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PENSOFT

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Occurrence of contact organs and sexual dimorphism in fishes of the family Profundulidae (Actinopterygii: Cyprinodontiformes)

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Abstract

The family Profundulidae is one of the few families of freshwater fishes endemic to the Mesoamerican region, and one of the least speciose within the order Cyprinodontiformes, with only 13 valid species. Sexual dimorphism is poorly developed and sparsely studied in this family. Herein, we report the presence of spicule-like organs of contact in scales and fins (anal and dorsal) in fishes of the family Profundulidae and discuss the use of spicules as a means to facilitate the identification of male and female in this family. Data from museum specimens of the 13 species of profundulid fishes were analyzed. We studied the following species: *Tlaloc labialis* (Günther, 1866); *Tlaloc candalarius* (Hubbs, 1924); *Tlaloc hildebrandi* (Miller, 1950); *Tlaloc portillorum* (Matamoros et Schaefer, 2010); *Profundulus punctatus* (Günther, 1866); *Profundulus guatemalensis* (Günther, 1866); *Profundulus oaxacae* (Meek, 1902); *Profundulus balsanus* Ahl, 1935; *Profundulus kreiseri* Matamoros, Schaefer, Hernández et Chakrabarty, 2012; *Profundulus parentiae* Matamoros, Domínguez-Cisneros, Velázquez-Velázquez et McMahan, 2018; *Profundulus mixtlanensis* Ornelas-García, Martínez-Ramírez et Doadrio, 2015; *Profundulus adani* Dominguez-Cisneros, Velázquez-Velázquez, McMahan, et Matamoros, 2021; *Profundulus chimalapensis* Del Moral-Flores, López-Segovia et Hernández-Arellano, 2020. Scales were observed and photographed with a Hitachi VP-MEB SU1510 scanning electron microscope; the spicules in fins were observed from specimens cleared and double-stained. This study revealed the presence of 1–10 spicules on the surface of the scales in males and its absence in females of all species analyzed. Numerous, small and thin spicules were also observed and they were restricted to the middle rays of the anal and dorsal fins of the males. The observation of spicules in the anal fins for the sexual identification of profundulid fishes constitutes an advantage of operational simplicity, speed, and potential for its application in taxonomic and ecological studies.

Keywords

Mesoamerica, endemic species, nuptial tubercles, profundulids fish, sexual differences, spicules

Introduction

Contact organs are structures composed of dermal bony outgrowths or spicules projecting from a fin ray or scale margin and surrounded by the epidermis, through which the bony outgrowths may protrude (Tripathi 2018). Such organs, present on scales or fin rays, exist primarily to facilitate contact between individuals during spawning (Wiley and Collette 1970). These structures are maintained through the continuous renewal of the most superficial keratinized layer for the entire life of the fish, playing an essential role in stimulating egg release during spawning

in zebrafish (Kang et al. 2013). In the majority of species, contact organs are present only in males or are more developed in males than in females (Wiley and Collette 1970).

The Profundulidae represents one of the few freshwater fish families endemic to Mesoamerica and constitutes a lineage with a limited geographical distribution that extends from southern Mexico to Central America (Miller 1955; Morcillo et al. 2016). Members of the Profundulidae are small (maximum standard length around 120 mm), and are often the only fish species found in the upper parts of river basins (Calixto-Rojas et al. 2021). Profundulids are among the least studied cyprinodontiforms. The sexual dimorphism, in this family of fishes, is poorly developed and inadequately studied.

Recently, we had the opportunity to describe a new species in the family Profundulidae (*Profundulus adani* Dominguez-Cisneros, Velázquez-Velázquez, McMahan, et Matamoros, 2021), a killifish distributed in the Papaloapan Basin in the upper reaches of the Papaloapan River in the Mexican state of Oaxaca (Domínguez-Cisneros et al. 2021). During the study, we noticed the presence of spicules in the scales and fins of males, which suggested a possible case of sexual dimorphism. Therefore, our objective was to describe the occurrence of secondary sexual dimorphism in the species of the family Profundulidae.

