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# Two new species of the genus *Notropis* Rafinesque, 1817 (Actinopterygii, Cyprinidae) from the Lerma River Basin in Central Mexico

## Dos nuevas especies del género *Notropis* Rafinesque, 1817 (Actinopterygii, Cyprinidae), de la cuenca del río Lerma, México central

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### RESUMEN

Diversos estudios sugieren la existencia de especies no descritas de ciprínidos en el centro de México. Las especies del género *Notropis* se distribuyen a lo largo del centro de México y regiones adyacentes, algunas especies llegan a cuencas del sur del país. Se han reconocido dos grupos: un clado sureño y uno en la parte central del país. En este último, *Notropis calientis* fue descrito como un complejo de especies que habita las partes altas de las cuencas donde se distribuyen. Con base en características morfométricas, merísticas y genéticas, se describen dos nuevas especies que emanan del complejo *Notropis calientis*. *Notropis marhabatiensis* sp. nov. se diagnostica por presentar: 7, rara vez 8 radios ramificados en la aleta pélvica (vs. 8 rara vez 7 o 9 en las otras especies dentro del complejo *N. calientis*) y 9 rara vez 8 escamas transversales (vs. 10 rara vez 11 o 9 en *N. grandis* y *N. calientis*). Una línea lateral oscura y delgada corre a partir del origen de la aleta pectoral hasta el pedúnculo caudal. Veintiséis posiciones nucleotídicas fijadas en el gen citocromo *b*. *Notropis grandis* sp. nov. presenta 6, rara vez 7 radios ramificados en la aleta anal (vs. 7, rara vez 8 o 6 en *N. marhabatiensis*, *N. calientis* y *N. aulidion*) y 8, rara vez 7 o 9 en *N. calabazas* y *N. amecae*; 42, rara vez 40-41 y 43-45 escamas en una línea lateral (vs. 40 rara vez 37-39 en *N. calabazas*; 35-36, rara vez 37-39 y 33-34 en *N. amecae*; 35, rara vez 31-34 y 36 en *N. marhabatiensis* y *N. calientis* y 34 rara vez 30-33 y 35 in *N. aulidion*) y 11, rara vez 10 o 12 branquiespinas en el primer arco branquial. La línea lateral se extiende a partir del origen de la aleta pectoral hasta el origen de la aleta dorsal, con un segmento ligeramente convexo. Los adultos de esta especie son de mayor tamaño (longitud estándar=  $\bar{X}$  = 42.6, SD= 4.69) (vs. *N. calientis* (n=55,  $\bar{X}$  = 33.3, SD=3.28) y *N. marhabatiensis* sp. nov. (n=30,  $\bar{X}$  =30.5, SD=7.57) (F = 16.87;  $p < 0.001$ )). Presentan treinta y una posiciones nucleotídicas fijadas en la secuencia del gen mitocondrial citocromo *b* y cuatro cambios aminoacídicos. Las distancias genéticas entre *N. marhabatiensis* y otras especies del complejo variaron entre  $\bar{D}_{GTR}$  = 6.1% a 7.4%. Para *N. grandis* fueron de  $\bar{D}_{GTR}$  = 6.3% a 8.1%.

**Palabras clave:** Nueva especie, *Notropis*, México central, Cyprinidae, especie en peligro crítico.

### ABSTRACT

Prior findings suggest the existence of undescribed species among the cyprinids of central Mexico. Within the genus *Notropis* distributed across central Mexico and adjacent areas sometimes reaching southern basins, two groups have been recognized: a Southern Mexican clade and a central Mexican clade. Within this last clade, *Notropis calientis* has been defined as a species complex of four small minnows inhabiting upland areas. Here we describe two new species of this complex based on morphometric, meristic and genetic characters. *Notropis marhabatiensis* sp. nov. was diagnosed according to the following set of characters: 7, rarely 8, branched pelvic fin rays (vs. 8, rarely 7 or 9, in other species of the *N. calientis* complex) and 9, rarely 8, scales in a transverse series (vs. 10, rarely 9 or 11 in *N. grandis* & *N.*

*calientis*); a dark, narrow lateral stripe running from around the pectoral fin to the caudal peduncle origin; and twenty-six fixed nucleotide positions in the cytochrome *b* gene. The diagnosis of *Notropis grandis* sp. nov. was based on: 6, rarely 7, branched fin rays (*vs.* 7, rarely 6 or 8, in *N. marhabatiensis*, *N. calientis* and *N. aulidion* and 8, rarely 7 or 9, in *N. calabazas* and *N. amecae*), 42, rarely 40-41, or 43-45 scales in a lateral series (*vs.* 40, rarely 37-39, in *N. calabazas*; 35-36, rarely 37-39, or 33-34 in *N. amecae*; 35, rarely 31-34, or 36 in *N. marhabatiensis* and *N. calientis* and 34, rarely 30-33, or 35 in *N. aulidion*) and 11 rarely 10 or 12 gill rakers in the first arch; a dark lateral stripe widening from approximately the pectoral fin origin to the dorsal fin origin, forming a slightly convex segment; adult animals larger ( $n=30$ ,  $\bar{X}=42.6$ ,  $SD=4.69$ ) relative to *N. calientis* ( $n=55$ ,  $\bar{X}=33.3$ ,  $SD=3.28$ ) and *N. marhabatiensis* sp. nov. ( $n=30$ ,  $\bar{X}=30.5$ ,  $SD=7.57$ ) ( $F=16.87$ ;  $p < 0.001$ ); and finally 31 fixed nucleotide positions in the mitochondrial gene cytochrome *b* sequence along with four amino acid changes. Calculated genetic distances between the new species and other species of the complex ranged between  $\bar{D}_{GTR} = 6.1\%$  to  $7.4\%$  for *N. marhabatiensis* and  $\bar{D}_{GTR} = 6.3\%$  to  $8.1\%$  for *N. grandis*.

**Key words:** New species, *Notropis*, Central Mexico, Cyprinidae, critically endangered species.

## INTRODUCTION

The central region of Mexico boasts a high diversity and endemism of its fresh water fishes, attributed to a complex geological and zoogeographic history (Barbour, 1973; Echelle & Echelle, 1984; Domínguez-Domínguez *et al.*, 2006). The Central Mexico is the northern limit for some neotropical families of freshwater fishes, whereas it constitutes the southern limit for others of nearctic origin. Thus, the region is a large zone of contact between nearctic and neotropical fish families. Cyprinid species are of nearctic origin, and the southern margin of their distribution range is Central Mexico and adjacent areas, such as the Balsas, Atoyac and Papaloapan river basins. Although cyprinids are among the most diverse group of fishes in the world, this southern limit of North American cyprinids shows less diversity, despite co-distributed fish groups (e.g. Goodeidae and Atherinopsidae) containing a high number of endemic species. The fish fauna of Central Mexico includes three genera of cyprinids, *Notropis* Rafinesque, 1818, *Algansea* Girard, 1856 and *Yuriria* Jordan & Evermann, 1896, totaling 15 species.

Several genetic approaches have revealed the presence of cryptic species among the cyprinids of Central Mexico (Schönhuth & Doadrio, 2003; Schönhuth *et al.*, 2008). Some of these have been recently described (Domínguez-Domínguez *et al.*, 2007). Within the genus *Notropis* inhabiting the Central Mexico and southern areas two groups have been identified (Schönhuth & Doadrio, 2003) based on complete sequences of the mitochondrial cytochrome *b* gene: a Southern Mexican clade, comprising the species *Notropis boucardi* (Günther, 1868), *N. moralesi* (de Buen, 1955), *N. imeldae* Cortes, 1968, *N. cumingii* (Günther, 1868) and an undescribed species from Oaxaca; and a Central Mexican clade, including the species *N. sallaei* (Günther, 1868), *N. calientis* (Günther, 1868) and *Yuriria alta* (Jordan, 1880). In a recent phylogenetic study by Schönhuth *et al.* (2008) based on four genes, the genus *Yuriria* was assigned a basal position with respect to the two *Notropis* clades (Southern and Central clades). The species *N. amecae* (Chernoff & Miller, 1986), *N. aulidion* (Chernoff

& Miller, 1986) and *N. calabazas* (Lyons & Mercado-Silva, 2004) were not included in either study (Schönhuth *et al.*, 2008).

