CYPRINODON ESCONDITUS, A NEW PUPFISH FROM LAGUNA CHICHANCANAB, YUCATAN, MEXICO (CYPRINODONTIDAE)

by

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ABSTRACT. - *Cyprinodon esconditus*, new species, belongs to the *Cyprinodon* species flock from Laguna Chichancanab, Yucatán, Mexico. The flock now consists of six species, which are hypothesized to have evolved sympatrically in the lake during the last 8,000 years. *Cyprinodon esconditus* is similar in meristic counts and overall shape and coloration to the other members of the flock. It differs in having a smaller eye, and shorter predorsal and prepelvic lengths. Also, the number of premaxillary teeth differs from that in the other members of the species flock: they are more numerous than in *C. beltrani*, *C. maya*, *C. verecundus* and *C. labiosus* but fewer than in *C. simus*.

RÉSUMÉ. - *Cyprinodon esconditus*, une nouvelle espèce de Cyprinodontidae de la lagune Chichancanab, Yucatan, Mexique.

Cyprinodon esconditus est une espèce nouvelle qui fait partie de l´ensemble *Cyprinodon* de la Laguna Chichancanab, Yucatan, Mexique. L'ensemble se compose de six espèces. On suppose que ces espèces ont évolué en sympatrie depuis 8,000 ans. *Cyprinodon esconditus* ressemble aux autres espèces de cet ensemble pour les caractères méristiques, la morphologie générale et la coloration. Les yeux sont plus petits et les longueurs prédorsales et prépelviennes plus courtes. Le nombre de dents sur le prémaxillaire diffère de celui des autres espèces : il est plus grand que chez *C. beltrani*, *C. maya*, *C. verecundus* et *C. labiosus* mais plus petit que chez *C. simus*.

Key words. - Cyprinodontidae - *Cyprinodon esconditus* - Mexico - Laguna Chinchancanab - Species flock - Sympatric speciation - New species.

An endemic species flock of *Cyprinodon*, which is hypothesized to have evolved by sympatric speciation (Strecker and Kodric-Brown, 1999, 2000) lives in Laguna Chichancanab, in the Yucatan peninsula, Mexico. The flock is composed of five species described from differences in morphological characters, especially in the structure of their viscerocranium (Humphries and Miller, 1981; Humphries, 1984a). These differences might suggest trophic divergence and exploitation of different feeding niches. The most abundant species, *C. beltrani* Alvarez, 1949, is a substrate feeder with a diet consisting primarily of detritus and algae. It is morphologically and ecologically similar to the coastal-dwelling Yucatan pupfish, *C. artifrons* Hubbs, 1936, the sister species of the Laguna Chichancanab pupfishes. Therefore, *C. beltrani* is assumed to be the basal member of the flock (Strecker and Kodric-Brown, 1999, 2000). The other four species, *C. maya* Humphries & Miller, 1981, *C. labiosus* Humphries & Miller, 1981, *C. verecundus* Humphries, 1984, and *C. simus* Humphries & Miller, 1981, have a significantly shorter gut indicating a carnivorous diet. This has been confirmed by gut content analysis (Stevenson, 1992; Strecker, 1993; Horstkotte, pers. com.).

Despite morphological differences among the five species, genetic divergence in allozymes (Humphries, 1984b) and mitochondrial DNA (mtDNA) sequences of the D-loop (Strecker, 1996; Strecker *et al*., 1996) is minimal or absent. From the mtDNA data a phylogenetic age of about 8,000 years was calculated (Strecker, 1996; Strecker *et al*., 1996). Geological data have shown that the lake was completely or nearly dry 8,000 years ago (Covich and Stuiver, 1974; Hodell *et al*., 1995). Thus it is hypothesized that the flock has evolved after this event (Strecker, 1996; Strecker *et al*., 1996). Whereas the samples of four of the five species could not be discriminated on the basis of mtDNA sequences, the sample of *C. maya* was fixed for a unique haplotype (Strecker, 1996; Strecker *et al*., 1996) found in all specimens examined. Thus only for *C. maya* is reproductive isolation assumed (Strecker, 1996; Strecker *et al*., 1996). In the laboratory all species are interfertile (Strecker, 1996). Mate choice experiments in *C. beltrani*, *C. labiosus* and *C. maya* indicate that the level of reproductive isolation varies. It is complete for *C. maya*, absent for *C. beltrani* and intermediate for *C. labiosus* (Strecker, 1996; Strecker and Kodric-Brown, 1999, 2000).

