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Morphological characterization of embryonic and larval stages in the viviparous fish *Poecilia reticulata* (Peters, 1859)

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Abstract: Knowledge of embryonic development in viviparous fishes is essential for understanding reproductive strategies, population dynamics and applications in ornamental aquaculture. Despite the ecological and economic importance of the guppy *Poecilia reticulata*, detailed descriptions of its embryonic development remain scarce. This study aimed to describe the embryonic development stages of *Poecilia reticulata*. For this purpose, adult females (n = 19) were collected, subjected to thermonarcosis, preserved and subsequently dissected to remove eggs, embryos and larvae. Developmental stages were recorded in photographs and classified according to specialized literature. A total of 379 images were analyzed, revealing the simultaneous presence of multiple developmental stages in the same female, consistent with superfetation. Three major developmental phases were recognized: gastrulation, organogenesis and larval development. Approximately 6% of the embryos were classified as gastrulation stage, characterized by a diameter of about 0.5 mm and the initial formation of the optic primordia and notochord. Organogenesis was the predominant phase (93%), with embryos averaging 1.3 mm in length and 1.5 mm in diameter, showing differentiation of the embryonic axis, pigmentation and development of major body structures. Larval stage embryos represented 1% of the observations and exhibited complete tail uncoiling, developed eyes, body pigmentation and mean dimensions of 1.7 mm in length and 0.3 mm in diameter. The results provide a morphological framework for identifying developmental stages in *P. reticulata* and confirm the occurrence of superfetation. The data contribute to the understanding of reproductive biology in viviparous poeciliids and provide useful information for ecological and ornamental aquaculture studies.

Keywords: Poeciliidae; Guppy; Lebiste; Reproduction of viviparous; Fish ecology.

1. Introduction

Knowledge of embryonic and larval development in fishes constitutes an essential foundation for studies in ecology and reproductive biology, particularly for identifying early life stages and understanding reproductive strategies (Andrade and Yasui, 2003; Fuiman and Werner, 2009; Reynalte-Tataje et al., 2020). However, research in this field faces significant methodological obstacles, ranging from the complexity of morphological identification to the scarcity of comparative literature and appropriate identification keys (Oliveira et al., 2012). In viviparous species, such as members of the family Poeciliidae, describing these stages is even more challenging, because embryonic development occurs within the maternal reproductive tract, which hinders direct observation under natural conditions and generally requires dissection (Turner, 1937; Turner, 1940; Mousavi-Sabet, 2014). This methodological limitation hinders the understanding of crucial aspects of these organisms' life cycles.

The family Poeciliidae, popularly known as guppies, lebistes, mosquitofish or guarus, comprises teleost fishes with approximately 274 species distributed among about 29 genera (Lucinda, 2003; Nelson et al., 2016; Fricke et al., 2019). Poeciliids exhibit a wide variety of adaptive reproductive strategies. These include internal fertilization, the capacity for clonal reproduction and developmental modes that encompass oviparity and viviparity. In addition, these fish exhibit different forms of embryonic nutrition, including lecithotrophy and matrotrophy. They may also undergo superfetation – where multiple gestations occur simultaneously at different developmental stages – as well as store sperm from their partners after copulation (Thibault and Schultz, 1978; Reznick, 2018; Facey et al., 2022). Males possess a distinctive morphological adaptation: the anterior rays of the anal fin are modified into a copulatory organ known as the gonopodium – formed by the third, fourth or fifth anal-fin ray – which is essential for the transfer of spermatophores (Evans and Meisner, 2019).

The family exhibits pronounced sexual dimorphism. In general, males are significantly smaller (approximately 4.0 cm) and display vibrant coloration and complex patterns, which play a decisive role in sexual selection (Houde, 1997). Females, in contrast, may reach up to 6.5 cm in length and exhibit more cryptic coloration and a more robust body, which contributes to protection against predators (Lucinda, 2003; Andrade et al., 2005; Shoji et al., 2006).

Poecilia reticulata is a Neotropical species native to Central America and northern South America. Omnivorous and highly adaptable, it has historically been introduced into various regions of the world, including Brazil, as part of biological control programs targeting disease-vector mosquitoes, due to its efficiency in consuming larvae (Lucinda, 2003; Deacon, 2023; El-Sabaawi et al., 2016). At the same time, its hardiness, the striking coloration of males and high fecundity have consolidated its position as one of the most popular species in the ornamental fish trade (Endler, 1980; Deacon, 2023; Patel et al., 2023).

Despite its ecological and economic relevance, knowledge of its early ontogenetic development remains fragmented. Most of the literature focuses on aspects of sexual selection, behavior or ecology (*e.g.*, Endler, 1980; Magurran, 2005), whereas detailed, standardized descriptions of embryonic and larval stages are scarce. This gap hinders comparative studies of life histories and the ecological impacts of their populations, which are often invasive.

