rio Victor San Juan at Villanueva Segundo, Oax. | 1 | 17°18.15′, 95°17.64′ | Yes | No | No | No | No | Yes | No | Yes | No |
| Arroyo Lirio, headwater of Rio Victor San Juan, Oax. | 1 | 17°13.31′, 95°17.76′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Headwater Rio Victor San Juan at San Antonio Tutla, Oax. | 1 | 17°11.24′, 95°17.23′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Tributary to Rio Arenalito at Los Fresnos, Oax. | 1 | 17°13.96′, 95°20.84′ | Yes | No | No | No | No | Yes | Yes | Yes | No |
| Stream S of Los Fresnos, Oax. | 1 | 17°13.60′, 95°20.60′ | Yes | No | No | No | No | Yes | No | Yes | No |
| Small stream flowing into Rio Arena, Oax. | 1 | 17°11.74′, 95°20.13′ | Yes | No | No | No | No | Yes | No | No | No |
| Tributary of Rio Arena, 2 km E of Santiago Tutla Nuevo, Oax. | 1 | 17°11.73′, 95°21.23′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Small brooks, tributaries to Rio Aguacatengo, 3–4 km SW of Santiago Tutla Nuevo, Oax. | 1 | 17°11.69′, 95°24.13′ | Yes | No | No | No | No | Yes | No | No | Yes |
| | 1 | 17°11.23′, 95°24.11′ | Yes | No | No | No | No | Yes | No | Yes | No |
| | 1 | 17°11.18′, 95°23.88′ | Yes | No | No | No | No | Yes | No | No | No |
| Rill flowing into Rio Aguacatengo, Oax. | 2 | 17°11.62′, 95°23.10′ | Yes | Yes | No | No | No | Yes | No | Yes | Yes |
| Rio Lagarto, 4 km SW of Santiago Tutla Nuevo, Oax. | 1 | 17°10.53′, 95°23.90′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Small stream, tributary to Rio Aguacatengo, Oax. | 1 | 17°10.73′, 95°24.12′ | Yes | No | No | No | No | Yes | No | No | Yes |
| Rio Lagarto at confluence with Rio Aguacatengo, Oax. | 1 | 17°11.54′, 95°23.08′ | No | No | No | No | No | Yes | No | No | No |
| Rio Cuchara, ~3 km E of Loma Santa Cruz, Oax. | 1 | 16°58.95′, 95°17.71′ | No | No | Yes | No | No | No | No | No | Yes |
| Tributary to Rio Cuchara and and Rio Aguacatengo, Oax. | 2 | 16°58.42′, 95°17.31′ | No | No | Yes | No | No | No | No | No | Yes |
| Rio Aguacatengo at Loma Santa Cruz, Oax. | 1 | 16°58.84′, 95°18.80′ | No | No | No | Yes | No | No | Yes | No | Yes |
| Arroyo Concha, tributary to Rio San Andres, 5 km WNW of Santiago Tutla Nuevo, Oax. | 1 | 17°12.34′, 95°25.22′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Rio Coyol, tributary to Rio San Andres, 7 km N of San Juan Mazatlan, Oax. | 2 | 17°05.10′, 95°25.77′ | No | No | No | No | No | No | No | No | Yes |
| Headwater of Rio Coyol 1.5 km NE of San Juan Mazatlan, Oax. | 1 | 17°02.24′, 95°26.11′ | No | No | No | No | No | No | No | No | No |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | *X. helleri* | *X. clemenciae* | *X. mixei* | *X. monticolus* | *X. maculatus* | *Priapella* sp. | *Poecilia* sp. | *Poeciliopsis* sp. | *Heterandria* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RIO JALTEPEC (*continued*) | | | | | | | | | | | |
| Rio Ovo, tributary to Rio San Andres, 3 km SW of San Juan Mazatlan, Oax. | 1 | 17°00.16′, 95°27.57′ | No | No | No | No | No | No | No | No | Yes |
| Tributary to Rio San Miguel near San Miguel, Oax. | 2 | 16°51.34′, 95°25.43′ | No | No | No | Yes | No | No | No | No | Yes |
| Rio San Miguel downstream from Nuevo Esperanza, Oax. | 2 | 16°50.59′, 95°24.83′ | No | No | No | Yes | No | No | No | No | Yes |
| Rio San Miguel at Nuevo Esperanza, Oax. | 2 | 16°49.80′, 95°25.07′ | No | No | No | Yes | No | No | No | No | Yes |
| RIO JUNAPAN | | | | | | | | | | | |
| Arroyo 6 km N of Jose Maria Morelos, Ver. | 2 | 17°16.78′, 94°59.34′ | Yes | Yes | No | No | No | No | No | No | No |
