Description of *Gambusia zarskei* sp. n. – a new poeciliid fish from the upper Rio Conchos system, Chihuahua, Mexico (Teleostei: Cyprinodontiformes: Poeciliidae)

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> Abstract

Gambusia zarskei, new species, is described from the upper drainage of Rio Conchos, Rio Grande system, Chihuahua, Mexico. It is clearly distinguished by metrics, gonopodial and other morphological characteristics from all other species of the genus. A mitochondrial DNA-sequence based molecular phylogenetic analysis revealed similar results on the status of the new species as a separate taxon and its relation to other closely related species.

> Zusammenfassung

Gambusia zarskei sp. n. aus dem oberen Einzugsbereich des Rio Conchos, Rio-Grande-System, Chihuahua, Mexico wird beschrieben. Sie unterscheidet sich deutlich durch ihre morphometrischen Merkmale, Besonderheiten des Gonopodiums und andere morphologische Charakteristika von den anderen Arten der Gattung. Eine auf mitochondrialer DNA-Sequenz basierende phylogenetische Analyse des betreffenden Taxons führte ebenfalls zum Status einer neuen Art. Die verwandtschaftlichen Verhältnisse von *Gambusia zarskei* sp. n. zu den am nächsten verwandten Arten werden dargestellt und diskutiert.

> Key words

Teleostei, Cyprinodontiformes, Poeciliidae, *Gambusia*, taxonomy, new species, Mexico.

Introduction

Since the systematic listing of the representatives of the Gambusiini by Rosen & Bailey (1963), numerous new descriptions and changes in nomenclature have occurred within the tribus, particularly in the case of the *Gambusia*. Rauchenberger (1989) designates a total of three subgenera for *Gambusia*, namely *Arthrophallus*, *Heterophallina* and *Gambusia*. The subgenera *Arthrophallus*, according to Rauchenberger (1989), includes three groups, namely the *affinis* species group, *nobilis* species group and *senilis* species group, whereby the here described new species is clearly a member of the later species group, together with *alvarezi* Hubbs & Springer, 1957, *amistadensis* Peden, 1973, *atrora* Rosen & Bailey, 1963, *gaigei* Hubbs, 1929, *geiseri* Hubbs & Hubbs, 1957, *hurtadoi* Hubbs & Springer, 1957, *longispinis* Minckley, 1962 and *senilis* Girard, 1859. It should be mentioned that due to cytochrome b sequence variations, Lydeard *et al*. (1995) developed differently weighted molecular-phylogenetic pedigrees from some Gambusiini groups. In contrast to Rauchenberger (1989), Lydeard et al. (1995) classify *hurtadoi* as an independent complex together with *vittata*, *marshi* and *panuco*, whereas the later three taxa are included in the subgenus *Heterophallina* and *hurtadoi* in the subgenus *Arthrophallus* by Rauchenberger (1989). Furthermore *geiseri* is not associated by Lydeard *et al*. (1995) with any member of the *senilis* species group, but with the *affinis* species group. In the light of this conflicting classification independent studies with additional material on the systematics of the genus *Gambusia* appear to be timely and necessary.

The genus *Gambusia* is distributed widely over continental and coastal habitats, from eastern North

America to northwestern South America, including the Antilles. The new species is found at an altitude of 1135 m and is thereby one of several endemic northern *Gambusia* species which live in thermal springs in the uplands of northern Mexico and southern USA. The present paper describes a new species of *Gambusia* from the headwaters of the Rio Conchos, Chihuahua, Mexico.

Materials and methods

All material of *Gambusia* used for the morphological comparison stemmed from the private fish collection of MANFRED MEYER, Bad Nauheim. *Gambusia alvarezi*, El Ojo de San Gregorio, Rio Parral drainage, Rio Conchos system, Chihuahua, Mexico; *Gambusia gaigei*, Graham Ranch Warm Spring near Boquillas Canyon in Brewster County, Big Bend National Park, Rio Grande drainage, Texas, USA (reared from Graham Ranch stocks in the laboratory in Austin, Texas); *Gambusia hurtadoi*, El Ojo de la Hacienda Dolores and Balneario Villa López at Villa López, Rio Florido drainage, Rio Conchos system, Chihuahua, Mexico; *Gambusia senilis*, small creek, Rio San Pedro drainage near Meoqui, Rio Conchos system, Chihuahua, Mexico.

