



## Two new species of the genus *Xenotoca* Hubbs and Turner, 1939 (Teleostei, Goodeidae) from central-western Mexico

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### Abstract

The subfamily Goodeinae (Goodeidae) is one of the most representative and well-studied group of fishes from central Mexico, with around 18 genera and 40 species. Recent phylogenetic studies have documented a high degree of genetic diversity and divergences among populations, suggesting that the diversity of the group may be underestimated. The species *Xenotoca eiseni* has had several taxonomic changes since its description. *Xenotoca eiseni* is considered a widespread species along the Central Pacific Coastal drainages of Mexico, inhabiting six independent drainages. Recent molecular phylogenetic studies suggest that *X. eiseni* is a species complex, represented by at least three independent evolutionary lineages. We carried out a meristic and morphometric study in order to evaluate the morphological differences among these genetically divergent populations and describe two new species. The new species of goodeines, *Xenotoca doadrioi* and *X. lyonsi*, are described from the Etzatlan endorheic drainage and upper Coahuayana basin respectively.

**Key words:** taxonomy, morphometric, meristic, conservation, Central Mexico

### Resumen

La subfamilia Goodeinae es uno de los grupos de peces de agua dulce más representativo y estudiado del centro de México, con aproximadamente 18 géneros y 40 especies. Estudios filogenéticos recientes han documentado una elevada diversidad y divergencias genéticas entre especies y poblaciones, sugiriendo una subestimación de la riqueza dentro del grupo. La especie *Xenotoca eiseni* ha presentado diversos cambios desde su descripción. *Xenotoca eiseni* es considerada una especie ampliamente distribuida a lo largo de las Cuencas Costeras del Pacífico Central de México, habitando seis cuencas independientes. Estudios moleculares recientes han sugerido que *X. eiseni* es un complejo de especies, representado por al menos tres linajes evolutivos independientes. Se realizaron análisis merísticos y morfométricos con el objetivo de evaluar las diferencias entre los linajes genéticamente divergentes, realizando la descripción de dos nuevas especies. Las nuevas especies de goodeines, *Xenotoca doadrioi* y *X. lyonsi*, son descritas de la cuenca endorreica de Etzatlán y la parte alta de la cuenca del río Coahuayana.

**Palabras clave:** taxonomía, morfometría, merística, conservación, Centro de México

### Introduction

One of the most representative groups of endemic fishes from central Mexico are the members of the subfamily Goodeinae, a group of small viviparous fishes (40 to 180 mm SL). Goodeinae is represented by 18 genera and 40 species, with most species being endemic to a specific basin or microendemic to a single water body in central Mexico (Domínguez-Domínguez *et al.* 2010). The subfamily has extraordinary adaptations for reproduction, such as internal fertilization, matrotrophy, and viviparity (Parenti 1981; Grudzien *et al.* 1992). These adaptations, linked with their Miocene origin (*ca.* 16 million years ago; Ma) and the complex geological and climatic history of

Central Mexico have largely been discussed as factors of their extraordinary radiation (Domínguez-Domínguez *et al.* 2010; Pérez-Rodríguez *et al.* 2015).

Although this subfamily has been largely studied and is considered a relatively well-known group (Girard 1859; Jordan & Evermann 1896–1900; Hubbs & Turner 1939; Domínguez-Domínguez *et al.* 2010), recent studies using molecular techniques have noted high genetic diversity and divergence among populations (Doadrio & Domínguez 2004; Domínguez-Domínguez *et al.* 2010; Piller *et al.* 2015), including the recognition of new species (Meyer *et al.* 2001; Rada & Meyer 2003; Doadrio & Domínguez 2004; Domínguez-Domínguez *et al.* 2008a). The genus *Xenotoca* was one of the most taxonomically troublesome groups within the Goodeinae. This genus was first identified by Hubbs & Turner (1939) based on characters of ovarian, trophotaenial, and external anatomy in order to differentiate the species *variatus*, formerly described as *Characodon variatus* Bean 1887. Recent molecular work (Doadrio & Domínguez *et al.* 2004; Webb *et al.* 2004; Domínguez-Domínguez *et al.* 2010) demonstrated that the genus is not monophyletic and that *Xenotoca variata* needs to be recognized as the sole species of *Xenotoca*, and the other two species recognized as *Xenotoca*, *X. eiseni* (Rutter, 1896) and *X. melanosoma* Fitzsimons, 1972, needed to be reassigned to a new genus.

*Xenotoca eiseni* was long considered a junior synonym of *Xenotoca variata* (Regan 1908; Hubbs 1926; Hubbs & Turner 1939). Mendoza (1965) was the first author to point out that the ovarian and trophotaenial structure of *X. variata* had important differences from those described for *X. eiseni* and questioned the validity of this synonymy, but Romero (1967) still used the synonymy made by Regan (1908). Finally, Fitzsimons (1972) re-established *Xenotoca eiseni* as valid species based on live male coloration and external anatomy. Recent molecular studies also confirmed the validity of *X. eiseni*, moreover these works found genetically divergent lineages within *X. eiseni*, suggesting the possibility that *X. eiseni* is comprised of multiple species (Doadrio & Domínguez 2004; Domínguez-Domínguez *et al.* 2010; Piller *et al.* 2015).

*Xenotoca eiseni* is a small, sexually dimorphic, colorful species (maximum SL 75 mm). Males possess orange-to-red coloration in the peduncle, anal and caudal fins and the lateral flanks possess iridescence scales. Females tend to be drab in coloration and lack the reddish coloration seen in males. There are also some coloration differences among populations.

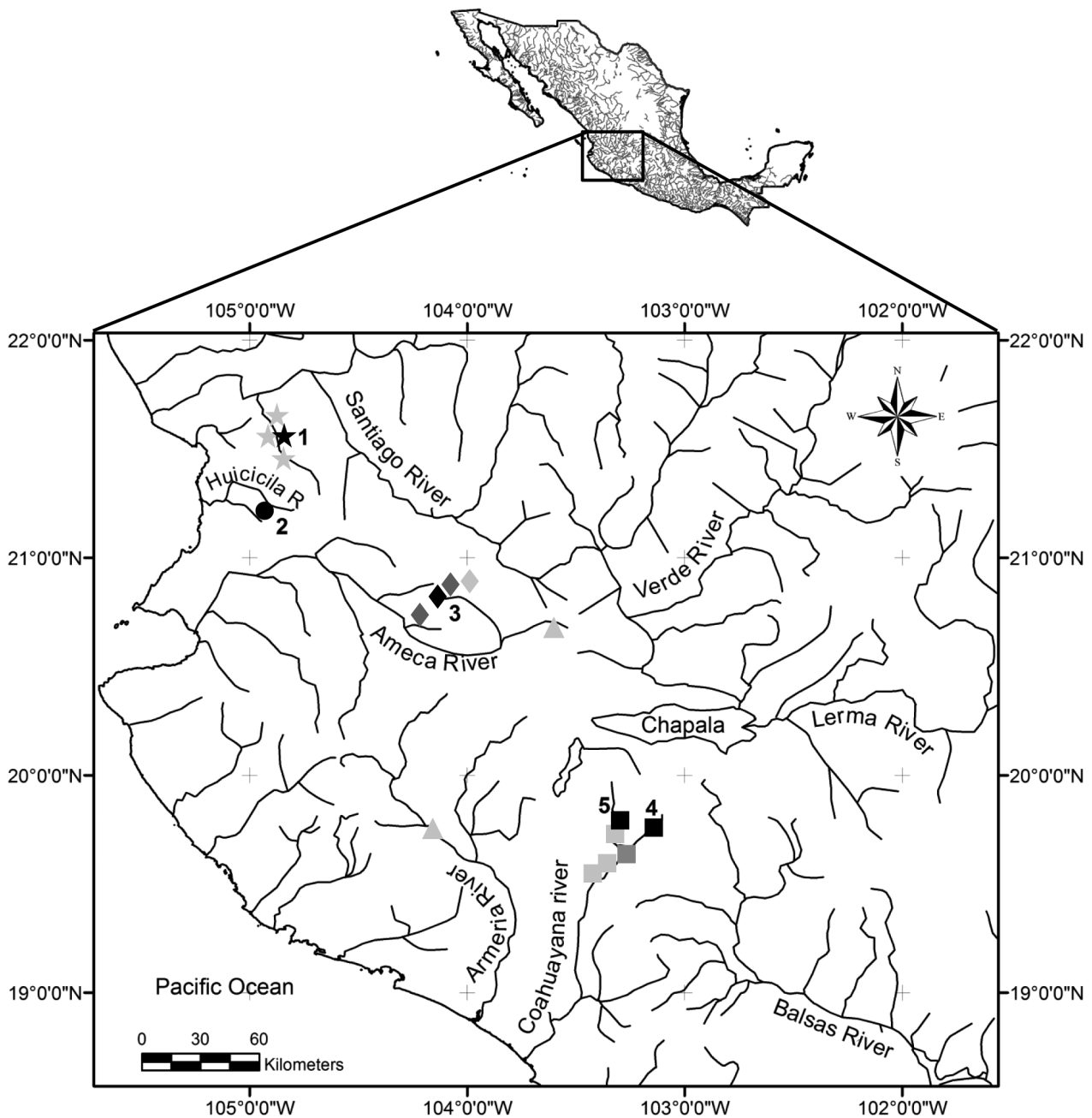
*Xenotoca eiseni* is distributed in central Mexico in the upper part of the Coahuylana, Armeria, Huicicila, and Ameca river drainages, in the Magdalena and Etzatlán endorheic basins, as well as in the springs and small streams of the northwest Santiago drainage, in the vicinity of Tepic city (Fig. 1). Domínguez-Domínguez *et al.* (2005) listed this species as endangered and a priority species for conservation. In the Mexican Official Norm of Ecology (SEMARNAT 2010), this species is listed as special protection, described as a species that could be threatened in the future if the negative impacts in their populations continue. It is not listed in the most recent IUCN red list (IUCN 2015). Recent studies recovered a 57% reduction in historical localities (De la Vega-Salazar 2006), whereas others report a 70% reduction in the historical distribution records (Domínguez-Domínguez *et al.* 2008b). Populations are seriously endangered, as in the case of those from the Santiago drainage, while in others, as in the cases of Ameca and Armeria drainages, entire populations have been extirpated presumably caused by pollution, habitat degradation, and introduction of not native species (Domínguez-Domínguez *et al.* 2008b; Kenway-Lynch *et al.* 2010, Pedraza-Marrón 2011).