Material and methods

The specimens deposited in the Fish Collection of the Zoology Museum of the University of Sciences and Arts of Chiapas were analyzed (collection code: 1138, MZ-UNICACH) (Sabaj 2020). The following species were studied: *Tlaloc labialis* (Günther, 1866); *Tlaloc candalarius* (Hubbs, 1924); *Tlaloc hildebrandi* (Miller, 1950); *Tlaloc portillorum* (Matamoros et Schaefer, 2010); *Profundulus punctatus* (Günther, 1866); *Profundulus guatemalensis* (Günther, 1866); *Profundulus oaxacae* (Meek, 1902); *Profundulus balsanus* Ahl, 1935; *Profundulus kreiseri* Matamoros, Schaefer, Hernández et Chakrabarty, 2012; *Profundulus parentiae* Matamoros, Domínguez-Cisneros, Velázquez-Velázquez et McMahan, 2018; *Profundulus mixtlanensis* Ornelas-García, Martínez-Ramírez et Doadrio, 2015; *Profundulus adani* Dominguez-Cisneros, Velázquez-Velázquez, McMahan, et Matamoros, 2021; *Profundulus chimalapensis* Del Moral-Flores, López-Segovia et Hernández-Arellano, 2020. The specimens were fixed in a 10% formalin solution and preserved in 70% ethanol. The sex of the fishes was determined through dissection; scales were carefully taken out from near the head region above the lateral line, and from other areas for microscopic observations. The scales were observed and photographed using a Hitachi VP-MEB SU1510 scanning electron microscope. The spicules in fins were observed in specimens of the 13 above-mentioned profundulids species. The samples were cleared and double-stained (bone alizarin and cartilage counter-stained with Alcian blue), according to the technique described by Taylor and van Dyke (1985).

Material examined. The list below includes material examined. Data on material is organized in the following sequence: species name, catalog number, number of specimens cleared and stained. *Profundulus balsanus*: MZ-UNICACH 6712, 7. *Profundulus oaxacae*: MZ-UNICACH 6714, 4; MZ-UNICACH 6715, 2. *Profundulus parentiae*: MZ-UNICACH 6575, 5; MZ-UNICACH 6576, 3. *Profundulus adani*: MZ-UNICACH 7382, 3; MZ-UNICACH 7342, 2. *Profundulus chimalapensis*: MZ-UNICACH 7559, 7. *Profundulus punctatus*: MZ-UNICACH 6632, 3; MZ-UNICACH 6578, 2. *Profundulus mixtlanensis*: MZ-UNICACH 6716, 9. *Profundulus guatemalensis*: MZ-UNICACH 7194, 4; MZ-UNICACH 7195, 2. *Profundulus kreiseri*: MZ-UNICACH 7205, 3; MZ-UNICACH 7214, 2. *Tlaloc hildebrandi*. MZ-UNICACH 4330, 3; MZ-UNICACH 5711, 2; MZ-UNICACH 2266, 2. *Tlaloc portillorum*: MZ-UNICACH 7220, 4; MZ-UNICACH 7222, 2. *Tlaloc labialis*: MZ-UNICACH 3467, 2; MZ-UNICACH 6740, 2; MZ-UNICACH 7233, 2. *Tlaloc candalarius*: MZ-UNICACH 6813, 4; MZ-UNICACH 7565, 2.

Results

The examined fish specimens were in the range of 26.59–101.93 mm standard length (SL) (Table 1). The males were slightly larger than females, the mean SL value was higher in males of 10 species except in *Profundulus punctatus*, *P. balsanus*, and *P. oaxacae*; the largest specimen recorded was a male of *Tlaloc hildebrandi* (SL = 111.49 mm); while the maximum size recorded in a female was in *Tlaloc labialis* (SL = 101.93 mm).

The contact organs were particularly prominent in the flank scales and dorsal and anal fins of males in all species of the genera *Tlaloc* and *Profundulus*. In the scales of the operculum and the caudal peduncle, the highest concentration of spicule-like organs of contact were observed (Fig. 1). The scales are large and cycloid, with numerous radii. The body and the head were entirely scaled, except on the anterior portion of the ventral surface of the head.

The study revealed the presence of 1 up to 10 spicules (commonly from 1 to 3) on the surface of the scales in males (Fig. 2). The spicules were absent in females of all species analyzed.