For the molecular phylogenetic analyses ethanol fixed syntypes of the new *Gambusia* species and *G. hurtadoi*, Balneario Villa López at Villa López, Rio Florido drainage, Rio Conchos system, Chihuahua, Mexico were used. For comparison sequences from *G. vittata* (acc.# EF017518), *G. affinis* (acc.# AP004422), *G. wrayi* (acc.# EF017516), *G. atrora* (acc.# EF017515) and *Belonesox belizanus* (acc.# EF017519) available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) were taken.

Measurements were made by vernier calipers, reading to 0.1 mm. Measurements and counts follow standard practice (MILLER, 1948). The length of distal tip of gonopodium ray 4a and 3 is measured on a horizontal line from the distal tip of ray 4p to distal tip of gonopodium hook. The determination of relative gonopodial serra length (SEL/SEW) follows the method of PEDEN (1973). The number of specimens for all counts is greater or equal to 5. The total gill-raker count of the first gill arch includes all gill rakers in the angle of the gill arch. The last two rays in the dorsal fin are counted as a single ray. Vertebral counts include the hypural plate as one vertebra. The nomenclature of the sensory canal system of the head follows the standard of GOSLINE (1949) and components of the gonopodial system follow ROSEN & GORDON (1953), ROSEN & KALLMAN (1959) and ROSEN & BAILEY (1963). Genomic DNA was isolated from dorsal fin clips according to ALTSCHMIED *et al*. (1997). Mitochondrial sequences were amplified by PCR with primers H16100 (5'ATGTAGGGTTACAYTACTTTAAATGG3') and L15513 (5'CTRGGAGACCCNGAAAACTT3'). The PCR was done under the following conditions: denaturation 95 °C for 120 sec, 35 cycles of denaturation 95 °C for 30 sec annealing 49 °C for 30 sec, extension 72 °C for 40 sec, followed by a final extension step at 72 °C for 120 sec. PCR cycles were run from less than 100 ng genomic DNA. In each case a single PCR product was obtained and sequenced directly. Nucleotide sequences were analysed using programs of the MEGA4.0 program package (TAMURA *et al*., 2007). Multiple sequence alignments were generated and phylogenetic trees were constructed using maximum parsimony, minimum evolution and neighbour-joining methods. Robustness of the trees was tested by bootstrap analyses using 100 replicas or in the case of neighbour-joining trees 1000 replicas. Pair-wise distances were calculated with the program M4 as part of MEGA4.0 conducted on the basis of the Maximum Composite Likelihood method using all distance correction methods implemented in the program. All DNA sequences generated in this study are deposited in GenBank under accession numbers GU 583794, GU 593795.

Abbreviations

APL	distance anus to pectoral fin
BD	depth of body
CPL	length of caudal peduncle
CPD	depth of caudal peduncle
ED	diameter of eye
F	female
GL	length of gonopodium
HL	length of head
HT	holotype
IOW	interorbital width
j	juvenil
M	male
MTD F	fish collection of the Museum für Tierkunde, Dresden, Germany
PDL	predorsal length
PL	length of pectoral fin
PT	paratype
SEL	serra length of gonopdium
SEW	serra width of gonopodium
SMF	fish collection of the Forschungsinstitut und Museum Senckenberg, Frankfurt/Main, Germany
SNL	length of snout
SL	standard length
TL	total length
VL	length of ventral fin.

Fig. 1. *Gambusia zarskei*; Mexico: Balneario San Diego de Alcalá, holotype, MTD F 31802, male, 23.1 mm SL.

Fig. 2. *Gambusia zarskei*; Mexico: Balneario San Diego de Alcalá, paratype, MTD F 31803, female, 28.6 mm SL.

Gambusia zarskei, new species

Figs. 1–5

Holotype. Male (MTD F 31 802), 23.1 mm SL; outlet of Balneario San Diego de Alcalá, near San Diego de Alcalá, Rio Chuviscar drainage, Rio Conchos system, Chihuahua, Mexico, K. Schneider & G. Teichmann *leg*., March 18th, 2006.
Paratypes. 2 females (MTD F 31803, 31924); same data as holotype. 3 males, 6 juveniles (SMF 32173), outlet of Tres Cabañas, near San Diego de Alcalá, Rio Chuviscar drainage, Rio Conchos system, Chihuahua, Mexico, K. Schneider & G. Teichmann *leg*., March 18th, 2006.

