

## Egg surface morphology in the Neotropical seasonal killifish genus *Leptolebias* (Teleostei: Aplocheiloidei: Rivulidae)

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### > Abstract

The egg surface of four species of the South American genus *Leptolebias* is examined under scanning electron microscopy. Two distinct morphological patterns are described: surface reticulate, lumen punctate, in *L. marmoratus*, *L. aureoguttatus*, and *L. itanhaensis*; and, surface insulate and not punctate, in *L. citrinipinnis*. Both the punctate lumen and the insulate pattern are features firstly recorded for rivulids. Phylogenetic inferences about evolution of unique characters states found are not postulated by the absence of data on chorion morphology for two congeners possibly extinct (*L. splendens* and *L. opalescens*). Mushroom-like projections about regularly distributed on egg surface were found in all examined species.

### > Key words

Killifishes, Seasonal fishes, *Leptolebias*, Egg Morphology, Chorion.

## Introduction

Seasonal (COSTA, 2002a) or annual (MYERS, 1942) fishes are members of the cyprinodontiform suborder Aplocheiloidei inhabiting temporary freshwater pools formed during the rainy seasons in South America (family Rivulidae) and Africa (Nothobranchiidae). During the dry seasons (once or twice a year, depending on the region) the pools dry completely and, consequently, the fishes die. Diapausing eggs survive for months until the next rainy season, when then eggs hatch. Seasonal fish eggs have a thickened chorion, often bearing some kind of ornamentation.

The elaborate chorion structure of aplocheiloid killifishes was first reported by CARVALHO (1957) and SIEGEL (1958a, 1958b), and its potential as a tool for taxonomic studies by SCHEEL (1968). COSTA (1990, 1998) used features of the chorion structure as phylogenetic characters, and more recently, a great variability in chorion morphology has been reported to some rivulid groups (LOUREIRO & SÁ, 1998; FAVA & TOLEDO-PIZA, 2007).

A special kind of egg ornamentation is found among fishes of the South American tribe Cynopoecilini (SIEGEL, 1958b; WOURMS, 1976; COSTA, 1990), which comprises the genera *Cynopoecilus* REGAN, *Campellolebias* VAZ-FERREIRA & SIERRA, *Leptolebias*

MYERS, and *Notholebias* COSTA (COSTA, 2008a, 2008b). WOURMS (1976) and WOURMS & SHELDON (1976) considered cynopoeciline egg as the most structurally complex among teleost fishes. It consists of hollow, cylindrical to conical projections distally terminating as a crown, with an external aperture on the center of the crown (WOURMS, 1976). These projections were named as mushroom-like projections (hereafter termed m-projections) due to their general appearance by COSTA (1990, 1998). The m-projections arise from a system of ribs forming pentagons or hexagons over the chorion surface, termed as reticulate pattern by COSTA (1998). WOURMS & SHELDON (1976) described the inner structure of the Cynopoecilini chorion as a continuous system of cavities and canals connected to the exterior by the apical aperture of the m-projections, constituting a chorionic respiratory system, similar to the plastron of insect eggs. This elaborate chorion may have other functions as to anchor eggs to substrate after first rains or act as a shock absorber (SIEGEL, 1958b; WOURMS & SHELDON, 1976).

Egg chorion morphology has been described for species of the genera *Cynopoecilus*, *Campellolebias* and *Notholebias* (SIEGEL, 1958a, b; WOURMS, 1976; WOURMS & SHELDON, 1976; FAVA & TOLEDO-PIZA, 2007), but not for species of *Leptolebias*. According to the most recent revision (COSTA, 2008a), *Leptolebias* comprises six valid species: *L. aureoguttatus* (CRUZ,