*Notropis calientis* has been defined as a species complex by Chernoff and Miller (1986), who described two of its species and portrayed the complex as a group of three small minnows inhabiting upland areas of Central Mexico: *N. aulidion*, *N. amecae* and *N. calientis*. All these species are characterized by their brilliant yellow to golden breeding coloration, small eyes, relatively few anal-fin rays and an incomplete pored-lateral line of scales (absent in *N. aulidion*). More recently, another species has been described, *N. calabazas* Lyons & Mercado-Silva (2004), from the upper Rio Verde in the Pánuco River Basin. This species was assigned by the authors to the *N. calientis* complex because of its reduced number of head and lateral sensory canals, which are also incomplete.

The species of the *N. calientis* complex mainly inhabit small mountain streams, springs and small spring-fed lakes and their outlets. The four species included so far are highly sensitive to environmental disturbances and their populations are in decline. The species *N. aulidion* is believed to be extinct, *N. amecae* (Chernoff & Miller, 1986) is considered very rare and *N. calabazas* is uncommon. *Notropis calientis*, on the other hand, is widespread throughout most of the Lerma and Verde de Santiago drainage, the Rio Santa María in Pánuco drainage and the Cuitzeo Lake and its tributaries, although the decline of populations in some drainages has also been reported (Lyons & Mercado-Silva, 2004).

In a recent phylogenetic study of the cyprinids of central Mexico, the populations of *Notropis* from Zacapu Lake and San Miguel spring showed high genetic divergence with respect to populations of *N. calientis* and have been proposed as new undescribed taxa (Schönhuth & Doadrio, 2003; Schönhuth *et al.*, 2008). The purpose of this study was to describe two new species within the *Notropis calientis* complex based on morphologic and genetic characters. These two new species inhabit the Zacapu Lake and San Miguel spring in Central Mexico.

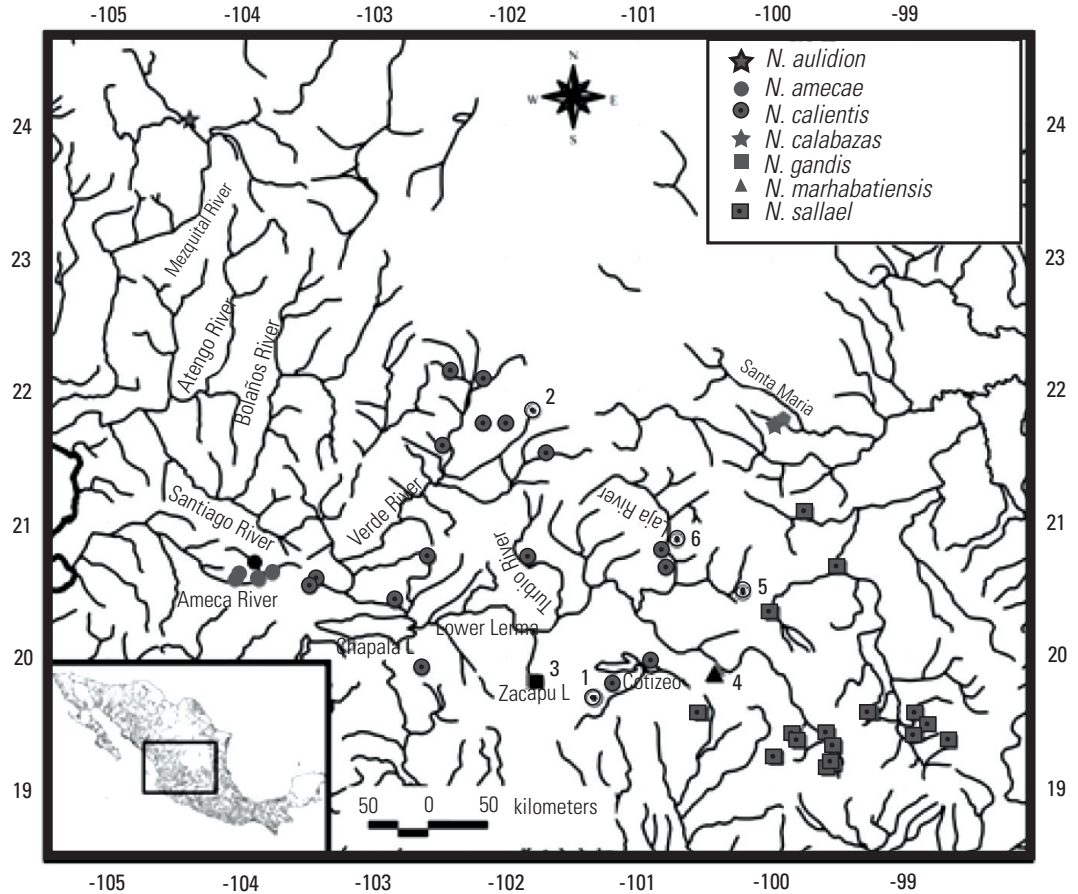


Figure 1.- Distribution ranges of *Notropis* spp. Symbols in gray correspond to know localities for the different species. Symbols in black and numbers indicate localities included in the study. Details of the localities are given in Table 1.

**MATERIALS AND METHODS**

The designations of the new species were based on morphological analyses of the type material listed in the species descriptions and the following additional material (see Table 1 and Fig. 1): *Notropis calientis*, 30 specimens, CPUM-1621 (La Paz Dam near the city of Aguascalientes, Verde River drainage, Santiago River Basin, state of Aguascalientes); and *N. calientis*, 25 specimens, CPUM-1622 (La Mintzita Spring near the city of Morelia, Cuitzeo lake drainage, Michoacan).

The holotype and paratype series of the two new species were deposited in the Colección de Peces, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico (CPUM), Colección Nacional de Peces, Universidad Nacional Autónoma de México (CNP-IBUNAM) and Museo Nacional de Ciencias Naturales de Madrid, España (MNCN).

**Morphological analysis.** Measurements were performed following Chernoff and Miller (1986), Doadrio *et al.* (2002) and Domínguez-Domínguez *et al.* (2007). Nineteen morphometric cha-

racters were determined using the computer program ImageTool 3.00, and seven meristic variables were counted with the help of a stereoscopic microscope. The following abbreviations were

Table 1. Localities and sample sizes of the *Notropis* populations used in the morphometric (Mor), meristic (Mer) and genetic (Gen) analyses.

	Sampling sites		Mor	Mer	Gen
	Locality	Drainage			
<i>N. calientis</i>	1.- La Mintzita Spring	Cuitzeo Lake	25	25	1+1*
<i>N. calientis</i>	2.- La Paz Dam**	Santiago River	31	31	2
<i>N. grandis</i>	3.- Zacapu Lake	Zacapu Lake	31	31	2*+1
<i>N. marhabatiensis</i>	4.- San Miguel Spring	Upper Lerma River	28	28	2*
<i>N. calientis</i>	5.- Pirules Dam	Middle Lerma River	----	----	1*
<i>N. calientis</i>	6.- Charco del Ingenio	Middle Lerma River	----	----	1*
<i>N. amecae</i>	7.- Chapulimita stream	Ameca River	----	----	2

\*Sequences obtained from Genbank. \*\*Type locality of *Notropis calientis*

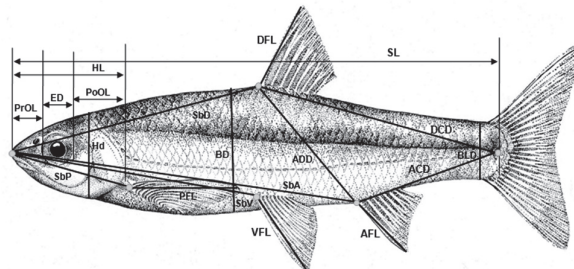


Figure 2.- Morphometric measurements included in the analysis.

used for morphometric and meristic characters: SL, standard length; HL, head length; PrOL, preorbital length; ED, eye diameter; PoOL, postorbital length; Hd, head depth; SbP, snout to origin of the pectoral fin; SbD, snout to origin of the dorsal fin; SbV, snout to origin of the ventral fin; SbA, snout to origin of the anal fin; DFL, dorsal fin length; PFL, pectoral fin length; VFL, ventral fin length; AFL, anal fin length; ACD, origin of the anal fin to origin of the caudal fin; DCD, origin of the dorsal fin to origin of the caudal fin; BD, body depth; BLD, body least depth; ADD, origin of the anal fin to origin of the dorsal fin (Fig. 2). Abbreviations for meristic characters are: C, caudal fin branched rays; D, dorsal fin branched rays; V, ventral fin branched rays; A, anal fin branched rays; SCS, scales in a central series; SLL, scales in a lateral line series; ST, scales in a transverse series according to Kottelat and Phreihof (2007); PS, pored scales; GR, gill rakers.