| Rock pool in ravine 1.5 km SE of Jose Maria Morelos, Ver. | 2 | 17°13.89′, 94°58.77′ | Yes | Yes | No | No | No | No | No | No | No |
| Stream ~3 km N of Palomares, Oax. | 2 | 17°07′.72′, 95°03.60′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Arroyo Quince Millas at La Esperanza, Oax. | 1 | 17°17.62′, 95°06.47′ | Yes | No | No | No | No | No | Yes | Yes | Yes |
| Arroyo Quince Millas, Oax. | 1 | 17°17.39′, 95°06.02′ | Yes | No | No | No | No | Yes | Yes | No | No |
| Arroyo Doce Millas, ~8 km NW of Donaji, Oax. | 1 | 17°17.01′, 95°04.45′ | Yes | Yes | No | No | No | Yes | No | No | Yes |
| Arroyo Doce Millas, Oax. | 1 | 17°16.78′, 95°04.27′ | Yes | Yes | No | No | No | Yes | Yes | Yes | Yes |
| Arroyo, tributary to Rio Tortuguero, Oax. | 1 | 17°14.83′, 95°07.22′ | Yes | No | No | No | No | Yes | No | No | Yes |
| | 1 | 17°14.05′, 95°07.00′ | Yes | No | No | No | No | Yes | No | No | No |
| Arroyo, tributary to Rio Junapan, 6 km W of Palomares, Oax. | 2 | 17°08.90′, 95°07.02′ | Yes | No | No | No | No | Yes | No | No | No |
| Rio El Robalo W of Palomares, Oax. | 3 | 17°09.43′, 95°09.18′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Headwater of Rio El Robalo, Oax. | 3 | 17°07.74′, 95°11.70′ | Yes | Yes | No | No | No | Yes | Yes | Yes | Yes |
| Rio La Colmena NE of La Mixtequita, Oax. | 2 | 17°12.85′, 95°11.78′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Tributary to Rio La Colmena at La Soledad, Oax. | 1 | 17°13.12′, 95°10.95′ | Yes | No | No | No | No | Yes | No | No | Yes |
| Rio Junapan at Revolucion, Oax. | 1 | 17°09.93′, 95°09.88′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Stream 1 km N of La Mixtequita, Oax. | 1 | 17°12.49′, 95°13.35′ | Yes | No | No | No | No | Yes | Yes | Yes | No |
| Stream at La Mixtequita, Oax. | 1 | 17°10.65′, 95°11.09′ | Yes | No | No | No | No | No | No | No | No |
| Stream at San Pedro, Oax. | 1 | 17°10.90′, 95°15.74′ | Yes | No | No | No | No | Yes | Yes | Yes | No |
| Small brook at Nuevo Centro, Oax. | 1 | 17°11.69′, 95°16.01′ | Yes | No | No | No | No | No | No | No | No |
| Pool below waterfall, Rio Junapan, 2 km NE Monte Aguila, Oax. | 1 | 17°08.52′, 95°14.20′ | No | No | No | No | No | Yes | No | No | Yes |
| Rill, ~300 m NW of top of waterfall, 2 km NE of Monte Aguila, Oax. | 1 | 17°08.40′, 95°14.29′ | No | ? | ? | ? | No | No | No | No | No |
| Rio Junapan, 0.5 km N of Monte Aguila, Oax. | 3 | 17°08.23′, 95°14.97′ | Yes | ? | ? | ? | No | Yes | Yes | Yes | Yes |
| | 1 | 17°08.98′, 95°15.24′ | No | ? | ? | ? | No | Yes | No | No | Yes |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | *X. helleri* | *X. clemenciae* | *X. mixei* | *X. monticolus* | *X. maculatus* | *Priapella* sp. | *Poecilia* sp. | *Poeciliopsis* sp. | *Heterandria* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RIO SARABIA** | | | | | | | | | | | |
| Brook flowing into Rio Sarabia at Rancho San Carlos, Oax. | 2 | 17°13.13', 94°54.85' | Yes | Yes | No | No | No | Yes | Yes | Yes | Yes |
| Arroyos, tributaries to Rio Sarabia, 8–9 km W of Cuauhtemoc, Oax. | 1 | 17°04.68', 94°57.68' | Yes | Yes | No | No | No | Yes | Yes | Yes | Yes |
| | 1 | 17°05.35', 94°57.71' | Yes | Yes | No | No | No | No | No | No | No |
| Pool in dry arroyo leading to Rio Sarabia, 5 km SE of Palomares, Oax. | 2 | 17°06.24', 95°01.37' | No | Yes | No | No | No | No | No | No | Yes |
| Pool in arroyo leading to Rio Sarabia, 4.5 km SE of Palomares, Oax. | 2 | 17°06.19', 95°02.30' | Yes | No | No | No | No | No | No | No | No |