Therefore, this study aims to describe the embryonic and larval development stages of *P. reticulata*. This research seeks to fill a gap in basic knowledge of this species' reproductive biology by providing a morphological reference framework that may support future studies in areas such as ecology, fisheries management, ornamental aquaculture and investigations into the reproductive plasticity of poeciliids.

2. Materials and Methods

Adult females of *P. reticulata* (n = 19) were collected from the Santa Fé River, in the municipality of Palotina, Paraná State, southern Brazil (24°17′65″ S; 53°50′53.72″ W). The sampled stretch is characterized by lentic conditions, predominantly sandy substrate, an average depth of approximately 50 cm and well-preserved riparian vegetation. Specimens were captured along the river margins using hand sieves with a 5 mm mesh size.

In the field, females presenting visibly distended abdomens, indicative of advanced reproductive condition, were selected for analysis. The specimens were transported alive to the Laboratory of Ecology, Fisheries and Ichthyology (LEPI), Federal University of Paraná, Palotina Campus, in aerated containers. All procedures were conducted in accordance with animal welfare guidelines and approved by the Ethics Committee on Animal Use of the Federal University of Paraná (CEUA/UFPR; Protocol No. 42/2015).

A total of 19 adult females were analyzed. Body mass (g) and total length (cm) were recorded before fixation. Females presented a mean body mass of 0.24 ± 0.11 g and a mean total length of 2.88 ± 0.39 cm. Subsequently, specimens were euthanized by thermonarcosis, following Freire and Gonçalves (2013), and fixed in Davidson's solution, following Pérez-Robles et al. (2016). After fixation, females were dissected under a stereomicroscope and all embryos present in the ovarian cavity were carefully removed. The embryos were preserved in 70% ethanol for subsequent analysis. Embryonic developmental stages were identified based on external morphological characteristics, following the criteria proposed by Sanches et al. (2001).

Images were obtained using an AVerMedia image capture system (AVerVision CP155) coupled to a stereomicroscope (Leica® DM1000). A total of 379 images from embryos and larvae were recorded and analyzed. For each developmental stage, morphological characteristics such as body shape, eye development, pigmentation pattern, notochord formation, yolk sac presence and tail position were evaluated. Whenever possible, embryo length and diameter were measured from digital images.

Data were analyzed descriptively. Female body mass and total length are presented as mean ± standard deviation. The frequency of each developmental stage was expressed as a percentage of the total number of embryos analyzed. Morphological descriptions were used to characterize and compare the observed developmental stages.

3. Results and Discussion

Females of *P. reticulata* possess a single gonad, formed by the anastomosis of the two primordial ovaries, constituting a pouch of germinative tissue that contains oogonia and oocytes (Betito, 2006). Development begins with the proliferation of oogonia through successive mitotic divisions, followed by the onset of the first meiotic division, which is arrested at prophase I (Wootton and Smith, 2014).

In studies involving already-fertilized females, as in the present study, direct observation of the initial stages (oogonia and primary oocytes) is hindered, leaving only advanced-stage oocytes visible. These post-copulatory oocytes are characterized by a significant accumulation of yolk, a typical adaptation of viviparous development that ensures embryonic nutrition (Campos, 1999; Reznick et al., 2002).

The difficulty of sampling females in the pre-copulatory phase is intrinsically linked to the species' reproductive singularity. Males of *P. reticulata* use a modified gonopodium to transfer spermatophores (spermatocysts). During copulation, this gonopodium may detach and remain attached to the female urogenital opening (Evans et al., 2011). This mechanism ensures successive fertilization of multiple batches of oocytes over several reproductive cycles without the need for additional copulations (Campos, 1999; Constantz, 1989). This strategy of internal fertilization and sperm storage represents an adaptive advantage in variable environments (Magurran, 2005).