Subgenus *Arthrophallus* Hubbs, 1926

The subgenus *Arthrophallus* is characterized by the derived character (S1) sections 4b to 6a and 6b to 7, disconnected infraorbital sensory canals Rauchenberger (1989).

The following species are included: *affinis* species group with *affinis* (Baird & Girard, 1854), *aurata* Miller & Minckley, 1970, *holbrookii* Girard, 1859, *lemaitrei* Fowler, 1950 and *speciosa* Girard, 1959; *nobilis* species group with *clarkhubbsi* Garrett & Edwards, 2003, *georgei* Hubbs & Peden, 1969, *heterochir* Hubbs, 1957, *krumholzi* Minckley, 1962 and *nobilis* (Baird & Girard, 1854); *senilis* species group with *alvarezi*, *amistadensis*, *gaigei*, *hurtadoi* and *senilis*; *sexradiata* species group with *eurystoma* Miller, 1975 and *sexradiata* Hubbs, 1936; following taxa are not included in any species group: *atrora, geiseri* and *longispinis*.

Diagnosis

Gambusia zarskei is a small sized species of the subgenus *Arthrophallus* (SL in males usually not exceeding 25 mm and in females 35 mm), which is distinguished from all other species of the *senilis* species group by the following characters: shafts of distal spines of gonopodium ray 3 much prolonged, lateral projection on elbow weekly developed, vs. well developed in

Table 1. Measurements (in mm) of holotype and paratypes of *Gambusia zarskei* sp. n.

	TL	SL	HL	SNL	BD	IOW	GL	ED	APL	CPL	CPD	PL	VL	PDL
01.HT(M)	28.80	23.10	5.80	1.80	6.20	2.30	7.50	2.00		6.20	4.20	4.80	1.80	13.90
02.PT(F)	34.90	28.60	7.80	2.00	8.40	3.60		2.20	10.10	6.80	4.90	5.70	2.70	19.30
03.PT(F)	34.30	28.30	7.70	1.90	8.80	3.50		2.10	9.80	6.70	4.80	5.50	2.50	18.80
04.PT(M)	22.40	17.80	4.80	1.50	4.50	1.80	6.10	1.80		4.90	3.30	3.70	1.40	11.10
05.PT(M)	21.20	16.90	4.70	1.40	4.40	1.70	5.90	1.70		4.70	3.20	3.50	1.40	10.90
06.PT(M)	18.10	14.40	4.10	1.30	4.00	1.50		1.60		4.20	3.00	3.30	1.30	10.40
07.PT(J)	19.10	15.20	4.30	1.30	4.20	1.60		1.60		4.40	3.10	3.40	1.30	10.60
08.PT(J)	18.40	14.60	4.20	2.10	4.10	1.50		1.50		4.20	2.90	3.30		10.30
09.PT(J)	16.40	13.10												
10.PT(J)	17.20	13.60												
11.PT(J)	16.60	13.20												
12.PT(J)	16.30	12.90												

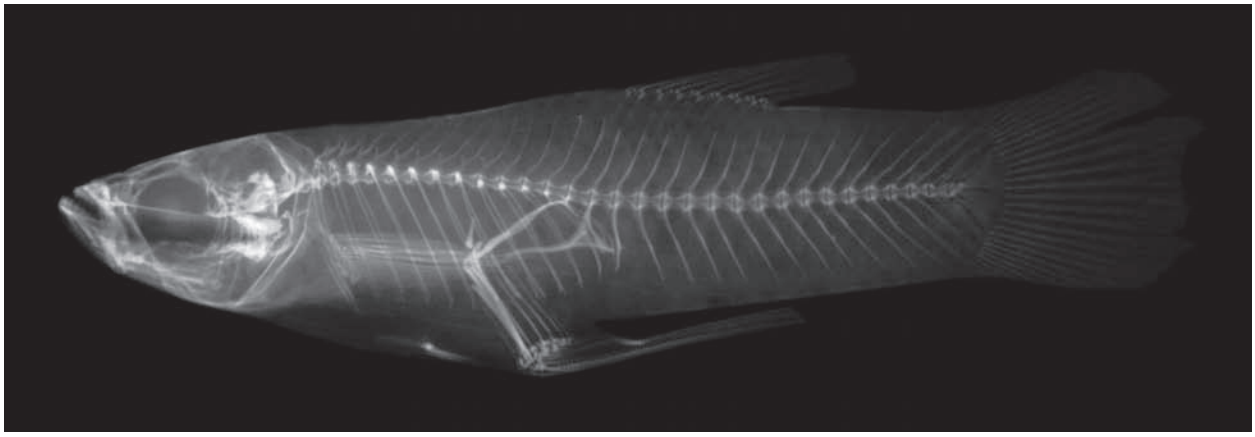

Fig. 3. X-ray picture of the holotype of *Gambusia zarskei*; Mexico: Balneario San Diego de Alcalá.