The widespread, disjunct distribution of *X. eiseni*, coupled with the recognition of several evolutionary independent genetic lineages (Piller *et al.* 2015), suggests that the taxonomic diversity within the species may be greater than is currently recognized. Therefore, we used morphometric and meristic data to analyze morphological variation across the range of *X. eiseni*. Herein, we describe two new species of goodeids (*Xenotoca*). Due to the conservation status of *X. eiseni* (De la Vega-Salazar 2006, Domínguez-Domínguez *et al.* 2008b, Kenway-Lynch *et al.* 2010, Pedraza-Marrón 2011), we also discuss the conservation implications of these taxonomic changes.

## Material and methods

Specimens were collected from four populations along the distributional range of *X. eiseni* (Table 1 and Fig. 1) and deposited at the Colección de Peces de la Universidad Michoacana (CPUM, MICH.PEC-227-07-09), Colección Nacional de Peces, IB-UNAM and Colección de Peces del Museo Nacional de Ciencias Naturales, Madrid. Data were taken from the left side of 30 specimens (15 males and 15 females) for all populations using a

stereomicroscope. Previous work has shown that there is a strong degree of sexual dimorphism and important differences between males and females in goodeines, therefore all the analyses were carried out by sex (Hubbs & Turner 1939; Miller & Fitzsimmons 1971; Fitzsimmons 1972; Rauchenberger 1988; Ritchie *et al.* 2007; Domínguez-Domínguez *et al.* 2008a).



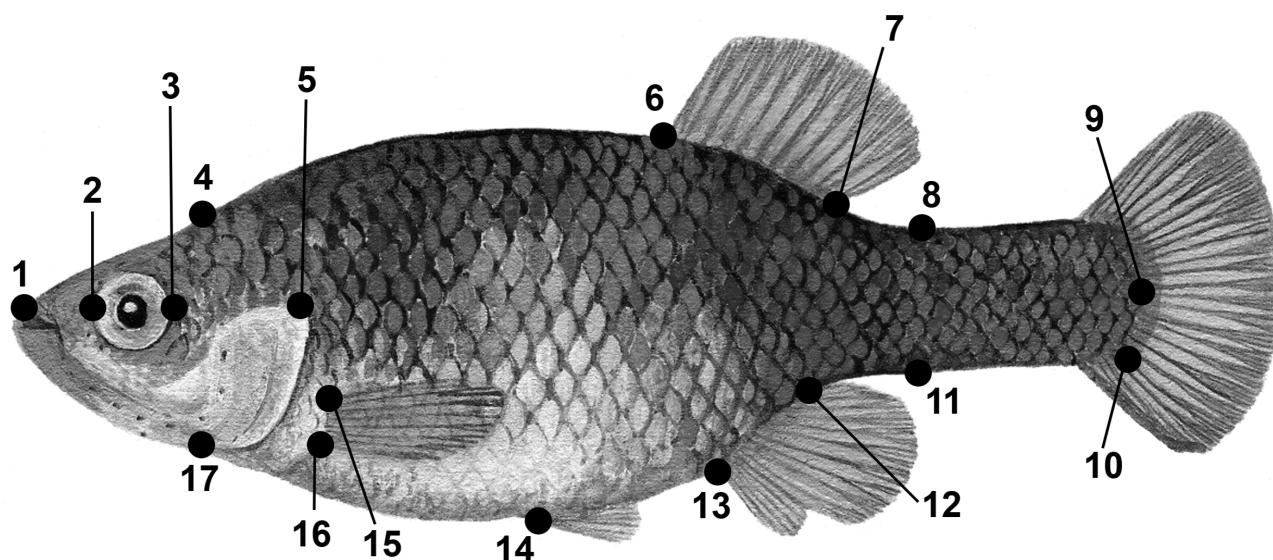
**FIGURE 1.** Distributional ranges of species in the *Xenotoca eiseni* group. Symbols correspond to specimens used in this study (black), localities of other known populations (dark gray), and historical localities that have not yielded specimens in the last 10 years or more (light gray). Stars correspond to *Xenotoca eiseni* from the Santiago River, circles for *Xenotoca eiseni* from the Compostela location, diamonds represent *X. doadrioi*, squares represents *X. lyonsi*, and triangles is for *X. eiseni* from Ameca and Armeria drainages never taxonomical or systematically analyzed. Numbers correspond to locations given in Table 1.

Meristic counts were taken following Fitzsimmons (1972) and included dorsal (D), anal (A) and pectoral (P) rays, lateral series scales (LSS), transverse scales (TS) (starting in the anterior insertion of the dorsal fin and ending at the posterior insertion of the anal fin) and scales along the peduncle (PS). The lacrimal pores (LPo), preopercular pores (POP), mandibular pores (MP) and supraorbital pores (SoP) were taken following Smith & Miller (1987). Principal Component Analyses (PCA), MANOVA, and Canonical Variance Analyses (CVA) were conducted for

meristic matrices separated by sex, in the program PAST v. 3.09 (Hammer *et al.* 2001). For the CVA, specimens were grouped by river basin in order to not bias the results by the species level hypothesis obtained by Piller *et al.* (2015). Frequency tables were also obtained for the meristic characters.

**TABLE 1.** Locality data and other specimen information.

Locality	Drainage	Coordinates	Catalog number (CPUM)
1. Manantial at 6 de Enero, near Tepic, Nayarit	Santiago River	21°31'34" N 104°48'18" W	5540
2. Asalto stream at Compostela, Nayarit	Huicicila River	21°13'56" N 104°53'59" W	9621
3. Pond at San Sebastian, North to Etzatlan, Jalisco	Etzatlan	20°49'25" N 104°7'11" W	5543, 9589
4. Tamazula river, North West to Tamazula town, Jalisco	Coahuayana River	19°43'25" N 103°12'05" W	5542, 9590
5. At Santa Cruz del Cortijo in Vista Hermosa Village	Coahuayana River	19°41'42.3"N 103°21'8.2"W	5541



**FIGURE 2.** Landmarks used for obtain the linear measurements, 1 to 9 standard length (SL); 1 to 5 head length (HL); 4 to 17 head high (HH); 1 to 2 preorbital length (PrOL); 3 to 5 postorbital length (POL); 2 to 3 eye diameter (ED); 8 to 11 body least depth (BLD); 13 to 14 pelvic-anal fin distance (PAD); 14 to 6 pelvic-dorsal fin distance (PDD); 14 to 15 pelvic-pectoral fin distance (PPD); 6 to 13 dorsal-anal fin distance (DAD); 6 to 12 dorsal fin origin to anal fin posterior extent distance (DOAE); 7 to 13 dorsal fin posterior extent to anal fin origin distance (DEAO); 7 to 9 end of dorsal fin-hypural plate distance (EDHP); 9 to 12 end of the anal fin-hypural plate distance (EAHP); 6 to 7 dorsal fin base length (DFL); 12 to 13 anal fin base length (AFL); 15 to 16 pectoral fin base length (PFL); 10 to 12 caudal peduncle length (CPL).