In addition to the five species described from Laguna Chichancanab the flock contains other specimens, which cannot be assigned unequivocally to one of the above species (Humphries and Miller, 1981; Strecker, 1996). Possible explanations include the existence of natural hybrids, morphological plasticity and/or unidentified species (Strecker *et*

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al., 1996). Here, I describe a new species that was discovered among such specimens.

MATERIALS AND METHODS

Morphology

The material examined is deposited in the Ichthyological Collections of the Zoological Institute and Zoological Museum of the University of Hamburg (ZMH), Instituto de Biología, Universidad Autonoma de Mexico (IBUNAM), Collección de Peces, El Colegio de la Frontera Sur, Unidad Chetumal (ECO-CH) and Division of Fishes, Museum of Zoology University of Michigan (UMMZ).

Counts and measurements (in thousands of standard length (SL)) follow Miller (1948) and Humphries and Miller (1981).

For a multivariate approach a canonical discriminant function (= discriminant coordinates) was performed (Seber, 1984) using SPSS (version 10.0). This analysis included 8 males and 8 females from each of three species that are most similar to the new species: *Cyprinodon beltrani*, *C. labiosus* and *C. verecundus*.

As comparative material the following specimens caught in Laguna Chichancanab, Yucatan, Mexico, were used. (For sampling site abbreviations (B-C) see figure 1. All specimens were collected by U. Strecker and H. Wilkens):

Cyprinodon beltrani. - ZMH 23178, 8 females 27.0 mm, 28.5 mm, 28.9 mm, 29.4 mm, 29.6 mm, 30.0 mm, 30.5 mm, 31.7 mm and 8 males 28.9 mm, 28.9 mm, 29.9 mm, 29.9 mm, 30.6 mm, 30.8 mm, 31.0 mm, 33.5 mm, (C), 17 Apr. 1997.

Cyprinodon labiosus. - ZMH 22929, 3 females 28.7 mm, 29.2 mm, 36.5 mm, and 4 males 31.9 mm, 32.6 mm, 33.7 mm, 34.7 mm, (C), 21 Dec. 1992. ZMH 23120, 5 females 28.4 mm, 30.1 mm, 30.6 mm, 30.7 mm, 32.2 mm and 4 males 26.6 mm, 28.6 mm, 29.8 mm, 32.4 mm, (C), 22 Jan. 1996.

Cyprinodon verecundus. - ZMH 22935, 3 females 27.8 mm, 27.9 mm, 31.3 mm, and 2 males 26.8 mm, 27.2 mm, (C), 21. Dec. 1992. ZMH 22961, 1 female 29.6 mm and 2 males 27.7 mm, 29.2 mm, (C), 11. Jan. 1993. ZMH 23868, 4 females 25.5 mm, 27.9 mm, 30.8 mm, 31.3 mm and 4 males 25.6 mm, 27.4 mm, 29.8 mm, 30.4 mm, (B), Jan. 1993.

Premaxillary teeth were counted in 10 cleared and stained specimens; values indicate left and right side for the upper and lower jaw. In one specimen scanning electron micrographs of the pharyngeal teeth were taken. For counts not included in tables the values for the holotype are indicated in bold type.

Collection sites are shown in figure 1. The description of body color was made on freshly preserved and aquarium-kept specimens.