| Rio Sarabia S of Palomares, Oax. | 1 | 17°06.00', 95°02.50' | Yes | No | No | No | No | Yes | No | No | No |
| Rio Sarabia, 7 km SW of Palomares, Oax. | 1 | 17°04.51', 95°05.00' | Yes | No | No | No | No | No | No | No | No |
| Arroyo, tributary to Rio Sarabia at Santa Ana, Oax. | 1 | 17°04.35', 95°05.60' | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Rock pool in arroyo at Santa Ana, Oax. | 1 | 17°03.35', 95°05.31' | Yes | No | No | No | No | No | No | No | No |
| Tributary to Rio La Polvora, 1 km ESE of San Juanita, Oax. | 1 | 17°05.05', 95°10.17' | Yes | Yes | No | No | No | No | No | No | No |
| Rio La Polvora at San Juanito, Oax. | 1 | 17°05.27', 95°10.81' | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Tributary to Rio La Polvora, 2.5 km NW of San Juanito, Oax. | 3 | 17°06.36', 95°11.66' | Yes | Yes | No | No | No | Yes | Yes | Yes | Yes |
| Rio del Sol, 2.5 km SE of San Juanito, Oax. | 1 | 17°04.14', 95°10.17' | Yes | No | No | No | No | No | No | No | Yes |
| Rio San Juan Viejo, 1 km NW of San Juan Viejo, Oax. | 1 | 17°02.84', 95°10.60' | No | No | No | No | No | No | No | No | Yes |
| Rio San Juan Viejo at San Juan Viejo, Oax. | 2 | 17°02.46', 95°10.22' | No | No | No | No | No | No | No | No | Yes |
| Tributary to Rio del Sol at Guasamann, Oax. | 1 | 17°02.31', 95°11.69' | No | No | No | No | No | No | No | No | Yes |
| Rio del Sol, 7.5 km N of village of Rio del Sol, Oax. | 1 | 17°00.24', 95°13.71' | No | No | Yes | Yes | No | No | No | No | Yes |
| Tributary to Rio del Sol, 5 km N of village of Rio del Sol, Oax. | 1 | 16°59.19', 95°13.71' | No | No | Yes | Yes | No | No | No | No | Yes |
| Tributary to Rio del Sol, ~1 km E of Platanillo, Oax. | 2 | 16°56.97', 95°14.80' | No | No | Yes | Yes | No | No | No | No | Yes |
| Tributary to Rio del Sol at Platanillo, Oax. | 2 | 16°57.29', 95°15.35' | No | No | Yes | No | No | No | No | No | Yes |
| Tributary to Rio del Sol W of Platanillo, Oax. | 1 | 16°57.41', 95°15.59' | No | No | Yes | No | No | No | No | No | No |
| Rio Cuautepec at Hierba Santa, Oax. | 1 | 16°54.92', 95°14.06' | No | No | Yes | Yes | No | No | No | No | Yes |
| Rio Las Uvas at St. Cruz Chahuitepec, Oax. | 5 | 16°53.995', 95°13.917' | No | No | Yes | Yes | No | No | No | No | No |
| Tributary to Rio Las Uvas at Las Flores, Oax. | 1 | 16°52.621', 95°14.25' | No | No | Yes | No | No | No | No | No | Yes |
| Arroyo Palizado at El Tejon, Oax. | 1 | 16°52.13', 95°13.07' | No | No | Yes | No | No | No | No | No | Yes |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | X. helleri | X. clemenciae | X. mixei | X. monticolus | X. maculatus | Priapella sp. | Poecilia sp. | Poeciliopsis sp. | Heterandria sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **RIO MALATENGO** | | | | | | | | | | | |
| Headwater of Rio Mogone at Arroyo Guajolote, Oax. | 2 | 17°02.14′, 95°08.37′ | No | No | No | No | No | No | No | No | No |
| Rio Huapinol, 1.5 km S of San Juan Guichicovi, Oax. | 1 | 16°57.03′, 95°05.29′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| Tributary of Rio Huapinol, Oax. | 1 | 16°56.19′, 95°05.12′ | No | No | No | No | No | No | No | Yes | Yes |
| Tributary to Rio Los Nanches at Zacatal, Oax. | 1 | 16°56.18′, 95°11.27′ | Yes | No | No | No | No | No | No | No | No |
| Arroyo La Canada, 3 km NW of St. Domingo Petapa, Oax. | 1 | 16°50.43′, 95°09.31′ | Yes | No | No | No | No | No | No | No | No |