G. amistadensis and *G. hurtadoi* or absent in *G. alvarezi*, *G. gaigei* and *G. senilis*.

G. zarskei is also distinguished by the following unique combination of characters: relative gonopodial ray 4p serra length of gonopodium long; frequency distribution of SEL/SEW radius 1.35–1.65, vs. 0.95–1.25 in *G. alvarezi*, 1.55–1.90 in *G. amistadensis*, 1.00–1.35 in *G. gaigei* and 0.75–1.05 in *G. hurtadoi*; elbow on gonopodium ray 4a simple and not rounded or distally hooked, vs. usually hooked in *G. alvarezi*, *G. gaigei*, *G. senilis*; 8 dorsal fin rays, vs. usually 9 in *G. alvarezi* and *G. hurtadoi*; black anal spot present vs. absent in *G. senilis*.

Description

Body deep, head pointed, 25.1–27.3 % of SL. Longitudinal scale series 28–30 (rarely 30); predorsal scale series 16–17; scale series around caudal peduncle 14–16. Number of vertebrae 30 to 31. Gill rakers on first arch 9 to 10.

Teeth of upper and lower jaws unicuspid and slightly recurved; those of outer row enlarged and spear-like shaped, not very numerous and widely spaced; spear-like shaped inner teeth small and not very numerous. Upper pharyngeal bones kidney-shaped. Teeth of the medial region somewhat enlarged. Lower pharyngeal bone (ceratobranchial 5) with a total of 80–100 unicuspid teeth, 9–11 on posterior rows, 5–7 on middle rows. Teeth of posterior region very large. Both halves of lower pharyngeal antler-shaped, closely together on a small part along midline. Arms of pharyngeal very long and split at the ends. Ceratobranchial 4 with teeth.

The cephalic sensory canals system consists of open canals and neuromasts. Supraorbital canal well developed, sections 1+2a, 2b–4a, 4b–6a, 6b–7 represented as open grooves; preopercular 7 neuromasts; preorbital 7 neuromasts; mandibular with 4 neuromasts.

Gonopodium short and compact, 2.9 to 3.2 times in SL; ray 3 broadly expanded, terminating in a well

Fig. 4. *Gambusia zarskei*; Mexico: Balneario San Diego de Alcalá , male.

Fig. 5. *Gambusia zarskei*; Mexico: Balneario San Diego de Alcalá, female.

developed spine series; distal spines of ray 3 very long, angular and projected beyond rays 4 to 5, 6 to 7 angulate spines long and pointed anterodistally, base of ray 3 somewhat tapered; ray 4a very short reaching to the middle of the large rounded terminal hook on ray 5a, ray 4a with an anterior swelling (elbow), elbow segments usually not fused, major elbow segment straight and not very long; ray 4p longer than 4a and terminating with a large rounded hook, 5 to 8 retrorse serrae, the lengths much longer than the widths of their bases; ray 5a shorter than ray 4p and longer than ray 4a. Ray 6 and 7 straight, ray 6 thickened distally.

Gonopodial suspensorium (fig. 3) with two well developed slender gonapophyses, gonapophysis I without uncini, gonapophysis II with uncini. Large ligastyle present. Gonactinost 1 without inferior wing-like appendage, gonactinostal complex 2 to 4 in front with a superior lateral wing, gonactinosts 5 to 9 without bony plates or outgrowths.