Genetics

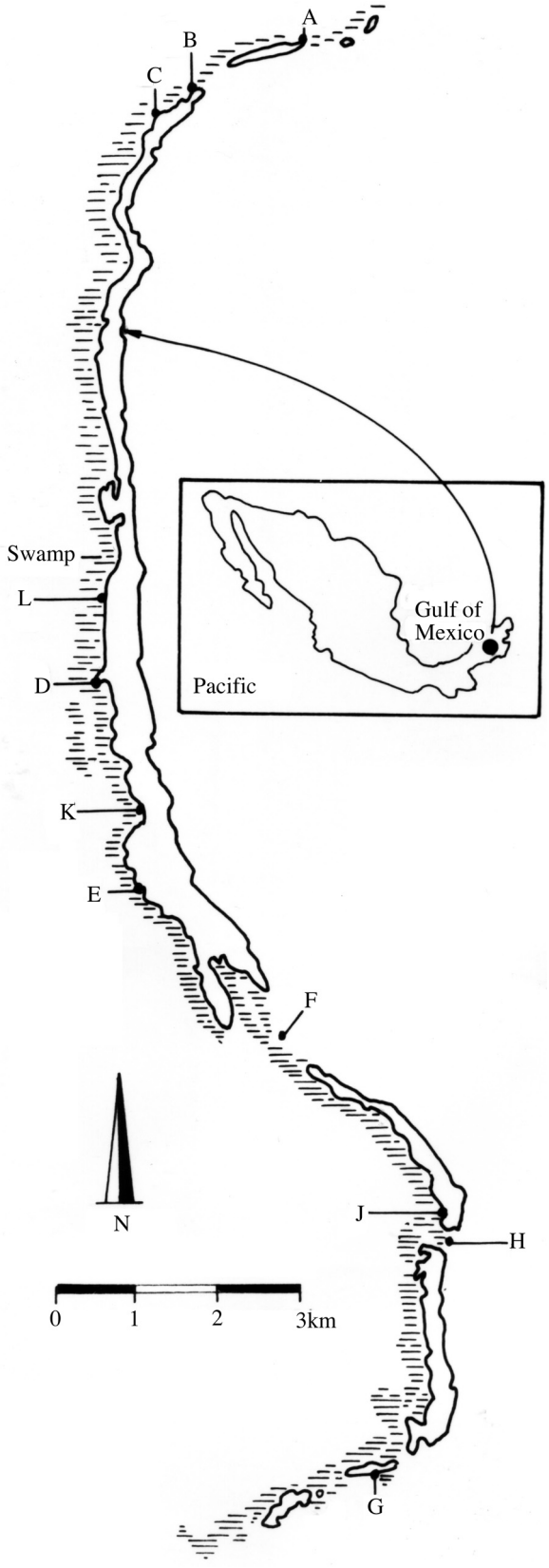
A 420-463 bp mtDNA segment comprising part of the Threonine tRNA gene, the Proline tRNA gene, and part of the control region was sequenced in 10 individuals of the new species. DNA extraction and PCR conditions were done as described in Strecker (1996) and Strecker *et al*. (1996). The PCR products were electrophoresed and the target DNA was recovered with magnetic beads (Boehringer Mannheimer) according to the manufacturer´s directions. Sequencing was performed with an ABI 3.1 automated sequencer. The sequences are available from GenBank under accession no. AY004308 and AY004309. Voucher specimens (ZMH 23966-23975) were cleared and stained for tooth counts.

CYPRINODON ESCONDITUS N. SP.

Material examined

Holotype. - IBUNAM-9488, adult male, 32.2 mm SL; Yucatan Peninsula, Quintana Roo, Mexico; southern end of Laguna Chichancanab at its broadest part, 19°51'10"N 88°45'58"W, U. Strecker and H. Wilkens, 6 Mar. 1999.

Paratypes. - Laguna Chichancanab, Yucatan, Mexico, for sampling site abbreviations (A-L) see figure 1. If not indicated otherwise all specimens were collected by U. Strecker and H. Wilkens. ZMH 23905, female, 30.4 mm, (A), 20 Dec. 1992; ZMH 23906, female, 28.1 mm, (C), 11 Jan. 1993; ZMH 23907, male, 29.3 mm, (C), 7 Mar. 1999; ZMH 23908, female, 30.2 mm, (C), 22 Jan. 1996; ZMH 23909, female, 31.4 mm, (C), 7 Mar. 1999; ZMH 23910, male, 32.3 mm, (C), 7 Mar. 1999; ZMH 23911, female, 31.0 mm, (C), 7 Mar. 1999; ZMH 23912, female, 35.0 mm, (C), Jul. 1991; ZMH 23913, male, 31.3 mm, (C), 7 Mar. 1999; ZMH 23914, male, 28.2 mm, (C), 7 Mar. 1999; ZMH 23915, male, 38.7 mm, (C), 7 Mar. 1999; ZMH 23916, male, 29.6 mm, (C), 7 Mar. 1999; ZMH 23917, male, 31.5 mm, (C), 7 Mar. 1999; ZMH 23918, male, 29.3 mm, (C), 7 Mar. 1999; ZMH 23919, female, 29.6 mm, (C), 7 Mar. 1999; ZMH 23920, male, 34.0 mm, (C), 7 Mar. 1999; ZMH 23921, male, 30.4 mm, (C), 7 Mar. 1999; ZMH 23922, male, 33.6 mm, (L), Dec. 1990; ZMH 23923, male, 30.9 mm, (A), Dec. 1992; ZMH 23924, male, 28.8 mm, (C), 14 Jul. 1991; ZMH 23925, female, 29.8 mm, (E), 9 Mar. 1999; ZMH 23926, female, 31.1 mm, (A), 1 Mar. 1998; ZMH 23927, female, 31.7 mm, (C), 1 Mar. 1998; ZMH 23929, female, 31.7 mm, (E), 19 Apr. 1997; ZMH 23930, female, 32.8 mm, (E), 19 Apr. 1997; ZMH 23931, female, 31.3 mm, (B), 18 Apr. 1997; ZMH 23932, male, 31.9 mm, (E), Dec.