To identify the variables contributing most to the differences between the populations sampled, principal component analyses (PCA) were performed on the meristic and morphometric data

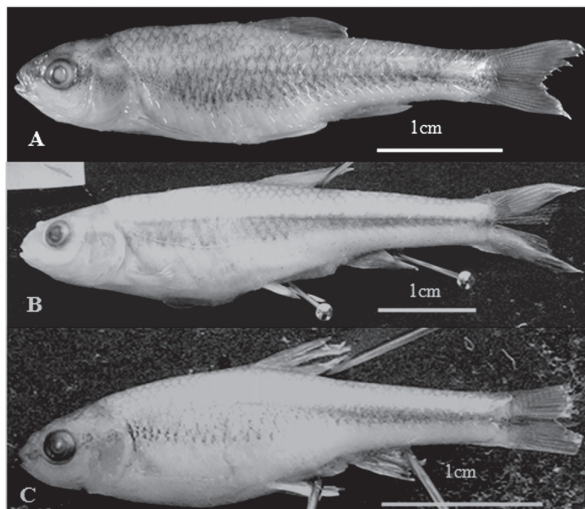


Figure 3A-C. A) Holotype of *N. marhabatiensis* sp. nov. B) Holotype of *N. grandis* sp. nov. C) Specimens of *N. calientis*.

Table 2. Statistical parameters for the morphometric (MPc) and meristic characters recorded in *Notropis marhabatiensis* sp. nov. and *Notropis grandis* sp. nov. For morphometrics, each variable is divided by standard length. Variable codes are given in the methods section. (SD = standard deviation).

MPc	<i>N. marhabatiensis</i>			<i>N. grandis</i>		
	Holotype	Range	Mean (SD)	Holotype	Range	Mean (SD)
SL	36.64	50.8-38.5	43.73 (3.2)	45.57	41.4-24	32.4 (4.4)
HL	0.25	0.22-0.29	0.26(.02)	0.22	0.21-0.25	0.23(0.01)
PrOL	0.06	0.05-0.08	0.07(.01)	0.05	0.04-0.08	0.06(0.01)
ED	0.07	0.06-0.08	0.07(.01)	0.06	0.05-0.07	0.06(0.01)
PoOL	0.11	0.10-0.14	0.12(.01)	0.11	0.10-0.12	0.11(0.01)
Hd	0.21	0.19-0.22	0.20(.01)	0.18	0.16-0.23	0.18(0.01)
SbP	0.27	0.25-0.42	0.29(.03)	0.26	0.23-0.28	0.26(0.01)
SbD	0.51	0.50-0.57	0.53(.02)	0.54	0.52-0.56	0.54(0.01)
SbV	0.56	0.52-0.59	0.56(.02)	0.56	0.45-0.56	0.53(0.02)
SbA	0.71	0.66-0.76	0.71(.02)	0.71	0.66-0.73	0.70(0.02)
DFL	0.16	0.15-0.24	0.20(.02)	0.14	0.14-0.22	0.19(0.02)
PFL	0.20	0.12-0.22	0.16(.02)	0.16	0.12-0.17	0.15(0.01)
VFL	0.16	0.11-0.19	0.15(.02)	0.14	0.10-0.21	0.13(0.02)
AFL	0.16	0.12-0.21	0.16(.02)	0.14	0.11-0.18	0.15(0.02)
ACD	0.32	0.27-0.35	0.31(.02)	0.30	0.29-0.36	0.32(0.02)
DCD	0.52	0.45-0.53	0.49(.02)	0.48	0.45-0.51	0.49(0.01)
BD	0.26	0.21-0.27	0.23(.02)	0.23	0.21-0.28	0.23(0.02)
BLD	0.12	0.08-0.12	0.10(.01)	0.09	0.09-0.11	0.10(0.01)
ADD	0.28	0.20-0.28	0.26(.02)	0.25	0.23-0.28	0.25(0.01)
Meristic characters						
D	7.00	8-8	8.00(.00)	7.00	7-9	7.94(.44)
V	8.00	7-8	7.4(.50)	8.00	8-8	8.00(.00)
A	7.00	6-7	6.86(.36)	6.00	6-7	6.10(.30)
SCS	36.00	34-36	35.00(.77)	42.00	40-46	42.16(.97)
ST	9.00	8-10	8.85(.39)	10.00	9-10	9.90(.30)
GR	7.00	6-8	7.11(.57)	11.00	10-11	10.90(.47)

collected from all the specimens, using a covariance matrix for the morphometric characters and correlation matrix for meristic characters. The PCA results for the morphometric data (not shown) indicated that all weighted characters on the first principal component (PC I) showed the same sign and were of similar magnitude, suggesting that this axis represents general size-related variation (Jolicoeur & Mosimann, 1960; Humphries *et al.*, 1981; Bookstein *et al.*, 1985). To test the possibility that one of the populations included in the dataset showed a larger size of specimens, as was empirically observed, the dataset was subjected to one way ANOVA and Tukey analysis. Burnaby's method was used to correct for the size effect (Burnaby, 1966; Rohlf & Bookstein, 1987; Doadrio *et al.*, 2002) and all subsequent

analyses were conducted on the corrected matrix. A second PCA was undertaken of the morphometric characters and subjected to canonical analysis of variance (CVA) to summarize differences among populations. The statistics package PAST v 1.75b was used for all the tests (Hammer *et al.*, 2001). Morphometric and meristic characters were analyzed independently.

**Genetic analysis.** Seven sequences of *Notropis* (AF469137-AF469143) were obtained from GenBank (see Table 1). Six specimens, including those of the type locality of *N. calientis* and *N. amecae* and the outgroup *N. sallaei*, were collected and sequenced (Table 1 and Fig. 1). Sequences were obtained as follows. Total cellular DNA was isolated from tissues by standard proteinase K and phenol/chloroform extraction procedures (Sambrook

Table 3. Statistical parameters for the morphometric and meristic characters recorded in *Notropis calientis*. For morphometrics, each variable is divided by standard length. Variable codes are given in the methods section. (SD = standard deviation).

Morphometrics	<i>N. calientis</i> (La Paz)		<i>N. calientis</i> (La Mintzita)	
	Range	Mean (SD)	Range	Mean (SD)
SL	34.7-28.22	31.4(1.6)	43.3-30.9	35.5(3.3)
HL	0.22-0.26	0.24(.01)	0.21-0.27	0.24(.01)
PrOL	0.04-0.06	0.05(.00)	0.04-0.07	0.06(.01)
ED	0.06-0.08	0.07(.00)	0.06-0.08	0.07(.01)
PoOL	0.10-0.13	0.11(.01)	0.10-0.14	0.11(.01)
Hd	0.17-0.20	0.18(.01)	0.17-0.20	0.19(.01)
SbP	0.22-0.27	0.24(.01)	0.23-0.30	0.25(.02)
SbD	0.50-0.54	0.51(.03)	0.49-0.55	0.53(.02)
SbV	0.51-0.54	0.52(.01)	0.48-0.55	0.51(.02)
SbA	0.67-0.69	0.66(.03)	0.64-0.70	0.67(.02)
DFL	0.18-0.25	0.23(.02)	0.12-0.19	0.15(.02)
PFL	0.14-0.23	0.20(.02)	0.17-0.25	0.22(.02)
VFL	0.10-0.19	0.16(.02)	0.08-0.18	0.15(.02)
AFL	0.14-0.22	0.18(.02)	0.14-0.20	0.17(.02)
ACD	0.31-0.38	0.35(.01)	0.32-0.37	0.35(.02)
DCD	0.49-0.55	0.53(.01)	0.47-0.54	0.51(.02)
BD	0.23-0.27	0.25(.01)	0.21-0.28	0.24(.02)
BLD	0.09-0.12	0.11(.01)	0.09-0.12	0.11(.01)
ADD	0.26-0.30	0.28(.01)	0.24-0.30	0.27(.02)
<b>Meristics</b>				
D	7-8	7.19(.40)	8-9	8.16(.37)
V	8-9	8.16(.37)	7-8	7.96(.20)
A	6-8	6.94(.44)	6-7	6.80(.41)
SCS	34-36	35.00(.45)	35-36	35.76(.44)
ST	9-10	9.87(.34)	9-10	9.76(.44)
GR	7	7(0)	6-7	6.64(.49)