Dorsal fin with 8, rarely 9 rays (first 2 rays simple, all others branched), origin of dorsal fin posterior to the insertion of anal fin; caudal fin with 31 to 33 (14–16 branched) rays; anal fin 9 rays (first 3 rays simple, all others branched); pectoral fin with 14 to 15 rays (top 2 and bottom 3 rays simple, all others branched);

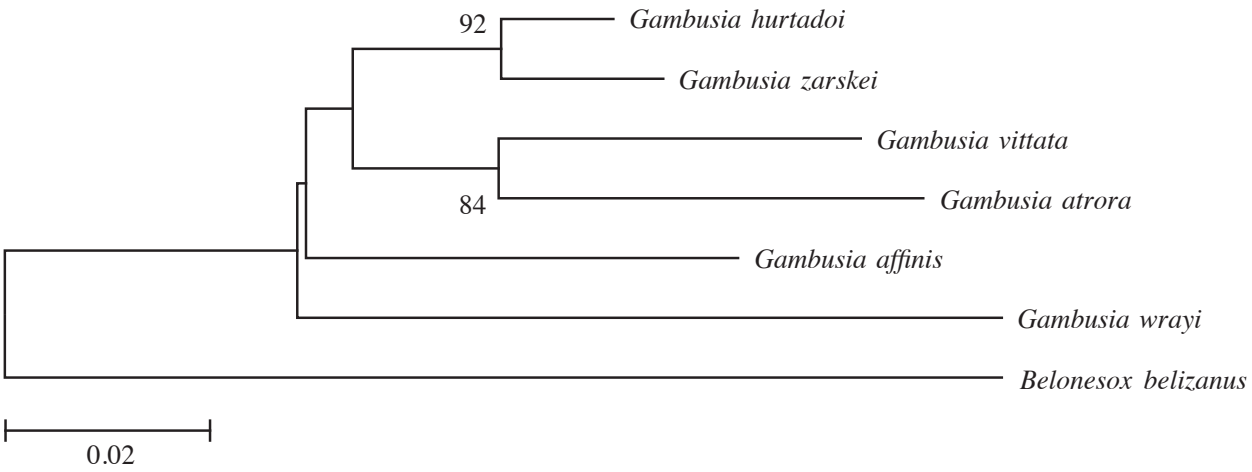

Fig. 6. Phylogram of *Gambusia zarskei* and related *Gambusia* species based on mitochondrial DNA sequences. 50 % majority rule consensus tree rooted on *Belonexos belizanus* as outgroup. Average bootstrap values obtained using different types of analysis (see Material and Methods) are indicated above the branches. Bootstrap values below 50 % are not shown.

ventral fin with 6 rays (first and last ray simple, all others branched), in females not reaching to the urogenital papilla and in males reaching to the base of the gonopodium, first ray thin and bent distally. Adult males with not very prominent keel, starting at the edge of caudal peduncle and ending near base of gonopodium. Males and females without sex specific coloration (Figs. 4–5). Body color of adult females and males greyish blue; lower posterior body sides intensely orange-yellow; iris black; short subocular dark bar leading posteroventrally across cheek from lower border of the eye. Border of dorsal fin black and subbasal row of a dark spot series present on lower third of dorsal fin, more intense in males. All fins hyaline and often light yellowish. Adult females with a black anal spot, the melanophores being mainly concentrated between the anus and urogenital papilla.

Etymology: We name this species after Dr. AXEL ZARSKE in recognition of his valuable contributions to discussions on the conservation biology and problems of endangered fishes such as *G. zarskei*.

Comparison and relationships: RAUCHENBERGER (1989) gives the following derived characters for the *senilis* species subgroup: medial anal spot in females and transversely enlarged segment distal to serrae on ray 4p. A third derived character for the *senilis* species group is the reduction of gonopodium ray 4a segments distal from the elbow. All these characters are present in *G. zarskei*. On the basis of these synapomorphies, *G. zarskei* is herein unequivocally attached to the *senilis* species group. The species *G. geiseri*, *G. longispinis* and *G. atrora* are not referred herein to the *senilis* species group because no synapomorphies could be found that unite them with *G. alvarezi*, *G. amistadensis*, *G. gaigei*, *G. hurtadoi*, *G. senilis* or *G. zarskei*.

By morphological criteria *G. zarskei* is most closely related to *G. amistadensis* and *G. hurtadoi*. There are several synapomorphies that unite *G. amistadensis*, *G. hurtadoi* with *G. zarskei,* namely: shafts of distal spines of gonopodium ray 3 much prolonged, lateral projection on elbow developed. However, *G. zarskei* is recognized as a separate species, because it does not share the following synapomorphies between *G. hurtadoi*: distal tip of gonopodium long. On the other hand there is one autapomorphy of *G. zarskei*: terminal spines of gonopodium ray 3 prolonged. *G. zarskei* is further distinguished from *G. hurtadoi, G. alvarezi* and *G. senilis* by fewer spines of gonopodium ray 3 (6–7 vs. 7–8), from *G. senilis* by the absence of an anal spot in females and a much shorter main segment of the gonopodium elbow, and from *G. alvarezi* and *G. hurtadoi* by fewer dorsal fin rays (8 vs. 9) and fewer anal fin rays (9 vs. 10).