| Arroyo Guigulana at St. Domingo Petapa, Oax. | 1 | 16°49.73′, 95°08.49′ | No | No | No | No | No | No | No | No | No |
| Arroyo La Campanario at Santa Maria Petapa, Oax. | 2 | 16°49.30′, 95°07.34′ | Yes | No | No | No | No | No | No | No | No |
| Arroyo La Campanario, Oax. | 1 | 16°47.66′, 95°07.81′ | Yes | No | No | No | No | No | Yes | Yes | Yes |
| Arroyo Pedro Gil S of | 1 | 16°44.91′, 95°08.52′ | No | No | No | No | No | No | Yes | Yes | Yes |
| El Barrio, Oax. | 1 | 16°45.32′, 95°08.52′ | No | No | No | No | No | No | No | Yes | Yes |
| Tributary to Rio Grande near Guivicia, Oax. | 1 | 16°57.00′, 94°58.50′ | Yes | No | No | No | No | No | No | No | No |
| Tributary to Rio Grande, ~6 km S of Matias Romero, Oax. | 1 | 16°49.24′, 95°01.94′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Rio Grande S of Matias Romero, Oax. | 1 | 16°47.60′, 95°00.94′ | Yes | No | No | No | No | Yes | Yes | Yes | Yes |
| Headwater of Rio Grande at Almoloya, Oax. | 1 | 16°45.27′, 95°03.96′ | Yes | No | No | No | No | Yes | No | Yes | Yes |
| 1st headwater stream of Rio Chichihua, Oax. | 4 | 16°45.92′, 94°50.73′ | Yes | No | No | No | No | No | No | No | No |
| 2nd headwater stream of Rio Chichihua, Oax. | 1 | 16°46.83′, 94°49.67′ | Yes | No | No | No | No | No | No | No | No |
| Rio Chichihua, Oax. | 1 | 16°48.33′, 94°49.47′ | Yes | No | No | No | No | No | No | No | No |
| Tributary to Rio Chichihua, Oax. | 1 | 16°48.89′, 94°48.26′ | Yes | No | No | No | No | No | No | No | No |
| **RIO VERDE** | | | | | | | | | | | |
| Tributary to Rio Verde, ~15 km SW of Cuauhtemoc, Oax. | 1 | 17°02.76′, 94°45.09′ | No | Yes | No | No | No | Yes | Yes | No | Yes |
| Tributary to Rio Verde, ~16 km SW of Cuauhtemoc, Oax. | 1 | 17°02.11′, 94°44.34′ | No | No | No | No | No | Yes | Yes | No | No |
| **RIO ESCOLAPA** | | | | | | | | | | | |
| Arroyo San Antonio, tributary to Rio Escolapa, Oax. | 2 | 16°49.40′, 94°47.07′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Arroyo Pita, tributary to Rio Escolapa, Oax. | 2 | 16°49.78′, 94°45.77′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Rio Escolapa, S of St. Maria Chimalapa, Oax. | 1 | 16°50.86′, 94°45.23′ | No | Yes | No | No | No | No | No | No | No |

APPENDIX 1
*(Continued)*

| Location | Samplings | Latitude N, longitude W | *X. helleri* | *X. clemenciae* | *X. mixei* | *X. monticolus* | *X. maculatus* | *Priapella* sp. | *Poecilia* sp. | *Poeciliopsis* sp. | *Heterandria* sp. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RIO NEGRO | | | | | | | | | | | |
| Arroyo, tributary to Rio Negro S of St. Maria Chimalapa, Oax. | 2 | 16°53.43′, 94°42.11′ | No | Yes | No | No | No | Yes | No | No | Yes |
| Rio Milagros near Santa Maria Chimalapa, Oax. | 1 | 16°53.86′, 94°41.60′ | No | No | No | No | No | No | No | No | No |
| RIO TRINIDAD, PAPALOAPAN DRAINAGE | | | | | | | | | | | |
| Rio Zapotal, Oax. | 1 | 17°22.27′, 95°27.57′ | Yes | No | No | No | No | No | No | No | Yes |
| SMALL INDEPENDENT TRIBUTARIES TO RIO COATZACOALCOS | | | | | | | | | | | |
| Rio Mojarras NE of Las Flores, Ver. | 1 | 17°25.18′, 94°43.83′ | Yes | No | No | No | No | No | Yes | No | Yes |
| Arroyo near San Miguel, Ver. | 1 | 17°19.27′, 94°55.14′ | Yes | No | No | No | Yes | Yes | Yes | Yes | Yes |
| Tributary to Arroyo La Barbacoa, 2.5 km E of Cuauhtemoc, Oax. | 1 | 17°06.45′, 94°51.86′ | No | Yes | No | No | No | No | No | No | No |
| Pool in Arroyo La Barbacoa, 4 km E of Cuauhtemoc, Oax. | 1 | 17°06.92′, 94°51.14′ | No | Yes | No | No | No | No | No | No | No |