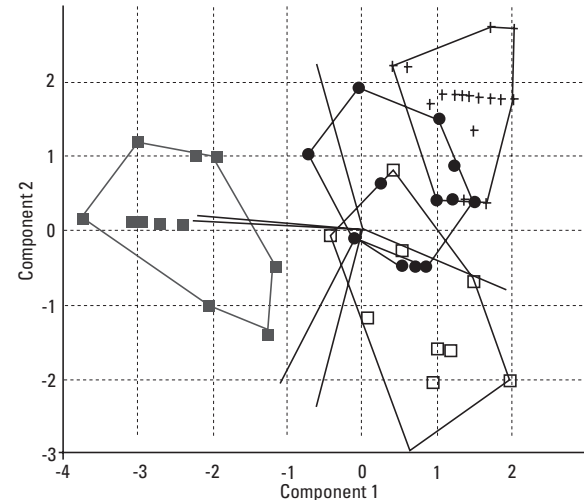


Figure 4.- Principal Component Analysis of meristic characters. Solid squares, *N. grandis* sp. nov.; open squares, *N. calientis* from La Paz; circles, *N. calientis* from La Mintzita and crosses, *N. marhabatiensis* sp. nov. from San Miguel spring.

*et al.*, 1989). Two overlapping fragments of the cytochrome *b* gene (1140 bp) were amplified via polymerase chain reaction (PCR) for each individual DNA sample. The primers used for cytochrome *b* in all species were those described in Zardoya and Doadrio (1998). The amplification process involved an initial denaturation step at 94°C for 2 min, and 35 cycles performed as follows: denaturation at 94°C (1 min), aligning at 48°C (1 min), and extension at 72°C (1.45 min), with a final extension of 7 min at 72°C. PCR mixtures were prepared in 25 µl reaction volumes with final concentrations of 0.4 µM of each primer, 0.2 mM of each dNTP, 1.5 mM MgCl<sub>2</sub>, and 1U of Taq DNA polymerase (Biotools). PCR products were checked on 1.5% agarose gels, and cloned using the pGEM-T vector (Promega) into *Escherichia coli* JM109. Positive clones were sequenced using the FS-Taq Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers. All samples were sequenced using an Applied Biosystems 3700 DNA sequencer following the manufacturer's instructions. Chromatograms and alignments were visually checked and verified. To obtain the model that best fitted our data we used a Bayesian information criterion implemented in the program Modeltest 3.7 (Posadas & Crandall, 1998). The aligned data were analyzed using the Bayesian inference method in the program Mr. Bayes 3.1.1 (Huelsenbeck & Ronquist, 2001), simulating four Markov chains for 1,000,000 cycles. Based on the GTR model provided by Modeltest, genetic distances (DGTR) between populations of *Notropis* and the new species were obtained using the program Sequencer 6.1.0 (written by B. Kessing and available at <http://nmg.si.edu/>). Accession numbers of the submitted sequences are GQ249850-GQ249856.

## RESULTS

### *Notropis marhabatiensis* sp. nov. (Figure 3A, Table 2)

Holotype: MNCN-205640, 36.64 mm SL. Ojo de Agua de San Miguel in Maravatio town, Lerma Basin, Michoacan, Mexico. Geographic coordinates: latitude 19.86667 N, longitude 100.4333 W. Coll. I. Doadrio, E. González-Luna, A. de Sostoa and O. Domínguez-Domínguez, 6 June 2000.

Paratypes: 14 specimens MNCN-205629-205641; 13 specimens CPUM-2299; 3 specimens CNP-IBUNAM 14622. Same data as holotype.

Diagnosis: *Notropis marhabatiensis* sp. nov. was diagnosed according to the following combination of characters: 7, rarely 8, branched pelvic fin rays (vs. 8 rarely 7 or 9 for the other species of the *N. calientis* complex; Table 3) and 9, rarely 8, scales in a transverse series (vs. 10, rarely 9 or 11, in *N. grandis* and *N. calientis*). A dark, narrow lateral stripe runs from around the pectoral fin to the origin of the caudal peduncle. Twenty-six fixed nucleotide positions, or autapomorphies, and one amino acid change were observed in the cytochrome *b* sequence with respect to *Notropis* sp., *N. calientis* and *N. amecae* (Table 4). Genetic distances for *N. marhabatiensis* were:  $\bar{D}_{GTR} = 6.1\%$  with respect to *N. calientis*;  $\bar{D}_{GTR} = 7.4\%$  with respect to *N. amecae*; and  $\bar{D}_{GTR} = 7.4\%$  with respect to *N. grandis* sp. nov. (Table 5).

Description: D= II 7; A= I-II 6 (7); V= I (7)8; GR= 6(7)8; SCS = 34(35)36; ST= (8) 9. As in the other species of the *Notropis calientis* complex, the lateral line is incomplete and curved, SLL = 0 to 7 pored scales. All morphometric and meristic measurements are provided in Table 2. Scales thin, moderately imbricate and deciduous. The body is moderately deep, laterally compressed and elongated. Deepest body section approximately at the origin of the dorsal fin, but its depth is similar in the anterodorsal part of the body, tapering to the posterodorsal portion. Maximum body depth is 3.7-4.9 ( $\bar{X} = 4.34 \pm 0.32$ ) times the standard length and

minimum body depth 8.5-12.6 ( $\bar{X} = 9.6 \pm 0.72$ ) times the standard length. Caudal peduncle relatively long, with least depth just before the hypural plate. Distance origin anal fin to origin caudal fin is 2.9-3.6 ( $\bar{X} = 3.2 \pm 0.17$ ) times the standard length and origin dorsal fin to origin caudal fin 1.9-2.4 ( $\bar{X} = 2.0 \pm 0.08$ ) times the standard length. Head slightly longer than deep, snout rounded to slightly pointed not projecting beyond the upper lip. Head length is 3.5-4.5 ( $\bar{X} = 3.9 \pm 0.26$ ) times the standard length and head depth 4.6-5.4 ( $\bar{X} = 4.9 \pm 0.21$ ) times the standard length. Eye centered at halfway head length. Eye diameter is 12.8-16.4 ( $\bar{X} = 14.7 \pm 1.16$ ) times the standard length. Dorsal fin about midway between snout and caudal peduncle at same level or slightly anterior to the ventral fin origin along the same axis. Distance from snout to the dorsal fin origin is 1.7-2 ( $\bar{X} = 1.88 \pm 0.05$ ) times the standard length and distance snout to ventral fin origin 1.7-1.9 ( $\bar{X} = 1.79 \pm 0.06$ ) times the standard length. Pectoral fin length is 4.6-7.6 ( $\bar{X} = 6.5 \pm 0.94$ ) times the standard length. Dorsal fin length is 4.2-6.6 ( $\bar{X} = 4.9 \pm 0.53$ ) times the standard length. Ventral fin length is 5.3-9.1 ( $\bar{X} = 6.9 \pm 0.94$ ) times the standard length. Anal fin length is 4.7-8.1 ( $\bar{X} = 6.2 \pm 0.77$ ) times the standard length. Snout slightly rounded. Mouth terminal to slightly subterminal and oblique. Barbels absent.

