

MORPHOLOGY AND HISTOLOGY OF THE TESTICLES OF QUEEN ANGELFISH *Holacanthus ciliaris* (LINNAEUS, 1758) (TELEOSTEI: PERCIFORMES: POMACANTHIDAE)

Morfologia e histologia dos testículos do peixe-anjo
Holacanthus ciliaris (Linnaeus, 1758) (Teleostei: Perciformes:
Pomacanthidae)

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ABSTRACT

*Aspects of the morphology and histology of the testicles of *Holacanthus ciliaris* were studied in this research. Monthly collections of living fish, totaling 39 males, were carried out between December, 2000 and November, 2001 on the coast of Ceará State, Brazil. The total length of the fish varied between 63.4 mm and 334 mm, the standard length between 50.9 mm and 270 mm, and the total weight between 6,70 g and 590 g. The testicles were bilobed and ribbon-like in shape, with firm texture and coloration varying between transparent and amber. In the histological study, male gametes were found in all spermatogenesis stages along the months of the year.*

Key words: *Holacanthus ciliaris, Pomacanthidae, reef fish, reproduction.*

RESUMO

*Aspectos da morfologia e histologia de testículos de *Holacanthus ciliaris* foram estudados nesta pesquisa. Coletas mensais de peixes vivos, totalizando 39 machos, foram realizadas entre os meses de dezembro de 2000 e novembro de 2001 na costa do Estado do Ceará. O comprimento total dos peixes variou entre 63,4 e 334mm, o comprimento padrão entre 50,9 e 270mm e o peso total entre 6,70 e 590g. Os testículos apresentavam-se bilobulados, em forma de fita, com textura firme e coloração variando entre transparente e âmbar. No estudo histológico foram encontrados gametas masculinos em todos os estágios da espermatogênese durante os meses do ano.*

Palavras-chaves: *Holacanthus ciliaris, Pomacanthidae, peixes recifais, reprodução.*

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INTRODUCTION

Holacanthus ciliaris (Linnaeus, 1758), commonly known as queen angelfish, belongs to family Pomacanthidae, which includes the marine angelfish and is distributed along reef areas of the Western Atlantic Ocean, from Florida to Southeast of Brazil (Randall, 1996; Menezes & Figueiredo, 1985; Humann, 1999; Lieske & Myers, 1999). Standing out for its extreme beauty and grace, the queen angelfish is the most captured and traded species at Ceará State, Brazil to supply the international market with ornamental sea fish (Nottingham *et al.*, 2000; Monteiro-Neto *et al.*, in press).

Studies of reproductive biology on Pomacanthidae are scarce and have been comprising mainly the feminine aspects or behavior (Lobel, 1978; Moyer & Nakazono, 1978; Bauer & Bauer, 1981, Moyer *et al.*, 1983, Hourigan & Kelley, 1985; Sakai, 1996; Sakai & Kohda, 1997). According to Thresher (1984), the testicles of pomacanthids resemble the ones from other teleosts, being bilobed, usually longitudinal, merging in the posterior region, and assuming a Y shape (Hoar, 1969). Still, the teleostean testicles frequently present flat texture and white-creamy coloration (Lagler *et al.*, 1977; Moyle & Cech, 1988).

Brief descriptions of the morphology of pomacanthid testicles can be found, for *Holacanthus ciliaris* and *H. bermudensis*, and their hybrids in Feddern (1968), *Centropyge multispinis* and *Geniactanthus caudovittatus* in Bruce (1980) and *H. tricolor* in Hourigan and Kelley (1985). As to *H. passer*, testicular maturation stages were described through the histological analysis and the development phases of the male germ cells (Arellano-Martínez *et al.*, 1999).

The present work aims at describing macro and microscopically the testicles of *Holacanthus ciliaris*, with the aim of giving information on sexual maturity stages and timing of reproduction.