Linear measurements were obtained from high resolution photographs taken for each specimen and all were taken with the same procedure and the same scale in order to minimize the error in the digitization process. Seventeen landmarks were used, from which linear measurements were obtained using the program MORPHEUS (Slice 2013) (Fig. 2). The linear measurements chosen were used in other taxonomic studies of fishes (Lyons *et al.* 2004; Domínguez-Domínguez *et al.* 2008a) but also included other measurements that were considered of interest in a preliminary comparison of specimens. The abbreviations used for morphometric variables are: SL, standard length; HL, head length; HH, head height; PrOL, preorbital length; POL, postorbital length; ED, eye diameter; BLD, body least depth; PAD, pelvic-anal fin distance; PDD, pelvic-dorsal fin distance; PPD, pelvic-pectoral fin distance; DAD, dorsal-anal fin distance; DOAE, dorsal fin origin to anal fin posterior extent distance; DEAO,

dorsal fin posterior extent to anal fin origin distance; EDHP, end of dorsal fin-hypural plate distance; EAHP, end of the anal fin-hypural plate distance; DFL, dorsal fin base length; AFL, anal fin base length; PFL, pectoral fin base length; CPL, caudal peduncle length. All measurements are presented in millimeters. For statistical analyses the body measurements were divided by SL, whereas head measurements were divided by HL. PCA, MANOVA and CVA analyses were carried out in the program PAST v. 3.09 (Hammer *et al.* 2001). For the CVA, the specimens were grouped by river basin in order to not bias the results by species level hypothesis obtained by Piller *et al.* (2015).

## Results

**Meristics.** For females, the PCA analyses shows three separated groups, corresponding to specimens from Compostela-Seis de Enero, San Sebastian, and the Tamazula River, with little overlap between specimens from Tamazula and Compostela (Fig. 3). PCI and II account for 64.5% of cumulative variance. For the CVA analysis, three well-separated groups were found, also corresponding to specimens from Compostela-Seis de Enero, San Sebastian, and the Tamazula River. The overall meristic variation among the four populations for females explains 79.6% of the variation in CV1 and 14% for CV2 (cumulative 93.6%). The plots show the existence of three separate groups for the three species and four studied populations (Fig. 4). The first CV axis separated Compostela-Seis de Enero specimens from those of Tamazula and San Sebastian. The second CV axis separated specimens from Tamazula and San Sebastian. Wilk's lambda values are significant ( $P < 0.001$ ), and Hotelling's paired comparisons show significant differences among Compostela-Seis de Enero, San Sebastian, and the Tamazula River, but not in the comparisons within Seis de Enero and Compostela specimens after Bonferroni correction (Fig. 4). The meristic characters that most account to the group formation were SoP, P and PS in the CV1 and LSS for the CV2 (Fig. 4). This result is in accordance with the counts, since the P (mode=\*13) and PS (\*9) are the same in Compostela and Seis de Enero, but differ from those of Tamazula and San Sebastian (\*12) and (\*8), respectively. Whereas LSS differ for the Tamazula (\*31) and SoP differ for San Sebastian specimens (\*10) (Table 2 to 4).

**TABLE 2.** Frequencies of fin rays in the studied populations. Comp = Compostela, Sn Seb = San Sebastian. Modal counts are in bold text.

	Dorsal Ray				Pectoral Ray				Anal Rays		
	12	13	14	15	11	12	13	14	13	14	15
Females											
Comp	0	<b>8</b>	6	1	2	4	<b>9</b>	0	0	<b>10</b>	5
6 Enero	0	6	<b>8</b>	0	0	1	<b>10</b>	3	1	<b>11</b>	2
Sn Seb	1	2	<b>11</b>	0	2	<b>10</b>	2	0	1	<b>13</b>	0
Tamazula	1	<b>8</b>	4	0	3	<b>7</b>	3	0	4	<b>9</b>	0
Males											
Comp	1	<b>8</b>	6	0	0	5	7	2	1	<b>13</b>	0
6 Enero	0	1	<b>13</b>	1	0	3	<b>10</b>	0	0	<b>15</b>	0
Sn Seb	0	6	<b>8</b>	0	1	<b>12</b>	1	0	0	<b>13</b>	1
Tamazula	0	<b>10</b>	5	0	0	<b>11</b>	4	0	4	<b>11</b>	0

For males, the first two PC axes explain 60.8% of cumulative variance, and showing three separate groups, corresponding to specimens from Compostela-Seis de Enero, San Sebastian, and the Tamazula River, with a small amount of overlap between Tamazula and Compostela specimens (Fig. 3). The CVA explains 59.3% of the variance for CV1 and 33.5% for the CV2 (cumulative 92.8%). This analysis also show the segregation of three groups, Compostela-Seis de Enero, San Sebastian area, and Tamazula River, with no overlap between the groups, but showing overlap between Compostela and Seis de Enero populations (Fig. 4). The first canonical function separated Compostela-Seis de Enero with respect to San Sebastian and Tamazula specimens. The second canonical function segregated San Sebastian and Tamazula specimens. Wilk's lambda values are significant ( $P < 0.001$ ). Hotelling's paired comparisons shown significant differences between the four populations analyzed for the three

species after Bonferroni correction (Fig. 4). The meristic characters that most account to the group formation are PS, SoP and P for the CV1 and LSS and TS in the CV2. This result are in accordance with the counts, since the P (mode=\*13) and PS (\*9) are the same in Compostela-Seis de Enero specimens, but differ from those of San Sebastian and Tamazula (\*12) and (\*8) respectively, TS (\*11) are similar in Compostela and San Sebastian specimens, whereas SoP (\*10) differ for San Sebastian (Table 2 to 4).

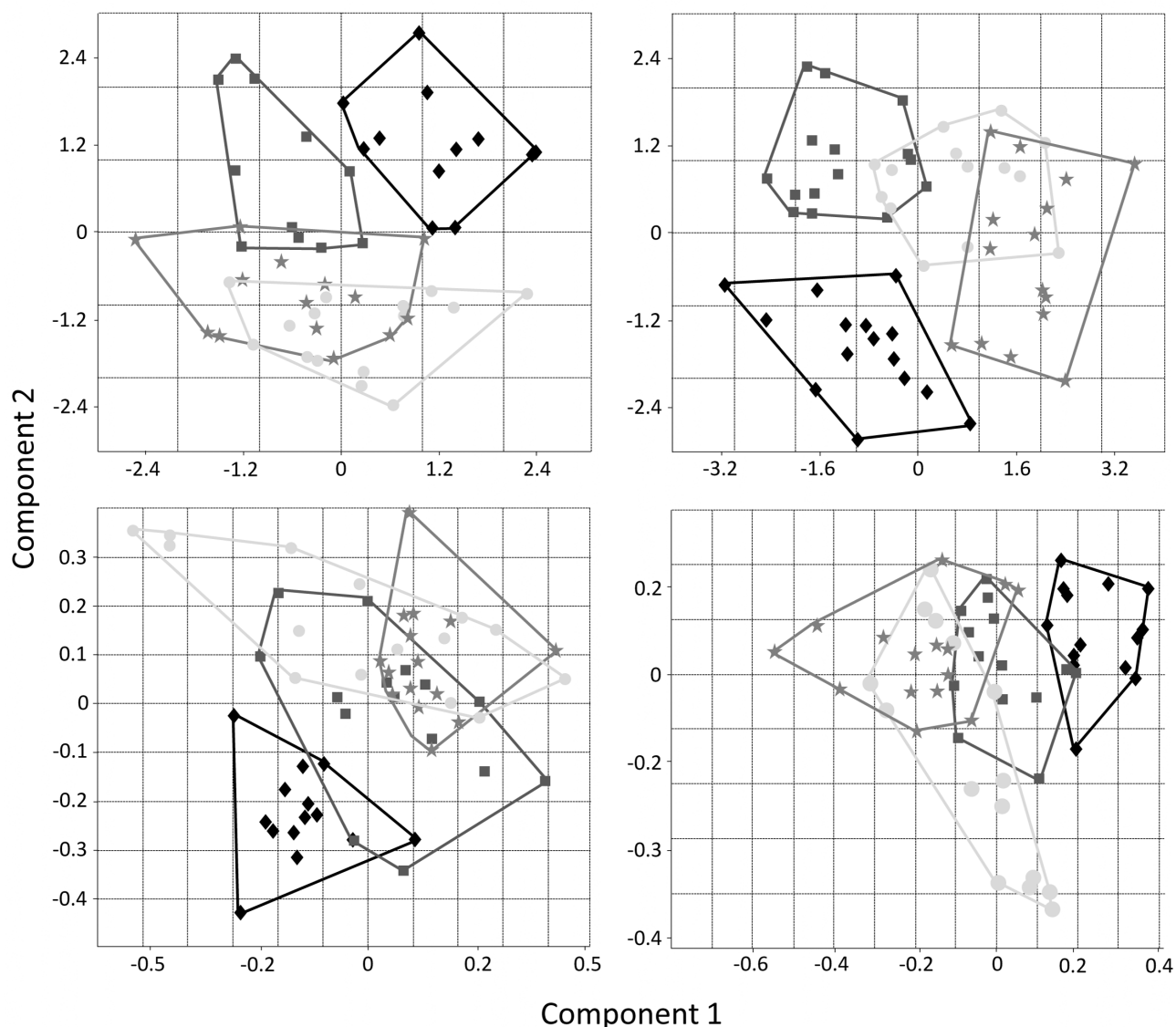
**TABLE 3.** Frequencies of the scales accounts in the studied populations. Comp = Compostela, Sn Seb = San Sebastian. Modal counts are in bold text.