Pigmentation pattern: Preserved specimens show a yellowish to light brown coloration of the body. A dark, narrow lateral stripe runs from approximately the origin of the pectoral fin to the caudal peduncle. In the predorsal region, a diffuse elongated spot appears in the middle body portion; in some specimens this spot is made up of a number of smaller spots. These spots occur across most of the body and are more conspicuous at the opercle. Some specimens show a small dark patch at the caudal peduncle and anterior part of the caudal fin. Fine pigmented predorsal stripes in the dorsal region. Upper head pigmented with small dark spots. Interorbital region darkly pigmented. All fins transparent and in some individuals the anal, caudal and dorsal fin show a dark coloration.

Table 4. Molecular diagnostic characters obtained for the cytochrome *b* gene in the genus *Notropis*. Ts= transitions and Tv= transversions within the same base position (BP).

Species /BP	33	36	48	49	50	54	62	71	78	87	88	106	108	111	115	127	132
<i>N. marhabatiensis</i>	G	G	T	G	C	A	T	C	T	G	C	T	G	A	C	A	A
aminoacid change																	<b>W</b>
<i>N. grandis</i>	A	G	C	G	C	A	T	C	T	A	T	C	A	G	T	G	G
aminoacid change																	<b>A</b>
<i>N. calientis</i>	G	G	T	G	C	A	T	C	C	A	C	T	A	A	C	A	A
aminoacid change																	<b>W</b>
<i>N. amecae</i>	G	A	T	C	G	C	A	T	T	A	C	T	A	A	C	A	A
aminoacid change																	<b>W</b>
Substitution type	Ts	Ts	Ts	Tv	Tv	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts

Table 4. *Continued*

Species/BP	141	162	213	216	225	240	282	303	306	318	324	333	348	354		
<i>N. marhabatiensis</i>	C	C	G	T	C	A	C	C	T	T	C	A	G	T		
<i>N. grandis</i>	A	T	A	T	T	A	C	T	T	C	T	G	A	C		
<i>N. calientis</i>	C	C	G	T	T	A	C	C	T	C	T	G	A	A		
<i>N. amecae</i>	C	C	G	C	T	C	T	C	C	C	T	G	A	T		
Substitution type	Tv	Ts	Ts	Ts	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts/Tv		
Species/BP	573	580	595	609	612	624	651	657	675	678	681	690	702	717	718	771
<i>N. marhabatiensis</i>	T	A	T	A	A	C	G	T	T	C	G	C	A	T	A	A
aminoacid change		<b>I</b>														<b>T</b>
<i>N. grandis</i>	T	G	T	G	A	C	A	C	C	T	A	C	G	T	A	G
aminoacid change		<b>V</b>														<b>T</b>
<i>N. calientis</i>	A	A	T	A	A	C	A	C	C	T	A	T	A	T	G	A
aminoacid change		<b>I</b>														<b>A</b>
<i>N. amecae</i>	T	A	C	A	G	T	A	C	C	T	A	C	A	C	A	A
aminoacid change		<b>I</b>														<b>T</b>
Substitution type	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts
Species/BP	783	789	813	816	823	825	831	843	841	846	849	852	867	880	883	885
<i>N. marhabatiensis</i>	G	C	A	G	T	A	C	C	C	A	T	C	G	T	T	A
<i>N. grandis</i>	A	C	G	A	T	A	C	C	C	A	A	C	A	C	C	A
<i>N. calientis</i>	C	G	A	A	T	G	T	C	C	A	T	C	G	C	T	A
<i>N. amecae</i>	G	T	A	A	C	A	C	T	T	T	T	T	G	C	T	G
Substitution type	Ts/Tv	Ts/Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts
Species/BP	903	924	930	939	957	958	978	979	993	997	1005	1035	1038	1041	1044	
<i>N. marhabatiensis</i>	A	T	G	G	A	A	G	G	C	A	G	C	G	C	C	
<i>N. grandis</i>	A	C	A	A	A	A	A	A	C	C	A	C	A	C	T	
<i>N. calientis</i>	G	C	A	A	G	A	A	A	C	G	G	C	A	T	T	
<i>N. amecae</i>	C	C	A	A	A	G	A	A	T	G	G	T	A	C	T	
Substitution type	Ts/Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts	Ts/Tv	Ts	Ts	Ts	Ts	Ts	
Species/BP	1050	1056	1059	1065	1066	1071	1095	1096	1101	1116						
<i>N. marhabatiensis</i>	T	G	C	G	A	G	C	G	C	A						
aminoacid change					<b>I</b>											
<i>N. grandis</i>	T	A	C	G	G	G	T	A	T	G						
aminoacid change					<b>V</b>											
<i>N. calientis</i>	T	A	C	G	G	G	C	G	T	A						
aminoacid change					<b>V</b>											
<i>N. amecae</i>	C	A	A	C	G	A	C	G	T	A						
aminoacid change					<b>V</b>											
Substitution type	Ts	Ts	Tv	Ts	Ts	Ts	Ts	Ts	Ts	Ts						



Table 5. Pairwise genetic distances among *Notropis* species. Uncorrected distances are given above diagonal. GTR distance appears below diagonal. Mean distance within species on the diagonal.

Species	<i>N. calientis</i>	<i>N. amecae</i>	<i>N. grandis</i>	<i>N. marhabatiensis</i>
<i>N. calientis</i>	0.013	0.071	0.064	0.065
<i>N. amecae</i>	0.068	0.003	0.082	0.077
<i>N. grandis</i>	0.063	0.081	0.029	0.076
<i>N. marhabatiensis</i>	0.061	0.074	0.074	0.006

**Etymology:** The name "*marhabatiensis*" is derived from its type locality, the town of Marhabatio (P'urepecha word of Maravatio). The P'urepecha word Marhabatio means beautiful thing or place.

**Common name:** Maravatio shiner.

**Distribution:** The type locality of *N. marhabatiensis* is the San Miguel Spring in the southeast region of Maravatio town in the upper Lerma River drainage, state of Michoacan. The species is only known in the San Miguel Spring (Fig. 1).

**Remarks:** The spring inhabited by *N. marhabatiensis* is highly degraded and used for recreation purposes. The main pool is around 2 m deep and 5 m wide. A small outlet stream runs along 200 m and then discharges into the town's (Maravatio) waste-water channel. The species is absent from the stream. Fish collected with *N. marhabatiensis* from the type locality were the native goodeids *Girardinichthys multiradiatus* (Meek, 1904) and *Goodea atripinnis* Jordan, 1880, and the introduced fish were the cichlid *Oreochromis* sp. and the poeciliids *Poecilia reticulata* Peters, 1859 and *Heterandria bimaculata* (Heckel, 1848). No vegetation was found around the pool. Within the spring grow aquatic plants of the genus *Ceratophyllum*. The substrate is composed mainly of sand and gravel with many boulders at the bottom of the pool and mud in the stream.

**Conservation status:** The species is only known in the type locality, San Miguel Spring, and only a small population was found in surveys conducted in 2000 and 2002. In recent visits to the area (2004 to 2007), the species was not found. This small spring is surrounded by the houses of Maravatio and suffers intense human pressure as a recreation and washing place. The recent introduction of the exotic species *P. reticulata*, *H. bimaculata* and *Oreochromis* sp. could explain the possible absence of this species from the spring. Because of its restricted distribution range, recent unsuccessful sampling efforts and threats to the habitat and species in the San Miguel Spring, we consider this species as Critically Endangered according to UICN criteria (CR B-1 b i, iii, iv). Further, according to the Risk Evaluation Method (Método de Evaluación de Riesgo-MER) (Sánchez et al., 2007), the species should be considered in danger of extinction (14 A,I; B,I; C,I; D,I).

### ***Notropis grandis* sp. nov.** (Figure 3B, Table 2)

**Holotype:** CPUM- 2300, 45.57 mm SL. La Angostura, Laguna de Zacapu, Zacapu, Michoacan, Mexico. Geographic coordinates: latitude 19.81667 N, longitude 101.78333 W. Coll. R. Pérez-Rodríguez and R. Rosas-Valdez, 9 June 2005.

**Paratypes:** 26 specimens CPUM-1623; 5 specimens CNP-IBUNAM 14624; 4 specimens MNCN-262768-262771. Same data as holotype.