1974), *L. citrinipinnis* (COSTA, LACERDA & TANIZAKI, 1988), *L. itanhaensis* COSTA, 2008, *L. marmoratus* (LADIGES, 1934), *L. opalescens* (MYERS, 1942), and *L. splendens* (MYERS, 1942). One species usually placed in *Leptolebias*, *L. leitaui* (CRUZ & PEIXOTO, 1992), is considered an incertae sedis taxon, possibly extinct in recent years, and three other species were transferred to *Notholebias* (*N. cruzi* (COSTA, 1988), *N. fractifasciatus* (COSTA, 1988) and *N. minimus* (MYERS 1942), according to Costa 2008a). Among the six species retained in *Leptolebias* by COSTA (2008a), one species is endangered (*L. itanhaensis*), one species (*L. marmoratus*) is critically threatened with extinction and two species (*L. opalescens* and *L. splendens*) are possibly extinct (COSTA, 2002b, 2008a), which impedes experiments with live specimens in captivity to obtain eggs for study. The objective of the present study is to first document the egg surface morphology in some species of *Leptolebias*, complemented by the analysis of ovules extracted from preserved specimens deposited in ichthyological collection.

## Material and methods

The analysis of the microstructure of egg surface was primarily based on fertilized eggs obtained from aquarium stocks of two species (10 eggs examined for each species): *L. aureoguttatus*, population from Ribeira do Iguape, São Paulo, Brazil, and *L. citrinipinnis*, population from Barra de Maricá, Rio de Janeiro, Brazil. As some species are rare and threatened with extinction, and consequently are not maintained in aquaria, data were complemented by the examination of mature ovules obtained from the dissection of specimens preserved in ethanol 70 % and deposited in the ichthyological collection of the Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ). Ovules of the following species were examined: *L. citrinipinnis*: UFRJ 6305 (15 ovules), *L. itanhaensis* UFRJ 5218 (4 ovules), *L. marmoratus* UFRJ 5355 (5 ovules). Compatibility among data obtained from fertilized eggs of living fish stocks and mature ovules of preserved specimens were tested analyzing both data sources for a single species (*L. citrinipinnis*). Comparative material included *Notholebias minimus* (10 fertilized eggs, population from Seropédica, Rio de Janeiro, Brazil) and *N. fractifasciatus* (10 fertilized eggs, population from Inoã, Rio de Janeiro, Brazil). Other comparisons were based on data and figures provided in the literature, as listed in the Introduction above.

Eggs were fixed in 1.5 % glutaraldehyde during 48 h. After fixation, eggs were washed three times in cacodylate sodium buffer. Immediately after washes,

samples were dehydrated through an increasing series of ethanol concentration: 50 and 90 %, 10 minutes each, and three additional 10 min changes in ethanol 100 %. After dehydration, material was submitted to the critical point technique in a Baltec apparatus model CPD 030 and metallized with gold in a Berlzer's metalizer model FL-9496. Upon metallization the material was mounted on aluminum stubs using a double-face carbon tape and observed in a Jeol's Scanning Electron Microscope (SEM) model JSM 5310. The images were digitally acquired by Jeol's Semafore Program. Nomenclature for egg surface ornamentation is according to COSTA (1998), which follows pollen grain nomenclature (e. g. PUNT *et al.*, 1994).

## Results

Eggs of all species of *Leptolebias* examined have similar general shape (spherical) (Figs. 1a, d) and size (diameter 896–1005  $\mu$ m). However, two distinct patterns of egg surface morphology are found in the genus, the first one in *L. marmoratus*, *L. aureoguttatus*, and *L. itanhaensis*, and the second one in *L. citrinipinnis*, as described below.