	Lateral line				Caudal peduncle				Transversal		
Females	30	31	32	33	7	8	9	10	9	10	11
Comp	7	5	3	0	0	2	12	1	1	<b>12</b>	2
6 Enero	1	5	<b>6</b>	2	0	0	13	1	0	<b>12</b>	2
Sn Seb	0	3	<b>9</b>	2	0	14	0	0	3	<b>11</b>	0
Tamazula	6	<b>7</b>	0	0	1	11	1	0	1	<b>12</b>	0
Males	30	31	32	33	7	8	9	10	10	11	12
Comp	4	<b>7</b>	3	0	0	0	<b>10</b>	4	3	<b>9</b>	2
6 Enero	0	3	<b>8</b>	4	0	0	<b>12</b>	3	0	6	<b>9</b>
Sn Seb	4	3	<b>6</b>	1	0	<b>14</b>	0	0	0	<b>10</b>	4
Tamazula	5	<b>8</b>	0	0	1	<b>12</b>	0	0	<b>13</b>	0	0

**TABLE 4.** Frequencies of head pores in the studied populations. Comp = Compostela, Sn. Seb = San Sebastian. Modal counts are in bold text.

	Opercular				Supraorbital			Mandibular			Preorbital		
Females	6	7	8	9	8	9	10	2	3	4	3	4	5
Comp	0	0	<b>15</b>	0	3	<b>12</b>	0	4	<b>8</b>	3	0	<b>15</b>	0
6 Enero	1	3	<b>6</b>	4	3	<b>9</b>	2	1	<b>11</b>	2	0	<b>14</b>	0
Sn Seb	0	0	<b>9</b>	5	0	2	<b>12</b>	7	7	0	0	<b>14</b>	0
Tamazula	0	3	<b>9</b>	1	0	<b>12</b>	1	2	<b>11</b>	0	0	<b>13</b>	0
Males	6	7	8	9	8	9	10	2	3	4	3	4	5
Comp	0	1	<b>12</b>	1	3	<b>11</b>	0	3	<b>8</b>	3	0	<b>14</b>	0
6 Enero	0	1	<b>8</b>	6	4	<b>11</b>	0	2	<b>12</b>	1	4	<b>10</b>	1
Sn Seb	0	0	<b>11</b>	3	0	4	<b>11</b>	4	<b>6</b>	4	0	<b>13</b>	1
Tamazula	0	0	<b>14</b>	1	4	<b>11</b>	0	3	<b>12</b>	0	1	<b>14</b>	0

**Morphometrics.** For linear measurements (PCA), the first two PC axes for females explain 55.2% of the variance. The plot shows a segregation of San Sebastian specimens from the others, however there is a small degree of overlap with respect to Tamazula, but a high degree of overlap among the Tamazula, Compostela and Seis de Enero populations (Fig. 3). For the CVA, 59.4% of the variance was accounted by CV1 and 29.3% for CV2 (cumulative 88.7%) and the Wilk's lambda value was significant ( $P < 0.001$ ). Hotelling's paired comparisons showed significant differences among the three species and four populations analyzed, except for the comparison between Tamazula and Seis de Enero. The plots show a clear segregation of the specimens from Compostela and San Sebastian, whereas specimens from Tamazula and Seis de Enero shown high degree of overlap (Fig. 4). The proportional measurements that account for the variation were SL/EDHP, HL/ED and SL/DFL in CV1. The specimens from San Sebastian possess a large distance from the end of dorsal fin to the hypural plate (EDHP), specimens from Tamazula showed a small eye diameter (ED), whereas San Sebastian specimens showed a high ED, and finally, San Sebastian and Tamazula specimens possessed a high dorsal fin base length (DFL). For CV2 the most important measurements were SL/BLD and HL/HH, where the Compostela specimens shown high body least depth distance and head high distance.



**FIGURE 3.** Principal Component Analysis for meristic characters. Upper left for females and right for males, and morphometrics; bottom left for females and bottom right for males. Diamond = San Sebastian, squares = Tamazula, dot = Compostela, stars = Seis de Enero.

The PCA analyses for linear measurements for males explain 57.3 of the variance (PCI and PCII), and the plot shows a segregation of specimens from San Sebastian with some overlap with respect to Tamazula. There is a high degree of overlap among the Tamazula, Compostela and Seis de Enero populations (Fig. 3). The CVA analyses recovered 71.6% of the variance along CV1 and 17.5% for CV2 (cumulative 89.1%). Wilk's lambda value is significant ( $P < 0.001$ ). Hotelling's paired comparisons shown significant differences between the four populations analyzed for the three species. The plots show a clear segregation of the specimens from San Sebastian whereas Seis de Enero, Compostela and Tamazula specimens show somewhat overlay (Fig. 4). The proportional measurements that account for the variation were HL/HH, SL/PPD and SL/EAHP in CV1. The specimens from San Sebastian showed shallower head height, pelvic-pectoral fin distance, and end of the anal fin-hypural plate distance. For CV2, the most important measurements were SL/BLD, SL/PAD, and SL/DOAE. The specimens from Seis de Enero showed less body least deep whereas Compostela specimens showed high body least deep, Tamazula and Seis de Enero specimens shown high dorsal fin origin to anal fin posterior extent distance, and for pelvic anal fin distances Seis de Enero specimens was the population that show the lowest values. The statistical data expressed as the ratio of the standard length or head length for all the linear measurements are shown in Table 5 and 6. As a result of the clear morphological differences, we describe two new species within the *X. eiseni* group.

**TABLE 5.** Morphometric data for *Xenotoca* females. Abbreviation of the measurements are given in method section. SL and HL in millimeters. N = sample size. Expressed as range (mean/Standard deviation).

	<i>X. eiseni</i> Compostela (N=15)	<i>X. eiseni</i> 6 Enero (N=15)	<i>X. doadrioi</i> sp. n. San Sebastian Paratypes (N=15)	<i>X. lyonsi</i> Tamazula Paratypes (N=15)
SL	30.7–55.8(42.4/7.1)	34.9–49.7(43.2/4.7)	33.2–47.0(40.8/4.2)	36.5–50(42/3.2)
HL	8.5–12.3(10.4/1.0)	9.9–14.3(12.0/1.4)	8.6–12.7(10.5/1.4)	9.4–14.5(11.2/1.2)
Ratio of the Standard Length (SL)				
DFL	6.3–7.1(6.8/0.27)	5.9–8.0(7.0/0.53)	5.8–7(6.5/0.38)	5.9–7.4(6.5/0.39)
AFL	9.2–11.6(10.2/0.73)	8.3–11.7(10.0/0.99)	8.3–11.2(9.7/0.88)	9.1–11.4(10.1/0.75)
PFL	14.1–17.2(15.7/1.1)	13.6–17.3(15.4/1.40)	14.8–17.1(17.1/1.14)	12.1–16.9(15.0/1.29)
CPL	4.5–5.2(4.9/0.25)	4.6–5.8(5.1/0.40)	4.2–5(4.7/0.23)	4.3–5.8(5.0/0.41)
BLD	5.9–6.4(6.1/0.16)	6.1–7.1(6.6/0.38)	6.2–7.2(6.4/0.29)	6–7.1(6.4/0.28)
PDD	2.2–3.1(2.7/0.30)	2.6–3.0(2.7/0.12)	2.3–2.9(2.6/0.13)	2.3–2.9(2.7/0.17)
PAD	4.2–6.6(5.3/0.69)	4.6–6.0(5.4/0.43)	4.5–6(5/0.39)	4.8–5.6(5.2/0.31)
DOAE	3.5–4.0(3.8/0.17)	3.7–4.0(3.8/0.10)	3.5–4(3.7/0.17)	3.5–4.4(3.8/0.22)
DEAO	3.3–3.8(3.5/0.17)	3.4–3.7(3.5/0.11)	3.1–3.7(3.3/0.18)	3.2–3.9(3.4/0.17)
PPD	3.1–4.5(3.9/0.44)	3.5–4.6(4.1/0.30)	3.8–4.8(4.2/0.24)	3.5–5.4(4.3/0.58)
EAHP	3.8–4.4(4.1/0.15)	4.0–4.6(4.2/0.19)	3.6–4.1(3.8/0.15)	3.7–4.4(4.1/0.19)
EDHP	3.9–4.7(4.2/0.23)	3.9–4.6(4.0/0.18)	3.3–3.9(3.6/0.13)	3.5–4.3(3.9/0.19)
Ratio of the Head Length (HL)				
ED	3.1–3.8(3.5/0.22)	3.0–4.0(3.5/0.24)	2.6–3.3(3.0/0.19)	3.4–4.0(3.7/0.2)
HH	1.0–1.3(1.1/0.09)	1.2–1.4(1.3/0.06)	1.2–1.4(1.3/0.06)	1.1–1.4(1.2/0.08)
PrOL	4.7–6.5(5.5/0.68)	4.4–5.6(4.8/0.31)	4.8–6.3(5.4/0.44)	4.1–6.2(5.0/0.57)
PoOL	1.6–2.6(2.1/0.27)	1.8–2.6(2.2/0.19)	2.0–2.5(2.2/0.15)	1.9–2.4(2.1/0.14)