In males of all species of profundulids studied, the dorsal and the anal fins had similar morphology, whereas, in females, the anal was slightly elongated. Contact organs of anal-fin rays were arranged in rows along the inner surface of fin rays; spicules were numerous, small, and thin, and restricted to the middle rays of the anal and dorsal fins, much more numerous on the anal fin (Fig. 3).

Discussion

Contact organs have long been known in the ichthyological literature under a bewildering variety of terms. The majority of such records have been concealed in taxonomic

Table 1. Minimal (Min) and maximal (Max) standard length (SL) in males and females of profundulid species (genera *Tlaloc* and *Profundulus*).

Species	Females					Males				
	Min	**Max**	**Mean**	**SD**	***n***	**Min**	**Max**	**Mean**	**SD**	***n***
T. labialis	39.63	**101.93**	49.56	13.26	68	37.70	79.94	50.54	11.96	38
T. candalarius	38.81	79.35	48.53	10.14	50	39.53	99.20	53.18	11.30	47
T. hildebrandi	37.94	92.76	56.80	11.02	42	40.97	**111.49**	64.68	22.35	16
T. portillorum	35.93	71.35	49.56	10.37	34	40.03	68.72	50.36	7.65	24
P. punctatus	32.50	77.50	52.80	9.89	76	36.40	82.20	51.90	9.65	75
P. guatemalensis	39.40	58.60	48.70	7.52	9	38.40	**83.90**	53.50	15.86	17
P. oaxacae	39.62	53.60	50.55	7.00	8	35.35	45.81	43.72	5.17	8
P. balsanus	38.76	66.04	53.92	8.60	8	38.87	58.83	49.25	6.98	10
P. kreiseri	37.20	66.10	48.00	7.23	27	38.50	72.70	50.40	9.72	23
P. parentiae	36.94	67.80	48.30	9.97	21	36.80	73.90	54.80	10.47	21
P. mixtlanensis	40.60	60.80	49.20	5.66	30	39.00	70.60	50.40	8.90	30
P. adani	36.70	**77.90**	53.50	10.45	35	38.70	83.70	54.50	12.49	39
P. chimalapensis	38.91	65.06	46.36	46.36	14	34.11	80.30	54.61	14.98	8

n = number of individuals, SD = standard deviation; **bold** font denotes maximum size values.