1992; ZMH 23933, male, 37.4 mm, (E), 4 Dec. 1993; ZMH 23934, male, 33.7 mm, (E), 6 Mar. 1999; ZMH 23935, male, 35.9 mm, (E), 4 Dec. 1993; ZMH 23936, male, 35.0 mm, (E), 6 Mar. 1999; ZMH 23937, male, 32.1 mm, (E), 4 Dec. 1993; ZMH 23938, male, 33.8 mm, (E), 6 Mar. 1993; ZMH 23939, male, 34.0 mm, (E), 6 Mar. 1999; ZMH 23940, male, 32.5 mm, (E), 4 Dec. 1993; ZMH 23941, male, 33.5 mm, (E), 4 Dec. 1993.

ECO-CH 4766, male, 30.5 mm, (C), 27 Feb. 1998; ECO-CH 4767, female, 33.3 mm, (E), 27 Feb. 1998; UMMZ 201752, female, 28.4 mm, 19°50'N 88° 45'W, 27 Jul. 1974, leg. J.M. Humphries and J.N. Taylor.

Diagnosis

Cyprinodon esconditus belongs to the endemic species flock of Laguna Chichancanab which is closely related to *C. artifrons*. As typical for pupfish most of the meristic and morphometric characters overlap. The new species can be distinguished from the other members of the flock by the following unique combination of characters (in percent of SL): Eye size smallest for the flock (8.9% and 8.3% for males and females, respectively, versus 12.1% in *C. verecundus*, 11.8% in *C. simus*, 10.6% in *C. maya*, 9.9% in *C. beltrani* and 9.6% in *C. labiosus*). Dorsal and pelvic fins are inserted more anterior correlated with a slightly shorter predorsal (53.6% versus 60.5% in *C. verecundus* and 57.5% in *C. beltrani* and 57.9% in *C. maya* but only 54.9% in *C. labiosus* and 55.3% in *C. simus*) and prepelvic length (51.6% versus 59.6% in *C. beltrani*, 59.5% in *C. maya* and 59.8% in *C. verecundus*, 58.3% in *C. labiosus* and 55.5% in *C. simus*). Caudal peduncle is longer (31.0% versus 22.2% in *C. verecundus*, 23.2% in *C. beltrani*, 23.5% in *C. maya*, 25.8% in *C. simus* and in *C. labiosus*) and slightly deeper than in *C. beltrani*, *C. maya* and *C. simus* (15.4% versus 13.1%, 14.5% and 13.9%, respectively) whereas caudal peduncle depth in *C. labiosus* and *C. verecundus* is higher (16.0% and 17.6%, respectively). Body depth (31,6%) is lower than in *C. beltrani* (36.1%) similar to *C. maya* and *C. labiosus* (31.2 and 31.8%, respectively) and much higher than in *C. simus* (28.4%). Numbers of premaxillary teeth range from 19-23 (mean 21.4) for the upper and 20-26

Figure 1. - Sampling sites of Laguna Chichancanab, Yucatan, Mexico. A: 19°57'18"N 88°44'52"W, northern end of north pond; B: 19°56'56" N 88°45'35"W, northern end; C: 19°56'46"N 88°45'52"W, dead end of road from Rancho Santa Cruz; D: 19°52'36"N 88°46'14"W, Balneario Dziuché; E: 19°51'10"N 88°45'58"W, southern end at broadest part; F: 19°50'00"N 88°44'48"W, bridge to San Diego; G: 19°46'54"N 88°44'17"W, southern most pond, 4 km NW of José Maria Morelos and 300 m East highway; H: 19°48'33"N 88°43'41"W, road crossing, east of La Presumida; J: 19°48'43"N 88°43'41"W, 500 m north of road crossing, east of La Presumida; K: 19°51'41"N 88°45'57"W, southern end, 500 m north of broadest part; L: 19°53'13"N 88°46'13"W, 1 km north of Balneario Dziuché.