***Xenotoca doadrioi*, Domínguez-Domínguez, Bernal-Zuñiga, and Piller, sp. n.**

(Figs. 5a, and Tables 2 to 6)

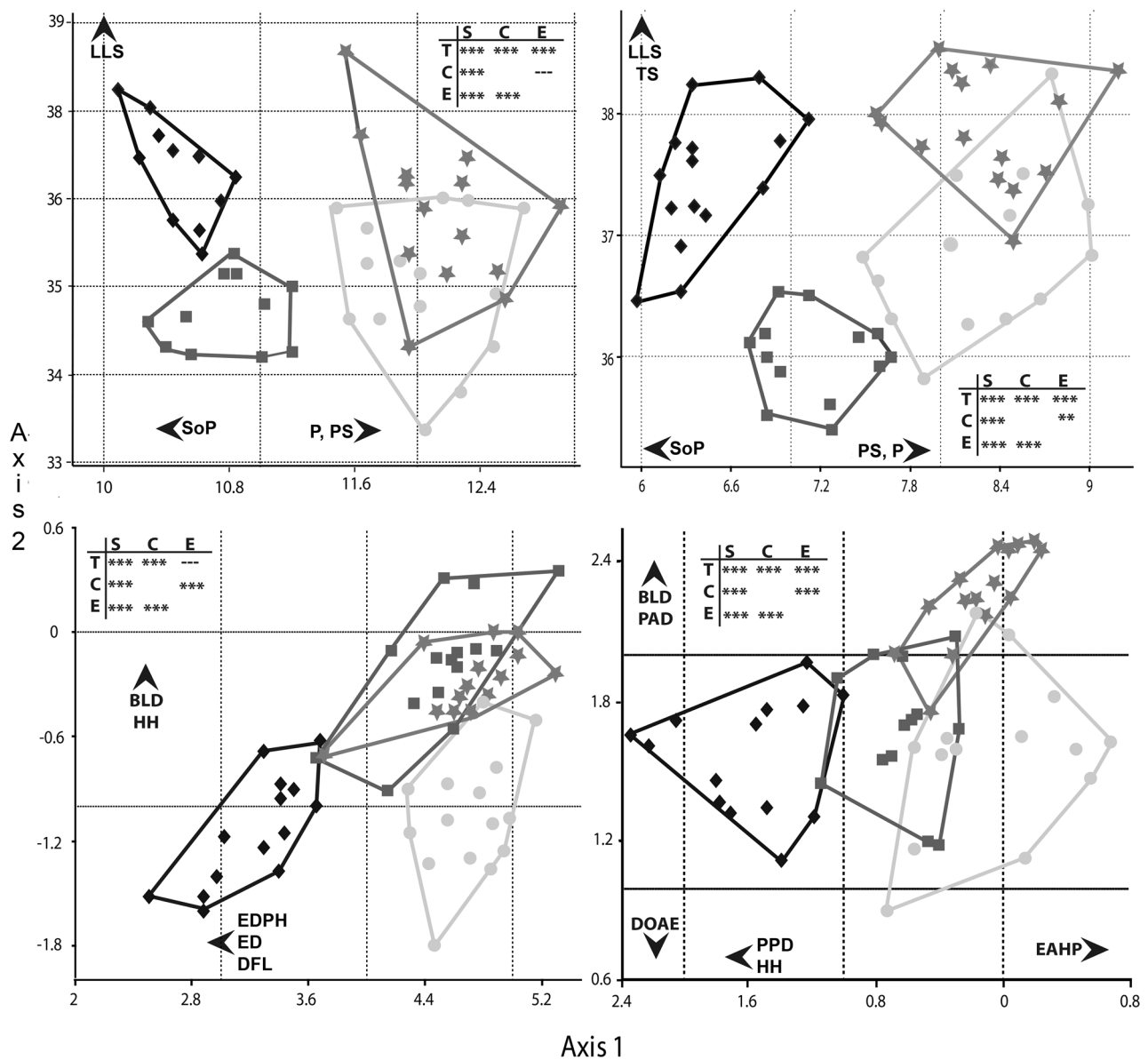
[Http://zoobank.org/urn:lsid:zoobank.org:act:EFE95E0B-674F-4E7E-ADFA-971A5CCCC0C20](http://zoobank.org/urn:lsid:zoobank.org:act:EFE95E0B-674F-4E7E-ADFA-971A5CCCC0C20)

**Type material: Holotype.** CPUM-9589, CPUM-T-41530, adult male 41 mm SL, Pond at San Sebastian village, North to Etzatlan, Jalisco, Mexico, Etzatlan endorheic drainage; 20°49'25"N and 104°7'10.8"W, collected 17 June 2010. **Paratypes.** CPUM-5543, 22 specimens, CPUM-T 11929–11933, same data as holotype. MNCN ICTIO 290.844 - 290.847, 4 specimens, same data as holotype. CNPE-IBUNAM20839, 4 specimens, same data as holotype.

**Diagnosis.** *Xenotoca doadrioi* sp. n. is distinguished from the other species of the *Xenotoca eiseni* group and other *Xenotoca* species inhabiting the Pacific Coast drainages by the combination of the following characters (none unique to the species): the females have 14 dorsal rays versus 15 or 16 in *X. melanosoma* and 13 in *X. lyonsi* sp. n., 14 anal fin rays versus 15 or 16 in *X. melanosoma*, 12 pectoral fin rays versus 13 in *Xenotoca eiseni*, 8 caudal peduncle scales versus 9 in *Xenotoca eiseni* and *X. melanosoma*, 32 scales in a lateral series versus 31 in *Xenotoca lyonsi* n. sp. and 10 suparorbital pores versus 9 in *Xenotoca eiseni* and *X. lyonsi* (Tables 2 to 4). Females of *X. doadrioi* show large caudal peduncle as is show by the  $\bar{x}$  SL/EAHP = 3.8 versus  $\bar{x}$  = 4.1–4.2 in *X. eiseni* and *X. lyonsi* and  $\bar{x}$  SL/EDHP = 3.6 versus 3.9–4.2 in *X. eiseni* and *X. lyonsi*, large eye as is show by  $\bar{x}$  HL/ED = 3 versus 3.5–3.7 in *X. eiseni* and *X. lyonsi* (Table 5). Males have 14 dorsal rays versus 15 or 16 in *X. melanosoma* and 13 in *X. lyonsi*, 14 anal fin rays versus 15 or 16 in *X. melanosoma*, 12 pectoral rays versus 13 in *Xenotoca eiseni*, 8 caudal peduncle scales versus 9 in *Xenotoca eiseni* and *X. melanosoma*, 11 transversal scales versus 9 in *X. lyonsi*, 32 scales in a lateral series versus 31 in *Xenotoca lyonsi* and 10 suprorbital pores versus 9 in *Xenotoca eiseni* and *X. lyonsi* (Tables 2 to 4). Poses a smaller head  $\bar{x}$  HL/HH = 1.4 versus 1.1–1.2 in *Xenotoca eiseni* and *X. lyonsi*, the



body is less high  $\bar{x}$  SL/PPD = 4.7 versus 4–4.4 in *Xenotoca eiseni* and *X. lyonsi* and large caudal peduncle  $\bar{x}$  SL/EAHP = 3.8 versus 4–4.2 in *Xenotoca eiseni* and *X. lyonsi* and  $\bar{x}$  SL/EDHP 3.8 versus 4.1–4.3 in *Xenotoca eiseni* and *X. lyonsi* (Table 6).



**FIGURE 4.** Canonical Variance Analysis for meristic characters; Upper left for females and right for males, and morphometrics; bottom left for females and bottom right for males. Abbreviations of measurements are given in method section. Diamond = San Sebastian, squares = Tamazula, dot = Compostela, stars = Seis de Enero. In the table are the Hotelling's paired comparisons. S = San Sebastian, T = Tamazula, C = Compostela and E = Seis de Enero. —NS, \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ .

**Description.** Frequency tables for each meristic character are shown in Tables 2 to 4. *Xenotoca doadrioi* has 12–14 dorsal rays, 13–15 anal rays, and 11–13 pectoral rays. Lateral scale series with 30–33, eight scales along the caudal peduncle, 9–11 transversal scales between dorsal and anal fin. The sensory pores of the lateral line system on the head are 8–9 opercular pores, 10–9 supraorbital pores, 2–4 mandibular pores and 4–5 preorbital pores (Tables 2 to 4). The females are large than males; maximum known size for females is 47 mm, compared to 37 mm for males. Morphometrics measurements are show in tables 5 and 6. Body measurements are given in times the standard length,  $\bar{x}$  = females/males. Body relatively deep, laterally compressed and elongated, anal fin inserted before the origin of the dorsal fin at same axis, PDD  $\bar{x}$  = 2.6/2.4, PAD  $\bar{x}$  = 5/5.2, DOAE  $\bar{x}$  = 3.7/3.4, and DEAO  $\bar{x}$  = 3.3/3.1, minimum body deep  $\bar{x}$  = 6.4/5.8 being the females slightly deeper than males. Relative large caudal

peduncle with respect to other species in the genus EAHP  $\bar{x} = 3.8/3.8$  and EDHP  $\bar{x} = 3.6/3.8$ . Dorsal profile markedly convex with a marked hump at the nape in large specimens. Dorsal fin length long  $\bar{x} = 6.5/5.6$ , being longer in males than in females. Head measurements are given in times the head length. The head is pointed, snout short, smaller than eye diameter, postorbital length HH  $\bar{x} = 1.3/1.4$ , PrOL  $\bar{x} = 5.4/6.1$ , PoOL = 2.2/2.5, eye relatively high, ED  $\bar{x} = 3/3.6$  being relatively bigger in females than in males. Mouth superior with the upper jaw slightly short than inferior.



**FIGURE 5.** a) *Xenotoca doadrioi*, Holotype male CPUM-9589 and female from San Sebastian b) *Xenotoca lyonsi*, Holotype male CPUM-9590 and female from Tamazula c) *Xenotoca eiseni*, male and female from Compostela population picture by Wolfgang Gessl [www.pisces.at](http://www.pisces.at)

**Pigmentation pattern.** When alive, the coloration varies with respect to the age and sex of the organism. Mature females display a general brownish coloration. Most mature females display dark blotches along the central part of the body, being bigger and conspicuous at the posterior half of the body; these blotches are formed by small black spots. Some scales show iridescent silver colorations in the body, being more evident in the postorbital and opercular region. Some females possess a dark stripe that runs along the middle part of the body, from the opercle to hypural plate. Scales are frequently rounded at their exterior margin by small black spots; a black blotch is present in the posteroventral region, between the pelvic and anal fins, which varies in depth and width (Fig. 5a). Juveniles have the same coloration as females, but as they reach  $\pm 20$  mm, they begin to differentiate to adult coloration. Males show the most colorful form of all *Xenotoca* species; this varies depending of the size and reproductive stage. In general, the caudal peduncle has an orange to almost red coloration combined with iridescent

blue scales, the intensity and coverage of each color along the caudal peduncle is highly variable, some specimens show a blue or green to dark blue or green scales in the anterior part of the peduncle, the blue or green coloration extends to the origin of the dorsal fin, and also the intensity and coverage is highly variable, the caudal fin and frequently the anal and dorsal fin also have orange to red coloration in the base and sometimes the dorsal fin shown a dark coloration in the base. The portion of the body from the origin of dorsal and anal fin to pelvic or pectoral fin is pale in coloration, with gray to yellowish coloration, in the pre-ventral region. Orange to red coloration exists frequently and extends to the inferior part of the head. Just up to the pectoral fin there is a black blotch with iridescent scales that also is highly variable in intensity and size. There is also blue iridescent coloration in the opercle and in some scales along the body (Fig. 5a). The coloration of preserved specimens varies with respect to fixation and time since fixation, but in general, female specimens preserved in 5% formalin possess clear brownish coloration. The blotches are less evident along the body, in larger females they are still present. Numerous dark small spots are found in all the upper half of the body. A silver stripe is present along the middle part of the body, being more evident in the posterior half. The dark blotch in the posteroventral region is still evident. The opercle shows a silver coloration. Males lose all coloration when preserved. The peduncle and pre-ventral region show a clearer brownish coloration. The rest of the body shown a more brownish dark coloration with numerous black spots distributed along the upper half of the body. Fins clear and unpigmented, a few specimens still show a dark blotch up to the pectoral fin and the scales are rounded by a numerous black spots.

**Sexual dimorphism.** As is the case with other members in the subfamily Goodeinae, sexual dimorphism is substantial, with males showing a reduced length on the first five to seven anal-fin rays (Hubbs & Turner 1939). Females are large than males. The base of the anal, dorsal and pectoral fins are larger in males than in females, (females/males) as show by the SL/DFL  $\bar{x} = 6.5/5.5$ , SL/AFL  $\bar{x} = 9.7/8.6$  and SL/PFL  $\bar{x} = 17.1/15.2$ . Males are deeper than females SL/BLD  $\bar{x} = 5.8/6.4$ , SL/PDD  $\bar{x} = 2.4/2.6$  and SL/DOAE  $\bar{x} = 3.4/3.7$ . The males have smaller eyes (females/males) HL/ED  $\bar{x} = 3/3.6$  (Tables 5 and 6). The most evident dimorphism is in coloration, with males much more colorful than females (Fig. 5a).

**Distribution.** The species is endemic to the endorheic region of Etzatlan, in the state of Jalisco, Mexico (Fig. 1). The type locality is a small and permanent pond just in the east end of the Hacienda San Sebastian, with around 6,000 m<sup>2</sup> fed by a spring (20°49'25" N, 104°7'10.8" W). Other known locations in the area are El Moloya spring, Estancia de Ayoles reservoir, Oconahua Dam around 3km west of Oconahua village, and the highly perturbed and seasonally affected streams along the federal road number 4, between Etzatlan and San Marcos Village, known as arroyo San Marcos and arroyo de la Granja Sahuaripa, but the last two locations have not yielded specimens since 2006, and in an extensive survey in 2015, these localities were found to be totally dry or full of *Pseudoxiphophorus bimaculatus* (Heckel 1848) when water was present.

**Etymology.** The name of the species, an adjective, is derived from the name of the prestigious ichthyologist Dr. Ignacio Doadrio, Museo Nacional de Ciencias Naturales, Spain, who has strongly contributed to the study and knowledge of Mesoamerican fish diversity.

**Habitat and ecology.** This species seems to be highly adaptable to variable habitat conditions. At the type locality, the species inhabits an area with turbid water, and was collected in a shallow water no more than 1.5 m deep. The pond is no more than 3 meters at its deepest part; the bottom is comprised of mud and gravel, and no water plants are present. Other fish species collected in the area were *Xenotoca melanosoma*, *Goodea atripinnis* Jordan 1880, *Poeciliopsis infans* (Woolman 1894) and the introduced *Xiphophorus variatus* (Meek 1904) and *Oreochromis* sp. Historically, other species reported from this pond include *Algansea amecae* Pérez-Rodríguez, *et al.* 2009, *Moxostoma austrinum* Bean 1880, and *Allotoca maculata* Smith & Miller 1980, but all of these species have not been collected in the area since 1970. In the El Moloya Spring, the species inhabits clear water with gravel to muddy bottom and water plants and this pond is used as a swimming pool. Other species inhabiting this pond are *X. melanosoma*, *Zoogoneticus purepechus* Domínguez-Domínguez *et al.* 2008a, *Ameca splendens* Miller and Fitzsimons 1971, *G. atripinnis*, *P. infans*, and the introduced *Oreochromis* sp. In Oconahua Dam, the water is turbid and contains a muddy bottom and with few water plants. Other species collected include *X. melanosoma*, *G. atripinnis*, *P. infans*, as well as the introduced *Lepomis macrochirus* Rafinesque 1818, and *Cyprinus carpio* Linnaeus 1758. The San Marcos stream is a seasonally fluctuating stream that is dry for most of the year, but when water is present the surface of the stream is totally cover with *Eichhornia crassipes* Martius, *Thypha* sp., and *Cyperus* sp. The water at this site is highly polluted by organic matter and is turbid, whereas the Sahuaripa stream is an irrigation channel totally modified and fed by a water pump; in 1999 and 2002, the species collected in both

places included *X. melanosoma*, *Allotoca* sp., *G. atripinnis*, *P. infans*, and *Oreochromis* sp. For the 2006 survey, *Allotoca* sp. was not collected; in the 2015 survey only *Pseudoxiphophorus bimaculatus* was found. Nothing is known about its biology in nature.

**Conservation.** *Xenotoca doadrioi* is known only from small springs and a dam in areas highly impacted by human activities that have been strongly modified for irrigation. It has been extirpated from more than 50% of the known historical localities (Pedraza-Marrón 2011). This species is found in small numbers in the three localities where it presently occurs, and these localities are under the influence of substantial irrigation pressure for agriculture. Introduced fish species pose a significant risk for the long-term survival of this species. It is recommended that *X. doadrioi* be considered a species in danger of extinction.

***Xenotoca lyonsi*, Domínguez-Domínguez, Bernal-Zuñiga, and Piller, sp. n.**

(Figs. 5b, Tables 2 to 6)

Http://zoobank.org/urn:lsid:zoobank.org:act:DC760909-BA97-40BD-A50E-0A6D92ADE46D

**Type material: Holotype.** CPUM-9590, CPUM-T-41522, adult male 55 mm SL, Tamazula River, Coahuayana drainage, just 5 km Northwest of the town of Tamazula town, Jalisco, 21°13'56.5"N and 104°53'58.7"W collected 12 July. **Paratypes.** CPUM-5541, 3 specimens; CPUM-T-12038–12040, Tamazula River, Coahuayana drainage, at Santa Cruz del Cortijo in Vista Hermosa Village, 19°41'42.3"N and 103°21'8.2"W, collected 20 June 2010. CPUM-5542, 19 specimens, CPUM-T-5217, 5221–5223, 5226, 5228, 5230–5232, 5234, 5236–5241, 5244–5245, 5249, same data as holotype. MNCN ICTIO 290.848 - 290.851, 4 individuals; CPUM-T- 12036–12038, same data as holotype. CNPE-IBUNAM20840, 4 specimens, same data as holotype.