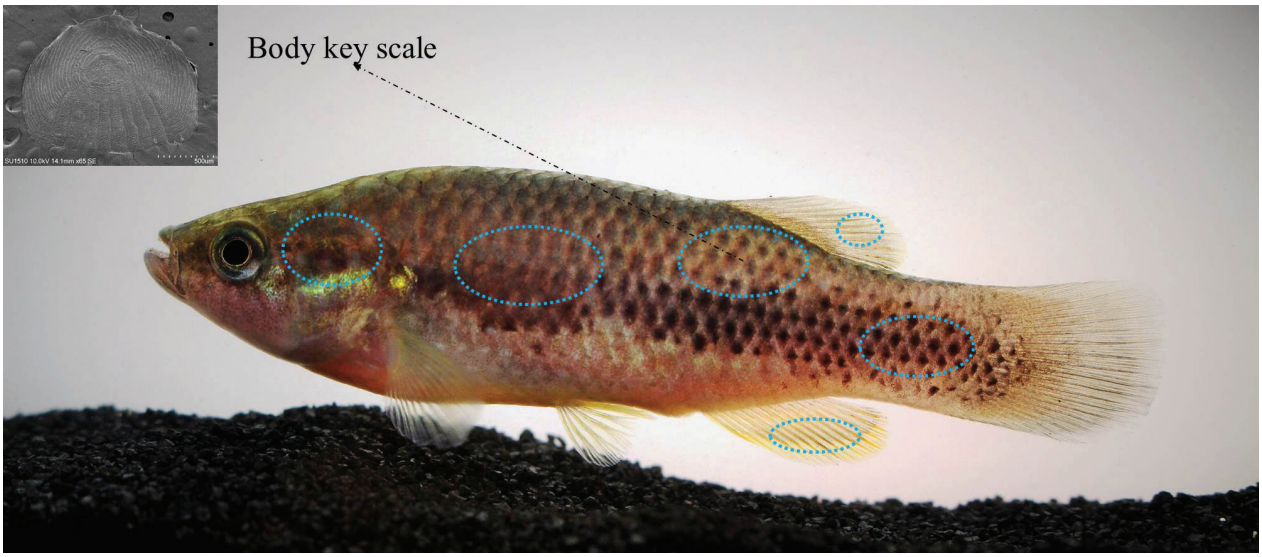

Figure 1. Left side of the fish specimen (Male of *Profundulus chimalapensis*) shows the location between the dorsal fin and lateral line, where the key scales were removed. Dotted circles indicate organs' contact clusters.

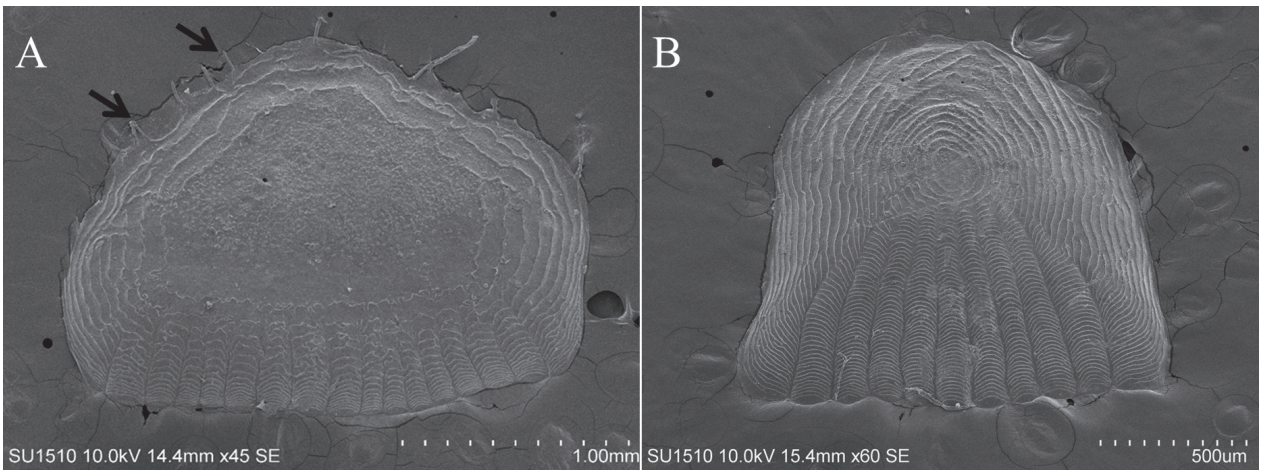

Figure 2. Scales of *Tlaloc portillorum* (male: **A**) and *Tlaloc candalarius* (female: **B**). The arrow points the spicules on the scales.

papers as parts of new species descriptions (Wiley and Collette 1970; Costa 2002; Volcan and Severo-Neto 2019). In the presently reported study, the examination of scales and fins in profundulid fishes revealed the presence of numerous spicules. The location, number, and extent of contact organ development varied intra and interspecifically.