**Diagnosis:** *Notropis grandis* sp. nov. was diagnosed according to the following combination of characters: 6, rarely 7, branched anal fin rays (vs. 7, rarely 6 or 8, in *N. marhabatiensis*, *N. calientis* and *N. aulidion* and 8, rarely 7 or 9, in *N. calabazas* and *N. amecae*), 42, rarely 40-41, or 43-45 scales in a lateral series (vs. 40, rarely 37-39, in *N. calabazas*; 35-36, rarely 37-39, or 33-34 in *N. amecae*; 35, rarely 31-34, or 36 in *N. marhabatiensis* and *N. calientis* (Table 3) and 34, rarely 30-33, or 35 in *N. aulidion*) and (10) 11 (12) gill rakers in the first arch. A dark lateral stripe widens from approximately the pectoral fin origin to the dorsal fin origin, forming a slightly convex segment. Adult animals large (n=30,  $\bar{X}$ =42.6, SD=4.69) relative to *N. calientis* (n=55,  $\bar{X}$ =33.3, SD=3.28) and *N. marhabatiensis* sp. nov. (n=30,  $\bar{X}$ =30.5, SD=7.57) (F=16.87; p<0.001). Twenty-three fixed nucleotide positions, or autapomorphies, and two amino acid changes were observed in the cytochrome b sequence with respect to *N. calientis*, *N. marhabatiensis* and *N. amecae* (Table 4). Genetic distances for *N. grandis* were:  $\bar{D}_{GTR}$  = 6.3% with respect to *N. calientis*;  $\bar{D}_{GTR}$  = 8.1% with respect to *N. amecae*; and  $\bar{D}_{GTR}$  = 7.4% with respect to *N. marhabatiensis* sp. nov. (Table 5).

**Description:** D=II 6(7)8; A=I-II (6)7; V=I 8; GR=10(11)12; SCS=40-41(42)43-45; ST=9(10). All morphometric and meristic measurements are provided in Table 2. As in other species of the *Notropis calientis* complex, the lateral line is incomplete and curved, SLL=1 to 17 pored scales. Scales thin, moderately imbricate and deciduous. The body is moderately deep, laterally compressed and elongated. Dorsal and ventral profiles moderately arched. Deepest body section approximately midway between the dorsal fin and the occiput. Maximum depth is 3.6-4.8 ( $\bar{X}$ =4.5±0.28) times the standard length and minimum body depth 9.2-11.7 ( $\bar{X}$ =10.4±0.48) times the standard length. Caudal peduncle relatively long and thick, and narrowest portion of the body occurs just at the end of the caudal peduncle. Distance origin anal fin to origin caudal fin is 2.8-3.4 ( $\bar{X}$ =3.1±0.16) times the standard length and origin dorsal fin to origin caudal fin 2-2.2 ( $\bar{X}$ =2.1±0.06) times the standard length. Head moderately longer than deep, snout rounded to slightly pointed not projecting beyond the upper lip, head length 4.0-4.7 ( $\bar{X}$ =4.3±0.16) times the standard length and head depth 4.4-6.12 ( $\bar{X}$ =5.6±0.36) times the standard length. Eye is relatively small and centered at halfway head distance. Eye diameter is 14.4-18.6 ( $\bar{X}$ =16.8±1.13) times the standard length. Dorsal fin appears about midway between snout and caudal peduncle at same level or slightly behind the ventral fin origin along

the same axis. Distance snout to dorsal fin origin is 1.8-1.9 ( $\bar{x} = 1.86 \pm 0.04$ ) times the standard length and snout to ventral fin origin 1.8-2.2 ( $\bar{x} = 1.9 \pm 0.08$ ) times the standard length. Pectoral fin length is 3.5-4.4 ( $\bar{x} = 3.9 \pm 0.19$ ) times the standard length. Dorsal fin is 4.7-7.3 ( $\bar{x} = 5.5 \pm 0.76$ ) times the standard length. Pectoral fin is 5.9-8.1 ( $\bar{x} = 6.84 \pm 0.55$ ) times the standard length. Ventral length is 4.8-9.83 ( $\bar{x} = 7.6 \pm 0.89$ ) times the standard length. Anal fin is 5.7-8.5 ( $\bar{x} = 6.9 \pm 0.73$ ) times the standard length. Snout slightly rounded. Mouth is terminal to slightly subterminal and oblique. Barbels absent.

**Pigmentation pattern:** Preserved specimens show a yellowish to light brown coloration of the body. A dark lateral stripe widens from approximately the origin of the pectoral fin to the dorsal fin origin forming a slightly convex segment; the stripe becomes narrow and more conspicuous in the postdorsal region of the body. The lateral stripe ends in a small dark patch in the caudal peduncle and caudal fin origin. Fine pigment stripes around the base of the dorsal fin. Upper part of the head pigmented. All fins clear and unpigmented.

**Etymology:** The name "*grandis*" refers to the larger size of this species relative to the other members of the *N. calientis* complex.

**Common name:** Zacapu shiner.

**Distribution:** The type locality of *N. grandis* sp. nov. is Lake Zacapu in the town of Zacapu, Lerma River drainage, state of Michoacán. It is only known in the Zacapu Lake and its outlet (Fig. 1).

**Remarks:** The water body is fed by at least 12 large springs that maintain the level of the lake. The water in the spring area is clean and sustains dense vegetation, mainly *Potamogeton illinoensis* Morong, *P. pectinatus* Linnaeus, *Myriophyllum* sp., *Sagittaria* sp., and *Ceratophyllum demersum* Linnaeus. The rest of the lake contains turbid water with less vegetation than the spring area. In the zone around the lake, grow *Taxodium*, *Salix*, *Berula erecta* Hudson, *Scirpus* sp., and *Typha latifolia* Linnaeus. The introduced *Eichhornia crassipes* Martius is also found. The fish species found are the goodeids *Hubbsina turneri* De Buen, 1940, *Goodea atripinnis*, *Xenotoca variata* (Bean, 1887), *Allotoca zacapuensis* Meyer, Radda & Domínguez-Domínguez, 2002, *Allophorus robustus* (Bean, 1892), *Skiffia lermæ* Meek, 1902, *Zoogoneticus quitzeoensis* (Bean, 1898), the poeciliid *Poeciliopsis infans* (Woolman, 1894), the atherinid *Chirostoma humboldtianum* (Valenciennes, 1835), the cyprinid *Algansea tincella* (Valenciennes, 1844), and the introduced cyprinids *Cyprinus carpio* Gldenstdt, 1773 and *Ctenopharyngodon idella* (Valenciennes, 1844).

**Conservation status:** The species is only known in Lake Zacapu. Although since 2003 the lake forms part of a protected area, the introduction of the exotic carp species *Cyprinus carpio* and *Ctenopharyngodon idella* and their parasite *Bothriocephalus*

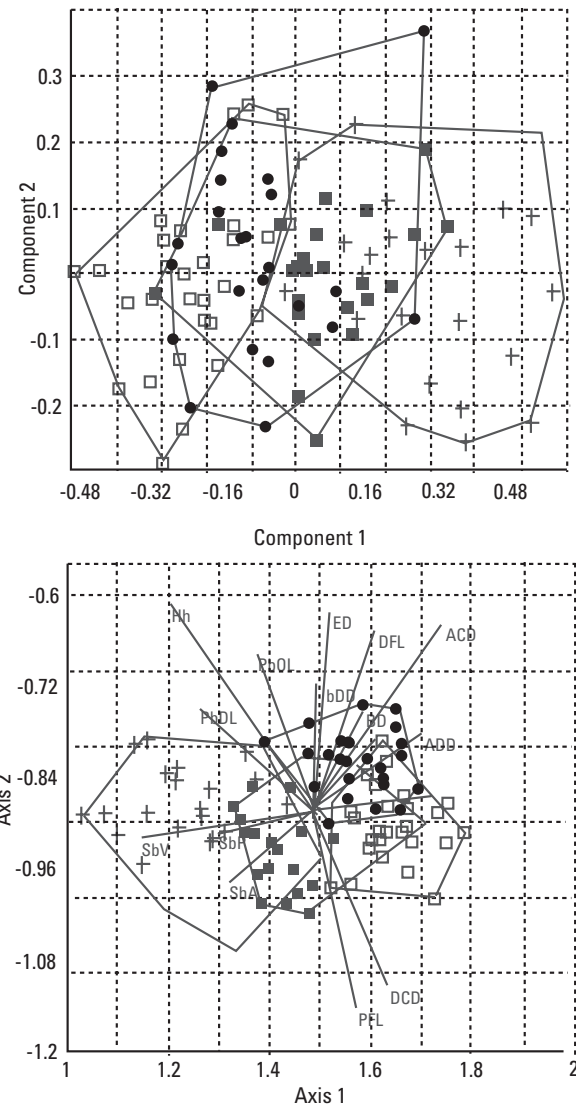


Figure 5.- Principal Component Analysis (top) and Canonical Variate Analysis (bottom) of morphometric characters. Solid squares, *N. grandis* sp. nov.; open squares, *N. calientis* from La Paz; circles, *N. calientis* from La Mintzita and crosses, *N. marhabatiensis* sp. nov. from San Miguel spring.