### Egg surface pattern I

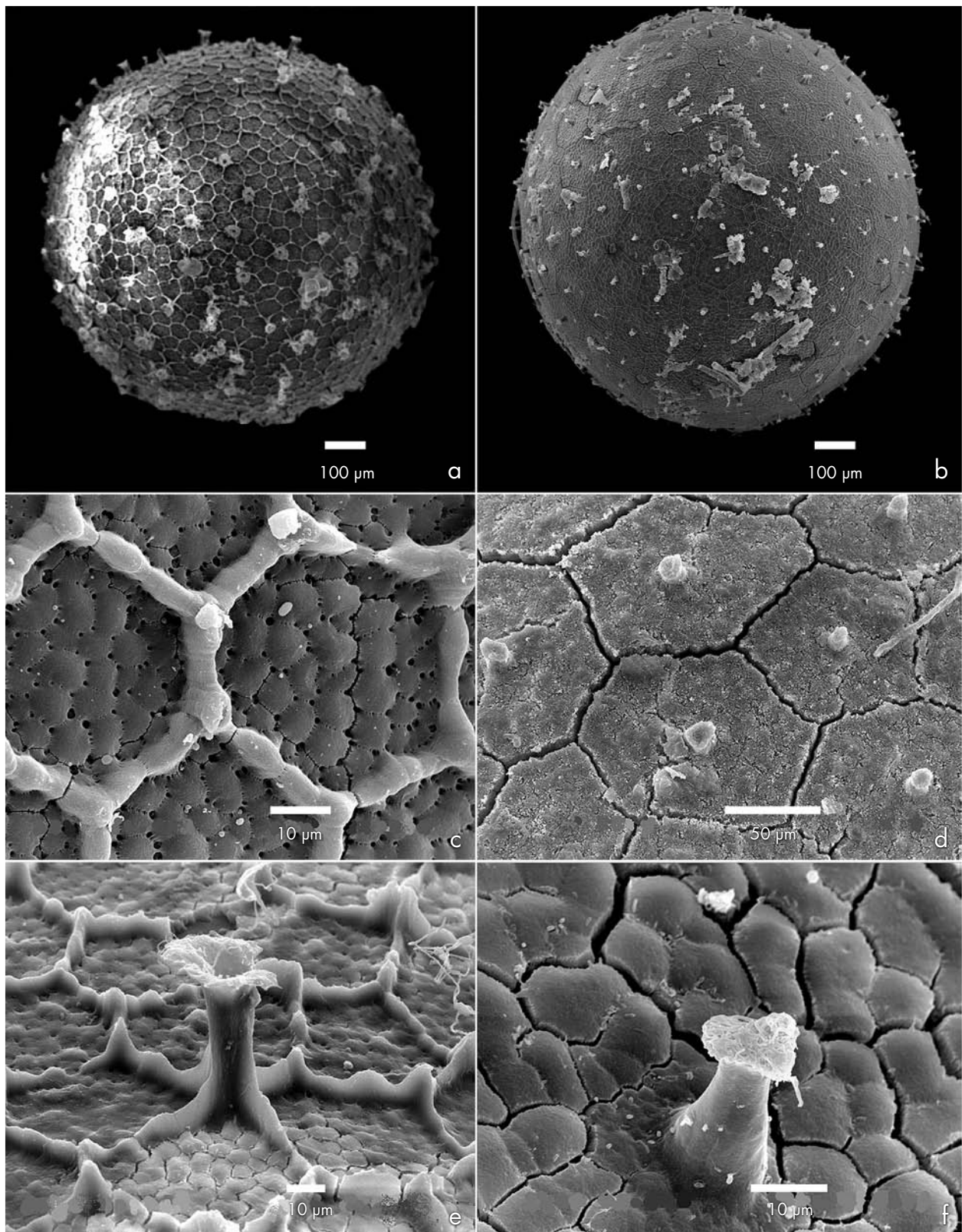
Figs. 1a–c

Egg surface reticulate; reticulum forming irregular pentagons, or sometimes hexagons, their greatest width 3.4–6.5 % ED (egg diameter). Reticulum muri narrow, width 0.4–0.6 % ED, sometimes with short interruptions. Distal margin of muri sinuous, sometimes presenting sharp projections. Lumen punctate. Mushroom-like projections about regularly distributed on egg surface, each projection inserted on reticulum vertex. Projection length 3.5–4.2 % ED, distal portion with rounded flap, its diameter 3.5–3.7 % ED. Projection stem nearly cylindrical, its width about one third of projection length.