MATERIAL AND METHODS

Holacanthus ciliaris specimens were collected monthly, between December, 2000 and November, 2001, totaling 39 males during the whole period. The fish were obtained from ornamental fish exporting wholesalers in Ceará State and were captured by fishermen on the coast of Fortim county (04°05'S - 37°36'W), in depths between 12 and 25 meters from the surface. The capturing process, which lasted two days on the average, as described by Nottingham *et al.* (2000), consisted of the manual collection in nets, decompression of the captured fish and stocking in the fishing boat tanks with constant water renewal.

The collected fish were measured (standard and total lengths to the nearest 0.02 mm) and weighed (total weight to the nearest 0.1 g). Testes were dissected and fixed in 10% buffered formalin solution for 24 hours (Vazzoler, 1982 and 1996), dehydrated in alcohol and embedded in paraffin following the routine pattern adapted from Junqueira and Junqueira (1983) and Silva (1999). Sections with 6 µm in thickness were cut and stained with haematoxylin and eosin, and examined under the light microscope.

RESULTS

The total length (TL) of the 39 collected individuals varied from 63.4 mm to 334 mm, the standard length (SL) from 50.9 to 270 mm and the total weight (W) from 6,70 g to 590 g.

Macroscopically, the *Holacanthus ciliaris* testicles appeared as two long, narrow, and little voluminous sheets, meaning that they were longitudinal and bilobed, presenting flat and compact texture, and coloration varying between translucent and amber (Figures 1 and 2). The larger the individual, the closer to the amber color were their testicles. The narrow, ribbon-like lobes merged in the posterior region to form the main sperm duct (*vas deferens*), giving a Y-shape to the organ. The left lobe, usually larger than the right one, was supported by a mesentery that was connected to the stomach and intestine. The right lobe was adhered to the mesentery that also supported the urinary bladder. Blood vessels irrigated the lobes starting from the dorsal region.

Histologically, the testicles did not present external muscular tunic, being wrapped up by a squamous epithelium layer (Figure 3-a), below which were the germ cells. The sperm sinuses were located in the gonad wall, and were longitudinal to the organ, being this disposition a characteristic of secondary sperm sinuses found in protogynous species (Figure 3-b).

The spermatogonia were isolated or in cysts; they were the largest cells of the germinative lineage, and presented a spherical and big nucleus in relation to the cytoplasm, with a central nucleolus (Figure 3-c). The spermatocytes were isolated or in groups; they had an spherical shape and nucleus with evident nucleolus and condensed chromatin that could be turned to one of the poles, in this case assuming a half moon shape (Figure 3-c/d). The spermatids were grouped in cysts; the eosinophile cytoplasm formed a narrow strip, its visualization was seldom possible, and the nucleus with condensed chromatin was uniform (Figures 3-d/e). The mature sperms were the smallest cells; they could be found in cysts or dispersed

in the seminiferous tubules, and had spherical and basophilous nucleus (Figures 3-e/f).

Some individuals contained testicles with previtellogenic oocytes, remnant lumen, and lamellar organization. Seven individuals with total length varying between 73.5 mm and 170 mm, presented

immature testicles, characterized by the absence of spermatogenic cysts and the presence of spermatogonia, and indifferent germ cells dispersed in the testicular tissue (Figure 3-e).

All the other analyzed individuals contained germ cells in all maturation stages (Figure 3-d).