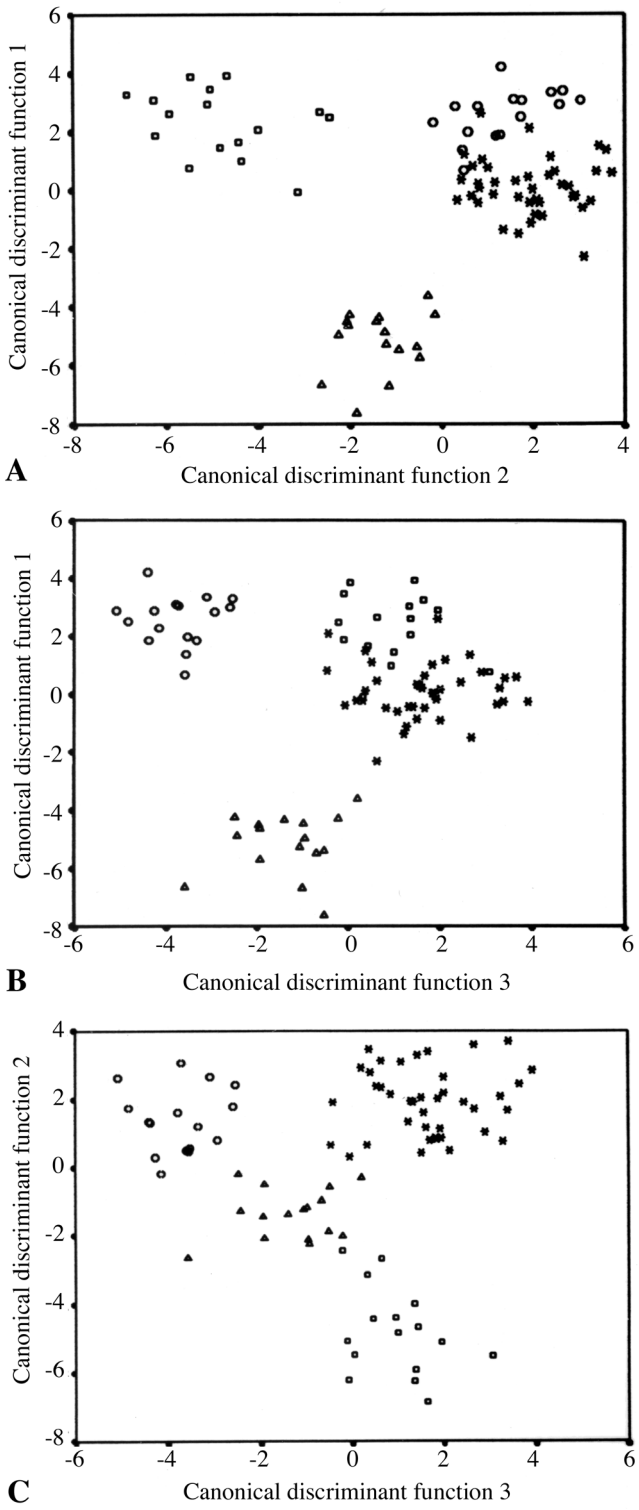


Figure 2. - Scatter plots of a canonical discriminant analysis of four *Cyprinodon* species from Laguna Chichancanab using 21 morphometric characters. Asterisks = *C. esconditus*, triangles = *C. beltrani*, circles = *C. labiosus*, squares = *C. verecundus*. **A**: Variables of function 1 *vs*. 2; **B**: Variables of function 1 *vs*. 3; **C**: Variables of function 2 *vs*. 3.

(mean 22.2) for the lower jaw. They are more numerous than in *C. beltrani* (14-18), *C. maya* (14-16 in specimens 43 to 56 mm SL, 18-24 in bigger specimens), *C. verecundus* (12-16) and *C. labiosus* (14-16) but less numerous than in *C. simus* (22-26) (Horstkotte, pers. com.).

Cyprinodon maya and *C. simus* are easily distinguished from *C. esconditus* based on their morphology but this is more difficult with *C. beltrani*, *C. labiosus* and *C. verecundus*. However, the canonical discriminant analysis shows that all species can be separated completely. Depending on the canonical discriminant function used, the cluster of *C. esconditus* overlap either with *C. labiosus* (Fig. 2A), with *C. verecundus* (Fig. 2B) or with *C. beltrani* (Fig. 2C).