### Egg surface pattern II

Figs. 1d–f

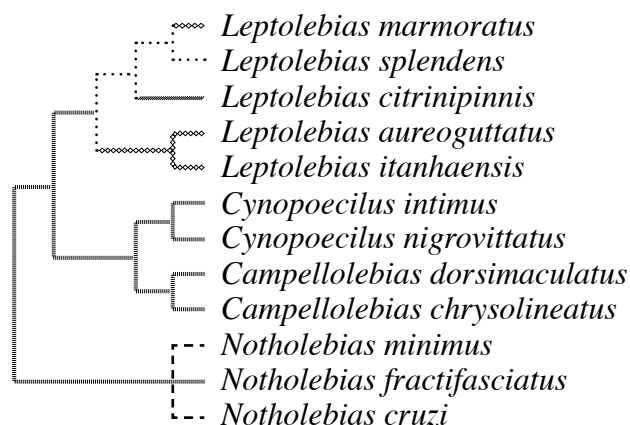
Egg surface insulate; islands forming irregular pentagons or hexagons, their greatest width 11.5–14.1 % ED. Space between islands narrow, width 0.3–0.5 ED. Mushroom-like projections about regularly distributed on egg surface, each projection inserted on



**Fig. 1.** Egg surface morphology in the genus *Leptolebias*. **a–c** Pattern I (**a**, **c** *L. aureoguttatus*; **b** *L. itanhaensis*); **d–f** Pattern II (*L. citrinipinnis*).

center of island. Projection length 2.2–3.1 ED, distal portion with rounded flap, its diameter 1.1–1.6 % ED. Projection stem subconical, width of proximal portion

slightly smaller than projection length, width of distal portion about half projection length.



**Fig. 2.** Most parsimonious phylogeny among cynopoeciline fishes according to COSTA (2008a) (tree length,  $L = 118$ ; consistency index,  $CI = 0.82$ ; retention index,  $RI = 0.85$ ), depicting two egg surface characters.

## Discussion

The egg surface of species of the genus *Leptolebias* are similar to those of the genera *Notholebias* and *Cynopoecilus* in having m-projections, a condition not occurring in other aplocheiloid killifishes, including the cynopoeciline genus *Campellolebias* (COSTA, 1990; FAVA & TOLEDO-PIZA, 2007). The presence of these peculiar structures has been considered as synapomorphic for the Cynopoecilini clade, reversed in *Campellolebias* (COSTA, 1990), a hypothesis corroborated by the occurrence of these structures in *L. marmoratus*, *L. aureoguttatus*, *L. itanhaensis* and *L. citrinipinnis* (Figs. 1c, f). Examination of five fertilized eggs of *L. splendens* by the first author in 1986, revealed that m-projections are also present in the egg surface of this species.

The second apomorphic character state of the egg surface used to diagnose the Cynopoecilini clade is the reticulate pattern, present in all cynopoeciline genera but absent in the remaining rivulids (COSTA 1990), which is corroborated by the occurrence of a similar structure in *L. marmoratus*, *L. aureoguttatus* and *L. itanhaensis* (Fig. 1b-c). However, uniquely among species analyzed, the egg surface of *L. citrinipinnis* is not reticulate, but insulate (Fig. 1e), a condition not reported to any other aplocheiloid killifish. Another firstly reported feature is the punctate lumen of *L. marmoratus*, *L. aureoguttatus* and *L. itanhaensis* (Fig. 1c), not present neither in *L. citrinipinnis* nor in other rivulids.

The origin of both the insulate pattern and the punctate lumen are presently unclear, since the distribution of these conditions among all species of *Leptolebias* is uncertain due to the microstructure of the egg surface being unknown for two species (*L. splendens* and

*L. opalescens*) not collected in recent years, possibly extinct. According to the most parsimonious tree topology of phylogenetic relationships among species of *Leptolebias* (COSTA, 2008) (Fig. 2), in which the clade comprising *L. aureoguttatus* and *L. itanhaensis* constitute the most basal lineage of the genus, the punctate lumen could be interpreted as a synapomorphic condition for *Leptolebias* with a reversal in *L. citrinipinnis*, whereas the insulate pattern seems to be autapomorphic for *L. citrinipinnis* (Fig. 2). However, depending on the condition occurring in the two not available species, *L. splendens* and *L. opalescens*, the latter also not included in the phylogenetic analysis by COSTA (2008a) (Fig. 2), different equally parsimonious interpretations could be supported (e. g., independent origin in *L. marmoratus* and in the clade *L. aureoguttatus* plus *L. itanhaensis*, if punctate lumen is not present in *L. splendens*).

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