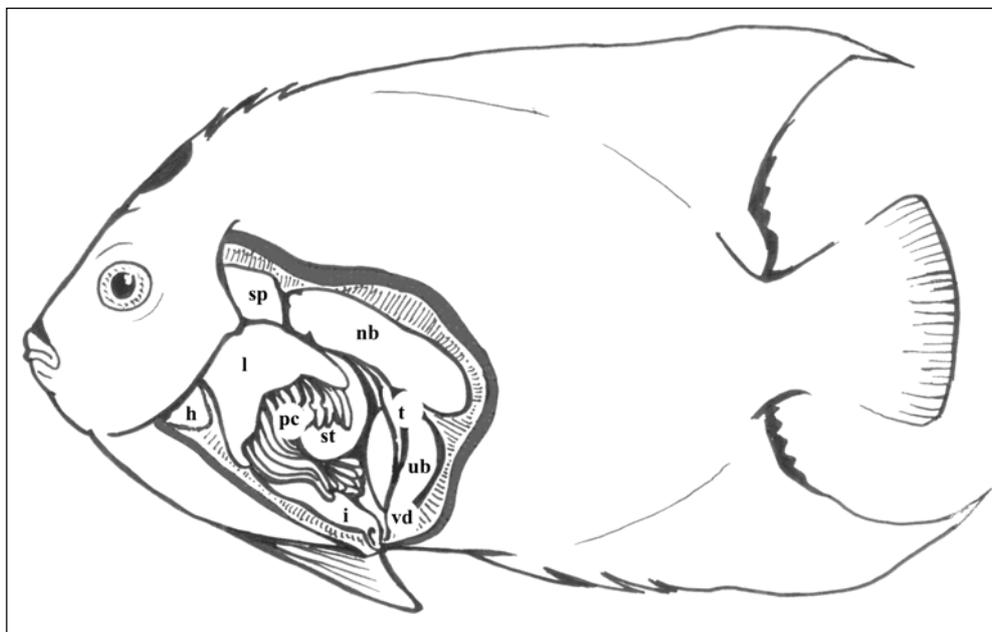


Figure 1 – Schematic drawing of partially dissected *Holacanthus ciliaris*, where the positioning of the internal organs can be observed. Spleen (sp), liver (l), heart (h), pyloric ceca (pc), stomach (st), intestine (i), natatory bladder (nb), testicle (t), urinary bladder (ub) and vas deferens (vd).

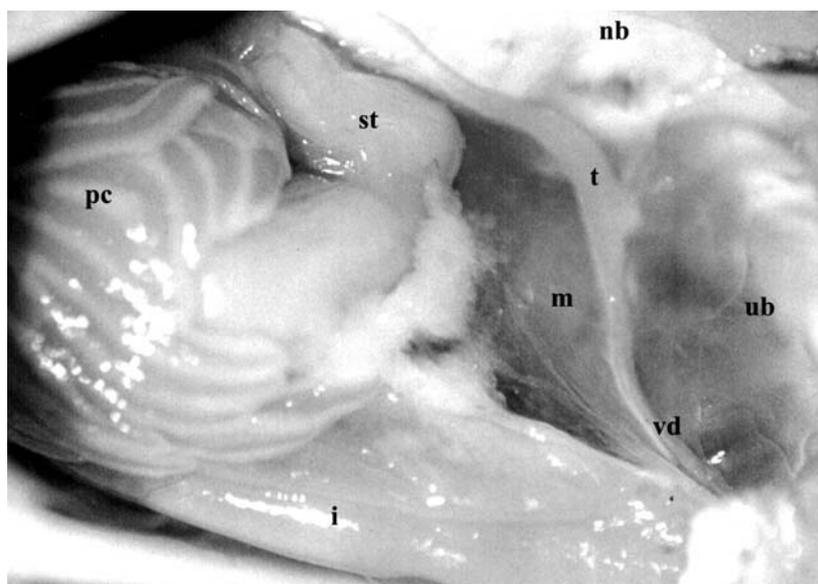


Figure 2 – Photographic registration of the left lobe of the testicle of *Holacanthus ciliaris*, evidencing its location, ribbon-like shape and amber coloration. Pyloric ceca (pc), stomach (st), intestine (i), natatory bladder (nb), testicle (t), mesentery (m), urinary bladder (ub), and vas deferens (vd).