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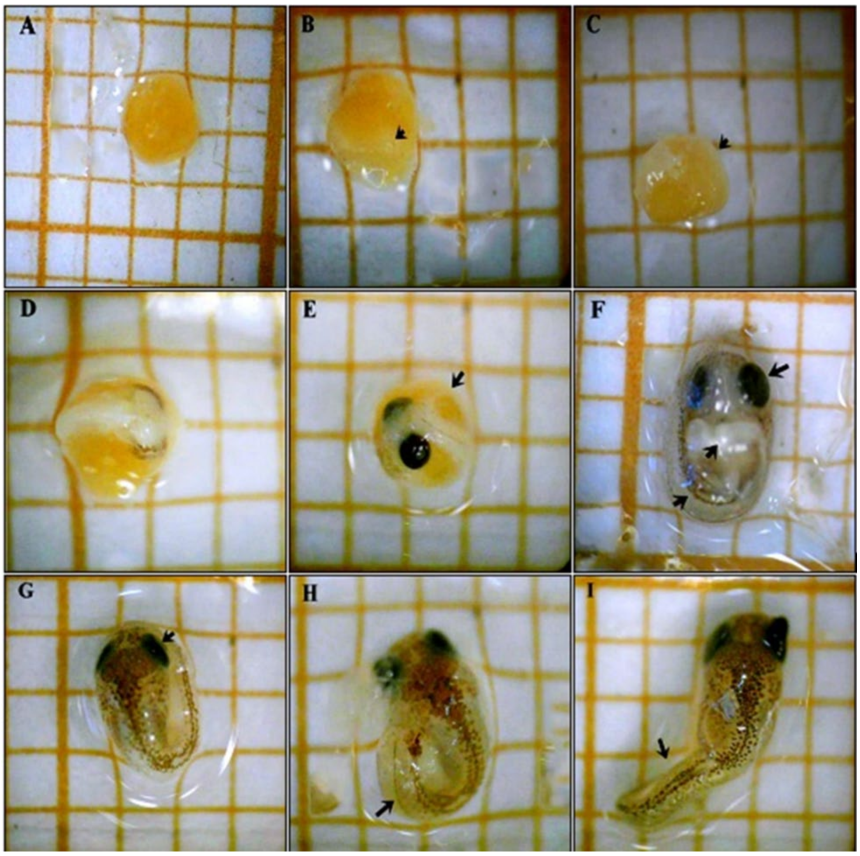

Figure 1 – Embryonic and larval development of *Poecilia reticulata*. A-C: Early stages of gastrulation – A) oocytes approximately 0.5 mm in diameter; B) optic primordia (arrows); C) indications of notochord formation (arrows). D-F: Organogenesis stages. Embryos approximately 1.5 mm in diameter and 1.3 mm in length. D) Development of the notochord (whitish structure) and the onset of eye formation, still with little pigmentation. E) Lateral view – tail coiled around the embryo's body. F) Ventral view – tail coiling, eye position, remnants of the yolk sac, and the onset of body pigmentation. G-I: Larval development. G) Embryo at the larval stage showing well-developed and pigmented eyes, with the tail beginning to uncoil. H) Advancement of tail uncoiling and definition of the pigmentation patterns previously described. I) Complete uncoiling of the tail. Structures indicated by arrows.

Among the 379 images recorded and analyzed, the simultaneous presence of different embryonic stages within the same female was observed, indicating superfetation. The presence of multiple embryonic developmental stages in a single female confirms superfetation in *P. reticulata*, a pattern described for Poeciliidae (Turner, 1937; Thibault and Schultz, 1978; Aranzábal et al., 2006). This condition is typical of Poeciliidae, in which the same gonad may simultaneously exhibit multiple embryonic stages, functioning both as germinative tissue and as an incubator (Aranzábal et al., 2006; Betito, 2006). This reproductive strategy allows greater adaptive success in unstable environments by ensuring continuous offspring release. Of the total embryos analyzed, approximately 6% were at the gastrulation stage (Figure 1 A-C), 93% at organogenesis (Figure 1 D-F) and 1% at the larval stage (Figure 1 G-I).
During gastrulation, embryos exhibited approximately 0.5 mm in diameter, bright yellow coloration and early indications of optic primordia and notochord formation (Figure 1 C). Gastrulation represents the final phase of cell division and may show early signs of eye formation (Sanches et al., 2001). During organogenesis, embryos reached approximately 1.3 mm in length and 1.5 mm in diameter, with the formation of the embryonic axis and differentiation of the cephalic and caudal regions (cephalo-caudal elongation). The notochord became well defined, eyes were pigmented, the yolk sac was reduced, the first somites were observed and the onset of body pigmentation occurred (Figure 1 D-F; Sanches et al., 2001; Gilbert, 2002; Faustino et al., 2011). The predominance of the organogenesis phase suggests it is the longest stage in the species' embryonic cycle.
The larval stage was characterized by complete tail uncoiling, full eye development and the presence of dark pigmentation distributed throughout the body (Figure 1 G-I). Individuals averaged 1.7 mm in length and 0.3 mm in diameter. According to the literature on other species, this stage corresponds to the point at which the larva, with the tail completely free, is ready to hatch. The predominant coloration is grayish, with melanotic pigments dispersed over the body surface, gills and a functional mouth (Sousa and Severi, 2000; Sanches et al., 2001; Andrade et al., 2014).

4. Conclusion

This study characterized the embryonic and larval developmental stages of *P. reticulata*, confirming superfetation and revealing morphological patterns consistent with those reported for other teleosts. These findings enhance the understanding of the species' reproductive biology and provide a basis for comparative and ecological studies, as well as for applications in ornamental aquaculture management.

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