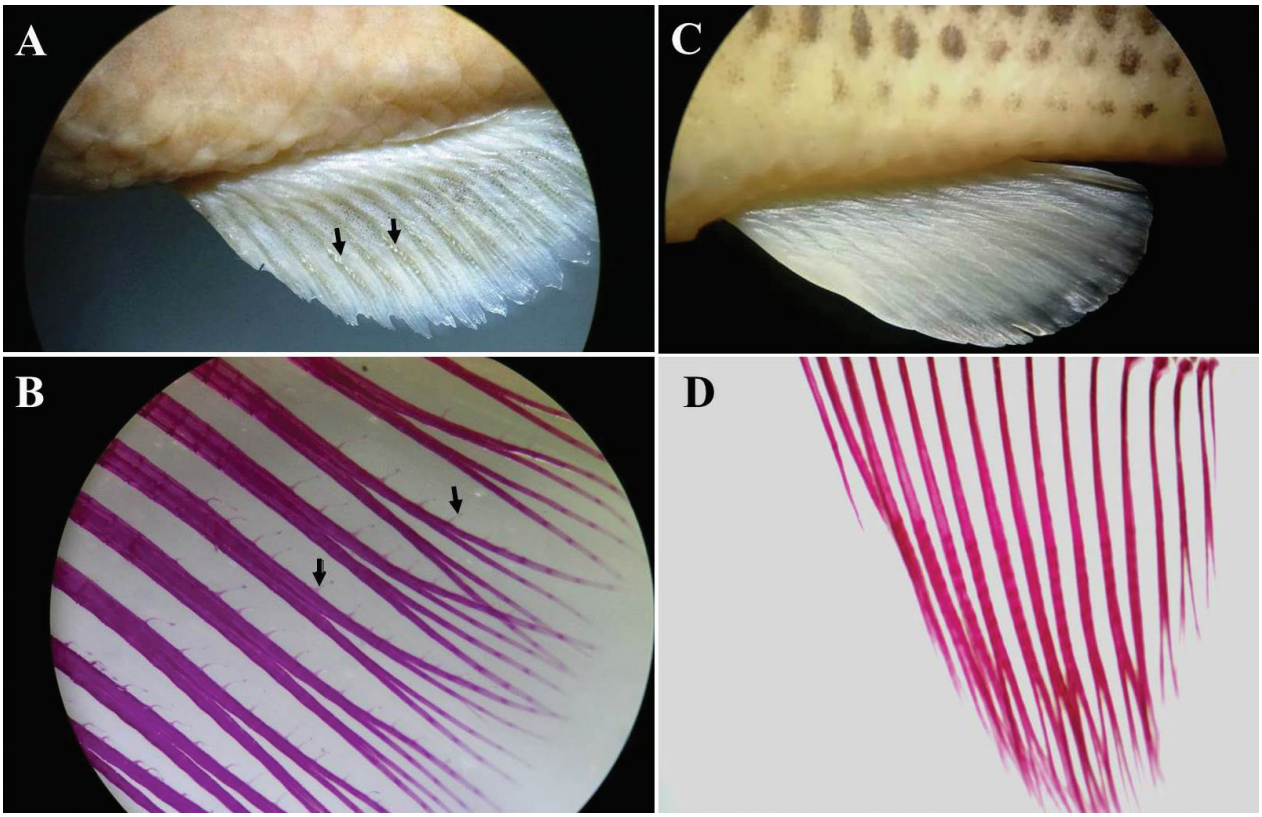

Figure 3. Anal-fins of *Tlaloc hildebrandi* (male: **A**–**B**) and *Profundulus punctatus* (female: **C**–**D**). Arrows indicate the spicules. **B** and **D** = anal fin rays (cleared).

Contact organs or spicules are common in atherinomorph fishes (Atheriniformes, Cyprinodontiformes, and Beloniformes). Possibly, they are important to the maintenance of body contact between sexes during reproductive behavior (Wiley and Collette 1970). Contact organs of the Cyprinodontiformes have been reported for numerous species of topminnows (Cashner et al. 2020), killifishes (Costa 2006), and pupfishes (Teimori et al. 2017). In profundulids, Miller (1950) described the presence of contact organs or ctenii as a difference (though not striking) between the nuptial male and female of the *Profundulus hildebrandi*; although he noticed only a few tubercules on the anal fin. In rivulids, they are present on the scales and fin rays in males of some taxa, during all the adult stage (Costa 2006). The occurrence of these structures in male fish of the family Profundulidae, seems to be throughout adult life since the spicules were observed in the cleared fish, which were collected at different times of the year. Velázquez-Velázquez and Schmitter-Soto (2004) pointed out that the reproductive period in *Tlaloc hildebrandi* is markedly seasonal, which occurs between February and June.

The determination of sex using contact organs requires the examination of the corporal surface and anal fin of the fish, which will reveal the presence or absence of spicules; these are clearly visible from above as thin elongated spicules along the central fin rays of the fins in males (Fig. 3A). Spicules are easily visible in adults with the help of a stereomicroscope. In particular, the observation of spicules in the anal fins, for the sexual identification of profundulid fishes, presents advantages of operational simplicity, speed, and potential for its application in taxonomic and ecological studies.

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