*acheilognathi* Yamaguti, 1934 seems to have had a negative impact on *N. grandis* sp. nov. Indeed, the repercussions of introducing carp species and their parasites on native fish species and their habitats in Mexico have been well documented (Lyons *et al.*, 1998; Soto-Galera *et al.*, 1998; Tapia & Zambrano, 2003; Salgado-Maldonado & Pineda-Lpez, 2003; Meja-Madrid *et al.*, 2005; Domnguez-Domnguez *et al.*, 2006). The lake's water quality has been affected by a drop in the water table reducing water inflow to the springs that feed the lake. Moreover, at the beginning of the XIX century, drying of the original marshy area (around 15,000 ha) for

Table 6. Frequency table for six species and five meristic characters of the *Notropis calientis* complex. For *N. aulidion* and *N. amecae*, data were obtained from Chernoff and Miller (1986) and for *N. calabazas* data were obtained from Lyons and Mercado (2004). Numbers in bold indicate mode.

	Pelvic-fin rays			Anal-fin rays			
	7	8	9	6	7	8	9
<i>N. grandis</i>		31		<b>28</b>	3		
<i>N. marhabatiensis</i>	<b>17</b>	11		4	24		
<i>N. calientis</i>	1	50	5	9	45	2	
<i>N. calabazas</i>	4	3			1	13	2
<i>N. amecae</i>	6	30			4	41	
<i>N. aulidion</i>	1	12		1	12		

	Scales in a central series															
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
<i>N. grandis</i>											1	2	<b>23</b>	2	1	1
<i>N. marhabatiensis</i>					8	12	8									
<i>N. calientis</i>		1	8	12	2	31	22									
<i>N. calabazas</i>								1	1	1	3					
<i>N. amecae</i>				1	1	7	7	5	5	5						
<i>N. aulidion</i>	2	1	1	2	3	1										

	Transverse scales				Gill rakers in the first arch								
	8	9	10	11	5	6	7	8	9	10	11	12	13
<i>N. grandis</i>		2	28	1						5	<b>24</b>	2	
<i>N. marhabatiensis</i>	5	<b>23</b>				3	19	6					
<i>N. calientis</i>		10	46			9	47						
<i>N. calabazas</i>											3	2	2
<i>N. amecae</i>						2	11	12	4	1			
<i>N. aulidion</i>									3	3	3	1	

agricultural purposes reduced the lake to a mere 32 ha (Guzmán, 1985). This decrease in the water mass had detrimental effects on the Zacapu Lake population of *Zoogoneticus quitzeoensis* (Bean, 1898), severely compromising genetic diversity and increasing the extinction risk of its fish populations (Domínguez-Domínguez *et al.*, 2007). According to these events and pending further population and genetic studies, we propose that the new species should be considered Critically Endangered according with the IUCN criteria (CR B-1 a i, iii, iv). Under Mexican environmental Law and following the criteria of MER (Sánchez *et al.*, 2007), the new species should be considered in danger of extinction (14 A,II;B;J; C,II, D,I).

## DISCUSSION

**Morphometrics.** As mentioned above, the specimens of *Notropis* from Zacapu and Ojo de Agua de San Miguel showed diagnostic meristic and genetic characters differentiating them from their

close relatives in the Ameca River (*N. amecae*), Mezquital River (*N. aulidion*), Pánuco River (*N. calabazas*) and Lerma and Santiago river drainages (*N. calientis*). Although only two juvenile specimens of *N. amecae* were included in our genetic analyses and no specimens of *N. aulidion* and *N. calabazas* were collected, we obtained some comparative meristic characters from the literature (Table 6). Another factor supporting our findings is the biogeographic scenario, since the rivers inhabited by *N. aulidion*, *N. calabazas* and *N. amecae* (Mezquital, Pánuco and Ameca, respectively) constitute a different drainage system and a different biogeographic region from the current habitat of *N. calientis*, *N. grandis* sp. nov. and *N. marhabatiensis* sp. nov. in the Lerma River and Verde River of the Santiago River drainage (Domínguez-Domínguez *et al.*, 2006).

In an exploratory PCA of morphometric measurements, PCI was able to explain 92.46 % of the variance and eigenvectors showed close values with the same sign, suggesting the influence of standard length on the results (Bookstein *et al.*, 1985; Doadrio *et*

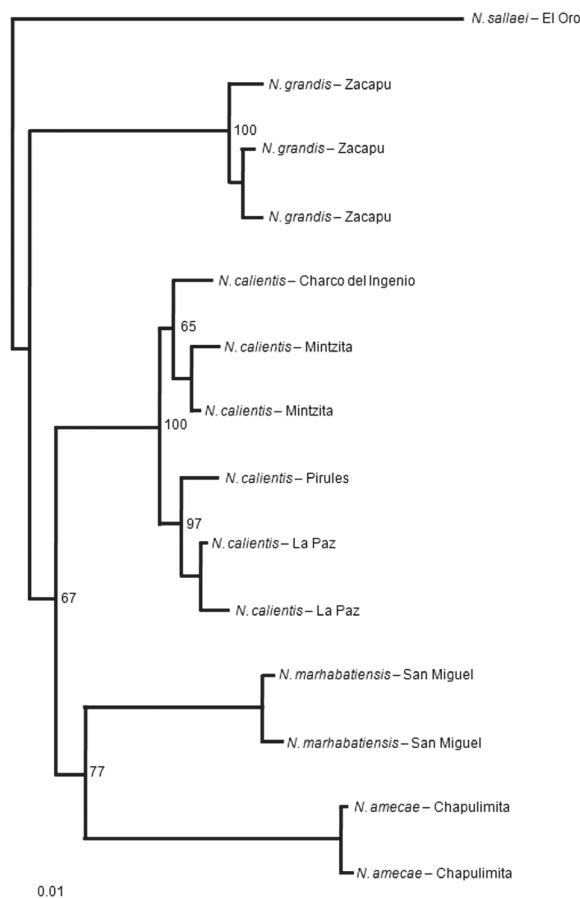


Figure 6.- Bayesian inference analysis of four species of the *Notropis calientis* complex. Numbers on branches indicate posterior probabilities.

*al.*, 2002). Accordingly, the original data matrix was size-corrected following Burnaby's method (Burnaby 1966; Rohlf & Bookstein 1987). Despite the differences found in the PCA and the use of the corrected matrix in subsequent analyses, one way ANOVA and Tukey analysis revealed the species *N. grandis* to be larger than the other two species examined (*N. marhabatiensis* and *N. calientis*). Moreover, according to the mean sizes given by Chernoff and Miller (1989) and Lyons & Mercado-Silva (2005), *N. grandis* also appears to be larger than *N. aulidion*, *N. amecae* and *N. calabazas*.

In a second PCA of morphological characters, PCI accounted for 41.26% of the variance and PCII for a cumulative variance of 53.02%. The characters that contributed most to this variance were PrOL and SbP for PCI and PFL and PVL for PCII (Table 6). Variance was more influenced by PCI and no well-defined groups emerged. Neither was there any evidence of diagnostic characters among the three species examined (*N. calientis*, *N. grandis* and *N. marhabatiensis*) (Fig. 5A). Although morphology has been largely used to describe fish species, several studies have shown that the morpho-

logic characters of cyprinids may not significantly differ between closely related species, and this seems to be a particular feature of the genus *Notropis* (Chernoff & Miller, 1986, Lyons & Mercado-Silva, 2005; Domínguez-Domínguez *et al.*, 2007). Moreover, it is widely recognized that phenotypic plasticity depends on habitat conditions and that allometry may affect morphometric characters (Hood & Heins, 2000; Trapani *et al.*, 2005). Thus, other sources of information are needed to identify all the species of the *N. calientis* complex.