A molecular phylogenetic analysis was performed using mitochondrial sequences (fig. 6, tab. 2). The resulting data set consists of 343 bases from the 3'part of the cytochrome b gene for each species. The genetic distance calculations revealed that there is an obvious closest genetic relationship of *G. zarskei* with *G. hurtadoi*.

The molecular data were analyzed with maximum parsimony, minimal evolution and neighbour-joining methods, which yielded almost identical phylogenetic results. The topology of the resulting trees was always the same. Two major branches supported by high bootstrap values were resolved, which unite *G. zarskei* with *G. hurtadoi* in one group and *G. vittata* with *G. atrora* in the other. The new species is clearly separated from *G. hurtadoi* similar to the split between *G. vittata* and *G. atrora*. Although the region that was analyzed is relatively short the phylogenetic information is robust and the separation of the species in percent changes is

Tab. 2. Pairwise distance matrix of *Gambusia* species and *Belonesox belizanus* mitochondrial sequences using the Maximum Composite Likelihood method.

	G. hurtadoi	*G. zarskei*	*G. vittata*	*G. affinis*	*G. wrayi*	*G. atrora*	*B. belizanus*
G. hurtadoi		0.027	0.081	0.064	0.102	0.084	0.155
G. zarskei			0.077	0.081	0.109	0.081	0.162
G. vittata				0.093	0.119	0.077	0.188
G. affinis					0.112	0.111	0.162
G. wrayi						0.130	0.195
G. atrora							0.182
B. belizanus							

Fig. 7. Habitat: Outlet of Balneario San Diego de Alcalá, type locality of *G. zarskei*.

well in the range of what has been reported for species of the genera *Xiphophorus* (Meyer & Schartl, 2002, Meyer & Schartl, 2003), *Poecilia* (Meyer, Schneider, Radda, Wilde & Schartl, 2004) and *Priapella* (Schartl, Meyer & Wilde, 2006).

Unexpectedly, the sequence of the 5' part of the cytochrome b gene of *G. zarskei* could not be used for the phylogenetic analysis, although this part of the mitochondrial genome has proven to be very useful for analysing phylogenetic relationships on the species level in all our previous studies of poeciliid fishes (Meyer & Schartl, 2002; Meyer & Schartl, 2003; Meyer, Schneider, Radda, Wilde & Schartl, 2004; Schartl, Meyer & Wilde, 2006). In this region the cytochrome b sequence of *G. zarskei* has several deletions and aminoacid changes at highly conserved positions (data not shown), which would result in a significantly altered protein whose functionality would have to be questioned. Given the conservation of the primary sequence of b-type cytochromes this is a puzzling result that cannot be explained without further studies. At least, this highly aberrant cytochrome b sequence is another molecular discriminator for the new species, as all other *Gambusia* species analyzed so far have the expected aminoacid conservation of cytchrome b.

Distribution. *Gambusia zarskei* is only known from the upper Rio Conchos system in the Sierra San Diego area, east of San Diego de Alcalá, Chihuahua, Mexico.

Habitat notes. *Gambusia zarskei* inhabits the waters of small warm springs and their outlets at the foot of Sierra San Diego on the northwestern mountain side. The collection sites are small outlets, approx. 0.5 to 2.0 m wide and 0.60 to 1.00 m deep, that flow into the Rio Chuviscar drainage, a tributary of the Rio Con-

chos, 2 km east of San Diego de Alcalá on an unpaved road. At the type locality (N 28° 35' 36.2", W 105° 33' 00.0") on March, 18th, 2006 before noon the water had a temperature of 24 to 26 °C, a conductivity of 1486 mS, pH 7.85, and a total hardness of 9. The water was crystal clear, fast flowing and without aquatic vegetation. The substratum consisted mainly of gravel and sand. The brook flows in an open area. Accompanying fishes were *Gambusia senilis* and *Cyprinodon* spec. *Gambusia zarskei* was mainly found in water depth of 0.05 to 0.10 m along the water edges, where the water temperature might rises to 34 °C near the hot spring.

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