**Diagnosis.** *Xenotoca lyonsi* sp. n. is distinguished from the other species of the group inhabiting the Pacific Coast drainages by the combination of the following characters (none unique to the species): females have 13 dorsal rays versus 15 or 16 in *X. melanosoma* and 14 in *X. doadrioi*. For males and females the differences are as follows; 14 anal fin rays versus 15 or 16 in *X. melanosoma*, 12 pectoral fin rays versus 13 in *Xenotoca eiseni*, 8 caudal peduncle scales versus 9 in *Xenotoca eiseni* and *X. melanosoma*, 30–31 scales in a lateral series versus 32 in *X. doadrioi*, 9 transversal scales versus 11 or 12 in *X. eiseni* and *X. doadrioi* and 11 suparorbital pores versus 10 in *X. doadrioi* (Table 2 to 4). Both sexes show a smaller eye diameter HL/ED  $\bar{x}$  = 3.7 versus 3.0 in *X. doadrioi* and 3.5 in *X. eiseni* for females, and HL/ED  $\bar{x}$  = 3.6 versus 3.3 in *X. eiseni* in males, high dorsal fin base SL/DFL  $\bar{x}$  = 6.5 versus 6.9 in *X. eiseni* for females and SL/DFL  $\bar{x}$  = 5.5 versus 5.9 in *X. eiseni* in males (Tables 5 and 6).

**Description.** Frequency tables for each meristic character are shown in Tables 2 to 4. 12–14 dorsal rays, 13–14 anal rays and 11–13 pectoral rays. Lateral scale series with 30–31, 7–9 scales along the caudal peduncle, 9–10 transversal scales between dorsal and anal fin, in females, and 9 in males. The sensory pores of the lateral line system on the head are 7–9 opercular pores, 8–10 supraorbital pores, 2–3 mandibular pores and 3–4 preorbital pores (Tables 2 to 4). The female are larger than males, maximum known size for females is 60 mm SL and 55 mm SL for males. Measurements are shown in tables 5 and 6. Body measurements are given in times the standard length,  $\bar{x}$  = females/males. Body relatively deep, laterally compressed and elongated. Anal fin inserted before the origin of dorsal fin at same axis, PDD  $\bar{x}$  = 2.7/2.4, PAD  $\bar{x}$  = 5.2/5.3, DOAE  $\bar{x}$  = 3.8/3.3, and DEAO  $\bar{x}$  = 3.4/3.1. Minimum body deep  $\bar{x}$  = 6.4/5.7 females are slightly deeper than males. Relative shorter caudal peduncle with respect to *X. doadrioi* EAHP  $\bar{x}$  = 4.1/4 and EDHP  $\bar{x}$  = 3.9/4.1. Dorsal profile markedly convex with a marked hump at nape in large specimens. Dorsal and anal fin are relatively longer in males than in females, DFL  $\bar{x}$  = 6.5/5.5 AFL  $\bar{x}$  = 10.1/8.8 (Tables 5 and 6). Head measurements are in times the head length. Head is pointed, snout short, smaller than eye diameter. Postorbital length HH  $\bar{x}$  = 1.2/1.2, PrOL  $\bar{x}$  = 5/4.8, PoOL  $\bar{x}$  = 2.1/2.5. Eye relatively high, ED  $\bar{x}$  = 3.7/3.6 (Table 5 and 6).

**Pigmentation pattern.** When alive, the coloration varies with respect to the age and sex of the organism. Mature females show a general brownish coloration with a dark pigmented strip along the body, from the opercle to the hypural plate region that varies in intensity and width. Dark blotches are not evident in big females, but being more evident in the posterior half of the body when present. Scales are frequently rounded in their exterior margin by small black spots. A black blotch is present in the posteroventral region, between the pelvic and anal fins, which varied in depth and width (Fig. 5b). Juveniles have the same coloration than females, but as they reach ±20 mm they start to differentiate to adult coloration. Juveniles show a brownish translucent coloration with small dark

blotches along the body. The fins are clear and unpigmented. Males show a coloration that varies depending on the size and reproductive stage, but in general the posterior half of the caudal peduncle has an orange coloration that extends to the caudal fin, and sometimes some iridescent blue scales are present. The anterior half of the caudal peduncle sometimes possesses blue iridescent scales that frequently extend anterior to the anal fin in the ventral region and to the middle of the dorsal fin, the intensity and coverage of each color is highly variable. Anal, pectoral and dorsal fins can show some pigmentation, being orange to dark coloration. The anteroventral portion of the body is normally with a brown to white coloration, the dorsal region is brownish in coloration, with blue iridescent scales in some males. Sometimes the dark blotch just up to the pectoral fin is present, and also is highly variable in intensity and size, and is less evident than in *X. doadrioi*. Blue iridescent coloration is often present on the opercle (Fig. 5b). The coloration of preserved specimens varies with respect to fixative and time since fixation, but in general, specimens preserved in 5% formalin show, for females, clear brownish coloration. The blotches are sometimes present along the body, but in bigger females are more evident in the dorsal half of the body, in young females are frequently present along the body. The ventral region is clear. Some females possess silver to dark stripe along the middle part of the body, being more evident in the posterior half. The opercle shows a silver coloration. Fins are unpigmented. Males lose all coloration, the ventral half of the body, including the head and pre-ventral region, and the posterior half of the peduncle show a clear brownish to beige coloration, the rest of the body shows a brownish dark coloration with the darkest coloration around the external part of the scales. Normally fins clear and unpigmented.

**Sexual dimorphism.** As with other members in the Goodeinae subfamily, sexual dimorphism is marked, with males showing a reduced length on the first five to seven anal-fin rays (Hubbs & Turner 1939). Females are larger than males, maximum known size for females 50 mm and 41 mm for males. The base of the dorsal and anal fins are relatively larger in males than in females (all measurements are given in time the standard length, females/males) as show the DFL  $\bar{x} = 6.5/5.5$  and AFL  $\bar{x} = 10.2/8.8$ . Males are deeper than females BLD  $\bar{x} = 6.4/5.7$ , PDD  $\bar{x} = 2.7/2.4$ , DEAO  $\bar{x} = 3.4/3.1$ , and DOAE  $\bar{x} = 3.8/3.1$  (Tables 5 and 6). But the most evident dimorphism is in coloration, with males much more colourful than females (Fig. 5b).

**Distribution.** The species is endemic of the Coahuayana River drainage, being reported only in the middle and upper part of the drainage, in the Tuxpan and Tamazula rivers at altitudes more than 1000 meters above the sea level, all localities within the state of Jalisco, Mexico. The type locality is in a highly seasonally changed Tamazula River, approximately 5 km northwest of the town of Tamazula town (19°43'24.9" N 103°12'05" W). The new species also has been reported in other locations along the Tuxpan and Tamazula rivers (Fig. 1) near Ferreria, Soyatlan de Afuera, San Rafael, Tuxpan and Atenquique villages, but most of these localities no longer harbour *X. lyonsi* and it is presumed to be locally extirpated.

**Etymology.** The name of the species, an adjective, is derived from the name of the prominent North American ichthyologist, Dr. John Lyons, who has made substantial contributions to our understanding of the distribution, ecology, diversity, and conservation status of fishes in Mexico, and to goodeids in particular.

**Habitat and ecology.** The type locality for *X. lyonsi* is an area with high seasonal changes in water clarity and volume, from a turbid and deep high flow running water in the rainy season to clear and low flow water other times of the year, sometimes reduced to a few shallow pools in the dry season. The bottom primarily is composed of mud and gravel, and water plants are only evident in the stream bed, which seems to change in composition and coverage depending on the season. The area is totally surrounded by sugar cane plantations. Other fish species collected in the area were *Xenotoca melanosoma* (now extirpated), *Ilyodon whitei* (Meek 1904), *Poecilia butleri* Jordan 1889, *Allodontichthys tamazulae* Turner 1946, and *Astyanax anaeus* (Günther 1860), as well as the introduced *Cyprinus carpio* and *Oreochromis* sp. Nothing is known about its biology in nature.

**Conservation.** *Xenotoca lyonsi* sp. n. is known only from a few sites along its original distributional range, and is reported to been extirpated from approximately 60% of the historical localities where it has been reported (Pedraza-Marron 2011). All the areas where the species originally occurred are highly impacted by human activities, being totally modified for agricultural purposes, with sugar cane plantations demanding high water resources and discharging polluted water from the production process. Also, un-treated urban waste water is a substantial ecological problem in the area. In several recent surveys for the species, it was never located downstream of the village waste water discharges, and, when found, it was always upstream of the discharge sites. Also, the species is not abundant in the few localities from where it is currently known to exist. This species should be considered as endangered of extinction.