Description

General appearance of male and female is shown in figure 3. Morphometric characters of holotype in bold and 39 paratypes (24 males, 15 females) of *C. esconditus* in table I. Precaudal vertebrae **11** (23), 12 (16). Caudal vertebrae 13 (15), 14 (22), **15** (2). Total vertebrae 24 (7), 25 (22), **26** (10). Head pores (both sides counted): mandibular always absent; lacrimal 3 (2), 4 (55), **5** (19), 6 (2); preopercular 6 (1), 7 (19), 8 (14), 9 (24), **10** (17), 11 (3).

Scale counts. - Lateral **25** (24-27, mean 25.4); dorsal to pelvic: **11** (10-12, mean 11.2); circular peduncle: **29** (26-31, mean 27.8); circular body **16** (14-16, mean 15.4). Fin-ray counts: dorsal **11** (10-12, mean 10.3); anal **11** (10-11, mean 10.5); pectoral left side **16** (14-16, mean 14.9); pectoral right side **15** (14-16, mean 14.8); pelvic left side **6** (6-7, mean 6.7); pelvic right side **7** (6-7, mean 6.8); caudal **14** (14-16, mean 14.2).

Number of teeth. - In upper jaw 20 (2), 21 (3), 22 (4), 23 (1), and in lower jaw 20 (1), 21 (1), 22 (4), 23 (3), 24 (1). Pharyngeal teeth of *C. esconditus* (Fig. 4) are intermediate in some aspects between those of *C. simus* and those of *C. beltrani*, *C. labiosus* and *C. maya* (see figure 5 in Humphries and Miller 1981): inner row with broad teeth similar to or even broader than those of *C. beltrani*, *C. labiosus* and *C. maya*, outer rows more acute as in *C. simus*. Intestine short, two to three times SL as in *C. labiosus*, *C. maya* and *C. verecundus*.

Sequencing of a part of the mtDNA control region revealed that eight of the individuals have the main haplotype H1 found in *C. beltrani*, *C. labiosus*, *C. simus* and *C. verecundus* as well as in the sister species of the flock *C. variegatus*. One new haplotype (numbered as H14) unique to this species occurred in two specimens. This haplotype differed from haplotype H1 (Strecker, 1996; Strecker *et al*., 1996) (GenBank accession no. L37113) at position 170 where C was substituted for T.

Sexual dimorphism

Males have deeper bodies and generally longer fins than

	Holotype	Males		Females	
		Range	Mean	Range	Mean
Standard length	32.2	28.1-38.7	32.4	28.1-34.9	31.1
Head length	315	295-353	316	298-338	317
Head width	209	193-238	209	192-240	216
Head depth	239	222-254	236	217-247	229
Postorbital length	119	112-144	123	108-133	125
Interorbital width	102	84-119	103	96-116	107
Snout length	88	76-108	88	69-101	87
Orbit length	93	80-100	89	81-99	83
Upper-jaw length	108	90-117	101	87-107	96
Mouth width	79	68-94	80	70-88	79
Predorsal length	534	496-570	536	532-569	548
Postdorsal length	546	492-568	540	511-548	524
Prepelvic length	507	488-556	517	493-550	527
Body depth	315	291-346	316	263-333	302
Caudal peduncle depth	151	136-168	154	134-156	143
Caudal peduncle length	313	281-334	310	275-330	301
Dorsal-fin length	285	253-338	289	233-277	252
Anal-fin length	241	217-281	241	178-221	200
Pectoral-fin length	256	226-271	248	206-255	238
Pelvic-fin length	116	103-131	119	95-125	112
Dorsal-fin base	181	149-186	172	144-178	158
Anal-fin base	113	95-124	107	81-102	90

Table I. - Proportional measurements of *Cyprinodon esconditus* from Laguna Chichancanab for 24 males and 15 females, expressed as thousandths of SL (except SL in mm).

females. The anal fins have a longer base and longer rays and the dorsal and to some extent the pectoral are prolonged. As in the other members of the species flock, a protuberance is developed in the region of the male genital opening.

Coloration

Live specimens. - Both, males and females are similar in coloration to the other members of the flock. Males in breeding coloration are largely black with metallic blue shoulders and yellow caudal peduncle. Females and juve-

Figure 3. - *Cyprinodon esconditus*, paratypes, **A**: Male 31.3 mm SL, ZMH 23913; **B**: Female, 30.2 mm SL, ZMH 23908.