The additional sources of information used here for species recognition were genetic and meristic characters. In our PCA analysis of meristic characters comparing *N. calientis* (two populations), *N. grandis* and *N. marhabatiensis*, PCI explained 45.59% of the variance, and PCII accounted for a cumulative variance of 72.95%. The characters that most contributed to the variance in PCI were transverse scales, gill rakers and branched anal fin rays, this component clearly separated the species *N. grandis* from *N. calientis* and *N. marhabatiensis*. The variables that contributed most to the variance in PCII were ventral fin rays (Table 8) and scales in a transverse series, with this component separating, although not too clearly, the species *N. calientis* and

Table 7. Eigenvectors and eigenvalues for the first three principal components obtained for 19 morphometric variables. Variable codes are given in the methods section.

Variable/eigenvectors	PCI	PCII	PCIII
SL	-0.0444	0.1258	-0.0158
HL	0.1092	-0.0445	-0.0808
PrOL	0.6234	-0.4412	-0.0208
ED	-0.0596	-0.0413	-0.2704
PoOL	-0.0177	0.1293	-0.0027
Hd	0.1003	0.0592	0.1335
SbP	0.2599	-0.0042	-0.0805
SbD	0.0487	0.2230	-0.0125
SbV	0.1330	0.0500	0.0697
SbA	0.0476	0.0896	0.1066
PFL	-0.3494	-0.3920	0.0203
DFL	-0.2211	-0.1163	-0.5659
VFL	-0.3332	-0.5050	0.5265
AFL	-0.2483	-0.3373	-0.3341
ACD	-0.2168	0.1625	-0.2581
DCD	-0.1573	0.1845	-0.0395
BD	-0.2036	0.2236	0.2368
BLD	-0.1153	0.1675	0.1332
ADD	-0.1534	0.1757	0.1659
Eigenvalues	0.0564	0.0160	0.0144
Percentage	41.26	11.76	10.55
Cumulative %	41.26	53.02	63.57

Table 8. Eigenvectors and eigenvalues for the first three principal components for 5 meristic variables. Variable codes are given in the methods section.

Variable/eigenvectors	CPI	CPII	CPIII
D	-0.158	0.575	-0.706
V	-0.150	-0.600	-0.419
A	0.490	-0.197	0.162
SCS	-0.577	0.035	0.208
ST	-0.271	-0.518	-0.315
GR	-0.553	0.042	0.396
Eigenvalues	2.73	1.64	0.676
Percentage	45.59	27.36	11.26
Cumulative %	45.59	72.95	84.21

*N. marhabatiensis* (Fig. 5). With respect to all other species within the *N. calientis* complex, the frequency tables (Table 6) indicate clear differences in *N. grandis* in terms of its lateral scales and gill rakers in the first arch, whereas *N. marhabatiensis* differed from other species in terms of its pelvic fin rays.

**Genetics.** Of the characters obtained for the *Notropis* cytochrome *b* sequences examined, 163 were variable, and 133 were parsimony informative. Third codon positions were the most informative (106 informative characters) followed by the first codon position (24 characters). Saturation of transition and transversion changes was checked by plotting the absolute number of changes of each codon position against patristic distances. There was no ingroup evidence of saturation at any of the three positions (not shown). The GTR+G model was selected as the model that best fitted the dataset. Rate matrix parameters were:  $-\ln L = 2622.7678$ ;  $K = 9$ ;  $AIC = 5263.5356$ . Base frequencies were:  $\text{freqA} = 0.2634$ ;  $\text{freqC} = 0.2805$ ;  $\text{freqG} = 0.1574$ ;  $\text{freqT} = 0.2987$ . The proportion of variable sites = 0 and gamma distribution shape parameter = 0.1655.

The phylogenetic hypothesis obtained through Bayesian analysis after burning 500 chains (Fig. 6) indicated four well-differentiated groups, each representing one of the species analyzed (*N. calientis*, *N. amecae*, *N. grandis* sp. nov. and *N. marhabatiensis* sp. nov.), although relationships among them were not resolved. This could be the outcome of the long genetic distances found among the species (Table 5) with an overall average  $\bar{D}_{\text{GTR}} = 5.5\%$ . Since most drainage and several populations within the Lerma-Santiago river system were surveyed, these high genetic differences could be the consequence of ancient isolation of the species in their respective water bodies, and a greater dispersal capacity of *N. calientis* inhabiting the Cuitzeo Lake and Lerma and Santiago river drainages.

The Zacapu Lake seems to have served as a diversification area for other species groups of aquatic fauna, as has been reported for the endemic goodeid *Allotoca zacapuensis*, the

amphibian *Ambystoma andersoni* (Krebs & Brandon, 1984) and the bivalve *Anodonta grandis* Say, 1829. However, divergences estimated for *N. grandis* were significantly greater ( $\bar{D}_{\text{GTR}} = 5.8$  to  $8.1\%$ ) compared to the other three *Notropis* species, than the divergences observed for *A. zacapuensis* ( $D_p = 2.5$  to  $2.6\%$ ) when compared to their closely related species (Aguilar-Medrano *et al.*, unpublished data), and even greater when compared to those shown by other goodeid population pairs living in Zacapu and contiguous regions;  $\bar{D}_{\text{GTR}} < 1\%$  for most species (Domínguez-Domínguez, *in press*). If we consider the molecular clock generally assumed for other cyprinids of  $1.05\%$  divergence per million years (Dowling *et al.*, 2002), the cladogenetic event for *N. grandis* would be dated around 6 Mya, during the upper Miocene (Messinian). This dating significantly exceeds that for the formation of Zacapu Lake, estimated to have occurred in the Upper Pliocene (Demant, 1992).

*Notropis marhabatiensis* sp. nov. is confined to a small spring in the upper Lerma River close to Maravatio city. The biogeographic differences between the upper Lerma and its lower reaches have been widely discussed (see Barbour, 1973; Domínguez-Domínguez *et al.*, 2006) and are supported by the species endemic to this region *Algansea barbata* Alvarez & Cortés, 1964, *Girardinichthys multiradiatus* and *N. sallaei*, the sister species of the *N. calientis* complex (Schönhuth and Doadrio, 2003). This region of the Upper Lerma was distinguished by Barbour (1973) as an ancient Maravatio basin, indicating the formation of an ancient lake in the region. Genetic distances between *N. marhabatiensis* and the other species of the *N. calientis* complex were  $\bar{D}_{\text{GTR}} = 6.1$  to  $7.4\%$ , and its divergence with respect to *N. sallaei* was in the range  $D_p = 8.5$  to  $10\%$  (Schönhuth and Doadrio, 2003). This information suggests that different events of isolation between and within the upper Lerma and the rest of the Lerma River occurred over 5 Mya. The former would have led to the formation of the *N. sallaei* species, possibly isolated in the ancient Aztatlan Paleolake ca. 10 Mya, and the latter related to the isolation and formation of *N. marhabatiensis* in the ancient Maravatio basin (Barbour, 1973) ca. 7 Mya.

Interestingly, the other two native species found in San Miguel Spring (*Goodea atripinnis* and *Girardinichthys multiradiatus*) displayed a smaller genetic distance,  $\bar{D}_{\text{GTR}} < 1.5\%$ , with respect to other populations inhabiting contiguous basins (Domínguez-Domínguez *et al.*, *in press*). This information, and the fact that *N. marhabatiensis* has only been identified at its type locality, would appear to indicate that the dispersal capacity or capacity to invade new areas of this species is lower than the capacity shown by its sympatric species.

Based on their endemism and on the threatened nature of the reduced water bodies inhabited by *N. grandis* and *N. marhabatiensis*, we recommend the two species are included as endangered in the Norma Oficial Mexicana (Mexican red list).

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