**TABLE 6.** Morphometric data for *Xenotoca* males. Abbreviation of the measurements are given in method section. SL and HL in millimeters. Expressed as range (mean/standard deviation). N = sample size. H = holotype.

	<i>X. eiseni</i>	<i>X. eiseni</i>	<i>X. doadrioi</i> sp. n.	<i>X. lyonsi</i> sp. n.
	Compostela (N=15)	6 Enero (N=15)	San Sebastian Paratypes (N=14)	Tamazula H Paratypes (N=14)
SL	31.1–43.8(39.1/3.1)	34.8–44.8(39.7/2.5)	32.3	35.8
HL	8.5–12.3(10.4/1.03)	10.2–13.3(11.8/1.0)	9	9.9
Ratio of the Standard Length (SL)				
DFL	5.4–6.5(5.9/0.42)	5.1–6.9(5.9/0.55)	5.4	5.7
AFL	8.1–9.6(9.0–0.43)	7.8–8.8(8.3/0.32)	8.1	9.4
PFL	10.7–15.5(13.3–1.45)	12.1–16.3(13.5/1.31)	16.1	14.3
CPL	4.5–5.7(4.8/0.32)	4.6–5.3(4.9/0.23)	4.3	4.5
BLD	5.3–5.9(5.6/0.21)	5.4–6.5(5.9/0.35)	6.3	6.2
PDD	2.2–2.6(2.4/0.14)	2.3–2.6(2.4/0.07)	2.5	2.3
PAD	4.7–6.3(5.2/0.54)	5.0–6.3(5.7/0.35)	6.1	4.8
DOAE	3.2–3.6(3.4/0.13)	3.1–3.4(3.3/0.09)	3.4	3.4
DEAO	3.0–3.5(3.2/0.14)	3.0–3.4(3.2/0.10)	3.3	3.4
PPD	3.5–5.0(4.0/0.45)	3.9–4.7(4.3/0.27)	5.1	4.3
EAHP	3.9–4.3(4.2/0.12)	4.0–4.5(4.2/0.16)	3.5	4.0
EDHP	3.8–4.7(4.3/0.24)	3.9–4.2(4.2/0.17)	3.6	4.1
Ratio of the Head Length (HL)				
ED	2.7–3.7(3.3/0.29)	2.8–3.6(3.3/0.25)	3.1	3.3
HH	1.0–1.2(1.1/0.06)	1.0–1.3(1.2/0.07)	1.3	1.1
PrOL	4.5–6.5(5.3/0.75)	4.1–6.2(5.0/0.75)	5.0	4.7
PoOL	1.8–2.3(2.1/0.15)	1.8–2.6(2.1/0.18)	2.0	2.0

## Discussion

Although the Goodeinae subfamily is considered one of the most studied and well-known groups of fishes in Mexico (Girard 1859; Jordan & Evermann 1896–1900; Hubbs & Turner 1939; Domínguez-Domínguez *et al.* 2010), the recent description of new species within the group, such as *Allotoca zacapuensis* (Meyer *et al.* 2001) and *Zoogoneticus purepechus* (Domínguez-Domínguez *et al.* 2008a), as well as the recognition of genetically divergent populations within certain species, suggests that the diversity of this group is underestimated (Doadrio & Domínguez 2004; Domínguez-Domínguez 2008; Domínguez-Domínguez *et al.* 2010; Piller *et al.* 2015). This indicated the necessity of the implementation of more systematic and taxonomic study in Mexican fish species.

The description of two new species from a previously considered widespread *Xenotoca eiseni* is an example of this underestimated diversity. Previous genetic analyses were corroborated with morphological data (Domínguez-Domínguez *et al.* 2010; Piller *et al.* 2015).

*Xenotoca eiseni* was previously considered a widespread species distributed in six independent drainages: the upper part of the Coahuayana, Armeria, Huicicila and Ameca river drainages, the lower part of the Santiago River basin, and the Magdalena and Etzatlan endoreic basins. With the description of *X. doadrioi* and *X. lyonsi*, *X. eiseni* is restricted to the upper Huicicila and lower Santiago basins, *X. doadrioi* **sp. n.** is endemic to a small area in the endorheic Etzatlan and Magdalena basin, and *X. lyonsi* **sp. n.** is endemic to the upper part of the Coahuayana basin. Unfortunately no specimens from the Ameca and Armeria river drainages were included in previous genetic and the present morphological analyses because of the absence of samples, in spite of the extensive sampling effort in these basins over the past several years. These populations are likely extirpated or exist in very small numbers (Pedraza-Marrón 2011). As a result, the taxonomic status of this population is still uncertain, and is recommended that it continue to be recognized as *X. eiseni*. The four species of *Xenotoca* inhabiting the Pacific drainages of Central Mexico, *X. eiseni*, *X. doadrioi*, *X. lyonsi*, and *X. melanosoma*, are clearly distinguished from one another. The coloration pattern and meristic counts clearly distinguish *X. melanosoma* from the other species, and *X. melanosoma* is the only species of *Xenotoca* that can be found in sympatry with *X. doadrioi* and *X. lyonsi*. The morphological differences between the new species and also with respect to *X. eiseni* are evident. *Xenotoca doadrioi* is the most morphological divergent species, whereas *X. eiseni* and *X. lyonsi* are close relatives, and this pattern have been also found in genetic analyses, showing the populations from Etzatlan-Magdalena area (*X. doadrioi*) as the most divergent, whereas populations of *X. eiseni* and *X. lyonsi* found as closest relatives even with some contradictory results depending of the marker used (Doadrio & Domínguez 2004, Domínguez-Domínguez *et al.* 2010; Piller *et al.* 2015).

Biogeographically, the three species share total or partial patterns that are similar to those observed with species of the genus *Algansea*, *Yuriria*, *Allodontichthys* and *Allotoca* (Domínguez-Domínguez *et al.* 2007; 2010; Perez-Rodríguez *et al.* 2009), and these patterns are the result of the intense tectonic and volcanic activity of this geologically active area of Mexico.

The newly recognized diversity within the *X. eiseni* group has deep conservation implications. Prior to the description of *X. doadrioi* and *X. lyonsi*, the widespread *X. eiseni* was considered as endangered by Domínguez-Domínguez *et al.* (2005) and a priority species for conservation in the Mexican Official Norm of Ecology (SEMARNAT 2010). However, after this study, *X. eiseni* remains endemic to the lower part of Santiago river and upper Huicicila river, two areas highly impacted by agricultural activities and the expanding of urban areas that cause the disappearing of more than 40% of the historical know records for the species in the two basins, even the spring El Sacristan, the type locality of this species, is now covered by an apartment complex in the city of Tepic (Domínguez-Domínguez *et al.* 2008b; Pedraza-Marrón 2011). *Xenotoca doadrioi* is endemic to a small portion of the Etzatlan and Magdalena endorheic basins, an area almost totally cover by culture fields and greenhouses, where water resources are scare and in high demand for agriculture. In our 2015 survey, three of the historical records where the species was captured in the last 10 years were totally dry, and in two others only the introduced *Pseudoxiphophorus bimaculatus* was found. *Xenotoca lyonsi* is a species with the bigger known area of distribution, but its distribution has also been reduced to 55% of its historical records (Pedraza-Marrón 2011) and this area is highly used for agricultural purposes as well. As a result, we highly recommend that these three species of *Xenotoca* need to be considered as critically endangered, and we encourage the incorporation of immediate conservation action.

**Comparative material examined.** Only for meristic comparisons; *Xenotoca eiseni*; CPUM 5540, Manantial

at 6 de Enero, near Tepic City, State of Nayarit, Santiago river basin, 21°31'34"N–104°48'18"W; CPUM 9621, Asalto Stream at Compostela, Nayarit, Huicicicla drainage, State of Nayarit, 21°13'34"N–104°53'59"W. *Xenotoca melanosoma*; CPUM 4187, Lago de Zapotlán endorheic basin, 19°42'14.1"N–104°8'37.9"W; CPUM 4345, Buena Vista, Lago de Atotonilco endorheic basin, 20°20'05.8N–103°45'19.7"W; CPUM 4201, San Sebastian, Etzatlán endorheic basin, 20°49'25"N and 104°7'10.8"W; CPUM 4300, Ahualulco de Mercado, Río Ameca basin, 20°43'17.5"N–103°57'53.1"W; CPUM 4302, Puente la Muerta, Río Ameca basin, 20°31'43"N–104°7'47.8"W; CPUM 4044, San Marcos endorheic basin, 20°20'32.9"N–103°34'47.6"W; CPUM 4351, La Purisima, Río Tamazula basin, 19°31'19.9"N–103°20'32.9"W; CPUM 5225, Sacachales, Río Ayuquila, 19°42'16.6"N–104°8'36.5"W.

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