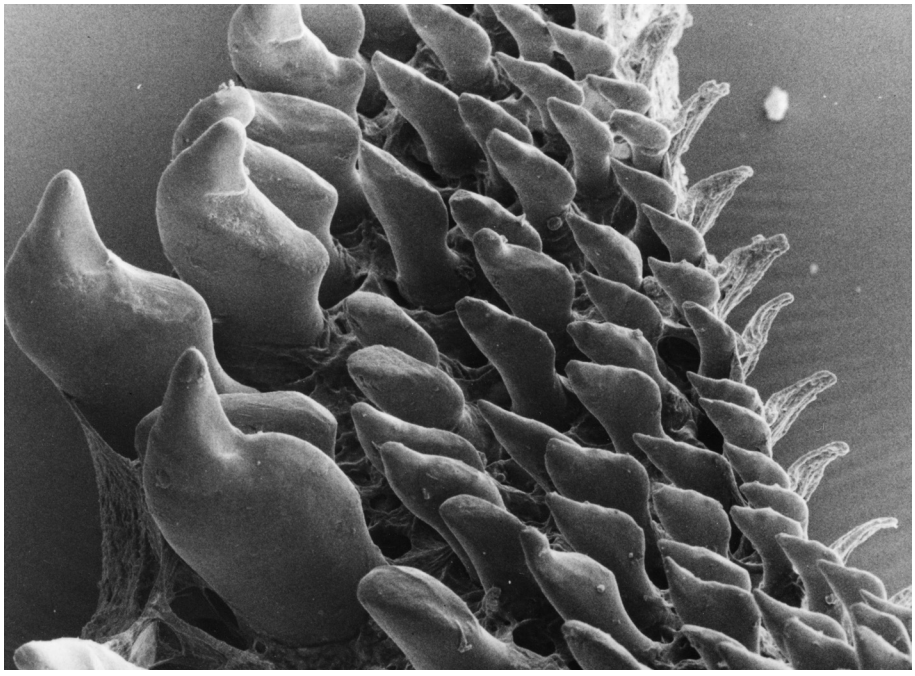


Figure 4. - Scanning electron micrographs of pharyngeal teeth of *Cyprinodon esconditus*.

niles show a cryptic colour pattern of buff background typical of most *Cyprinodon*, mostly with a black ocellus in the dorsal fin. Sides of body in both sexes are usually covered by 6 to 7 vertical brown bars.

Preserved specimens. - In preserved specimens only the melanine pigments are visible. Both sexes show the 6 to 7 vertical brown bars and in juveniles as well as in females the dorsal fin ocellus is visible.

Habitat and associates

Biotic and abiotic conditions in Laguna Chichancanab have been described (Humphries and Miller, 1981; Strecker, 1996; Strecker and Wilkens, submitted). *Cyprinodon esconditus* was first noticed by the author during a field trip in 1999. However, it was subsequently discovered in earlier samples from the 1970s (UMMZ 201752). In the last decade it was regularly observed at an abundance of about 2% of the total catch of *Cyprinodon* (Strecker and Wilkens, submitted).

Besides pupfishes, the only other native fish in Laguna Chichancanab is the live bearing tooth carp *Gambusia sexradiata* (Rosen & Bailey, 1963), a Poeciliidae. In 1988 an African cichlid, *Oreochromis* sp., that escaped from aquacultural facilities got access to the lake (Stevenson, 1992; Strecker, 1993, 1996; Strecker *et al*., 1996; Schmitter-Soto and Caro, 1997). Eight years later in 1996 the characid *Astyanax fasciatus* (Cuvier, 1819) (= *A. aeneus*), a fish species widely distributed in Mexico, invaded the lake (pers. obs.; Schmitter-Soto, 1998). These events were associated with the hurricanes Gilbert and Roxana, respectively (Strecker and Wilkens, submitted).

Etymology

The adjective *esconditus* is from the Latin word ex(s) condere (to discover) and refers to prior failure to detect the species among specimens not assignable to one of the other pupfish species in Laguna Chichancanab.

Distribution

Cyprinodon esconditus belongs to an endemic species flock only known from Laguna Chichancanab. This species was collected at sampling sites A, B, C, E, G, H and L (Fig. 1) and thus it is widespread throughout the lake.

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