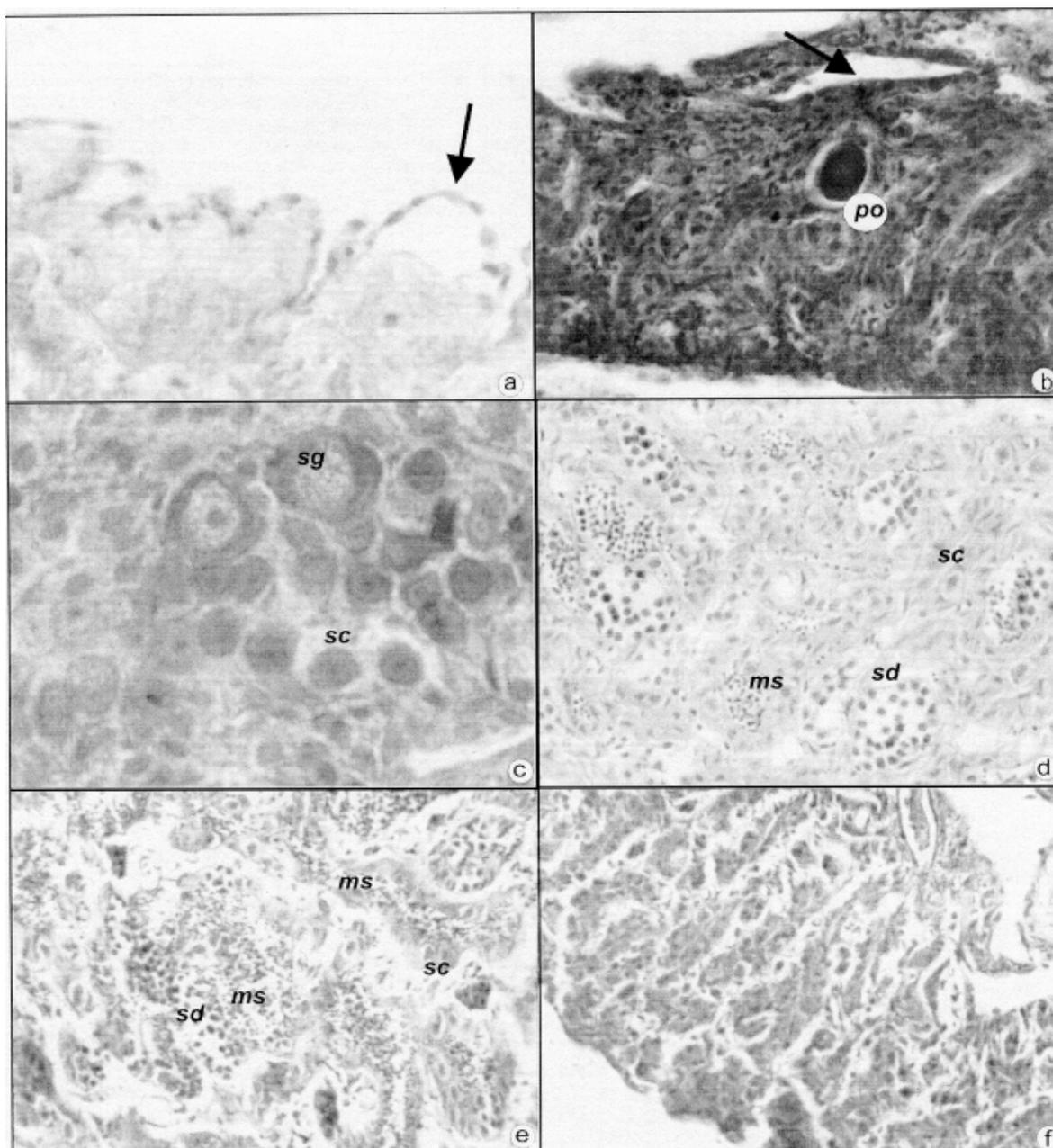


Figure 3 – a) Details of the squamous epithelium (arrow) that involves the testicle externally (2400x); b); immature testicle presenting previtellogenic oocyte and secondary sperm sinuses (2400x); c) detail showing testicular tissue with spermatogonia and spermatocytes (6000x); d) testicle with germ cells in several development phases (600x); e) testicle detail with spermatocytes, spermatids and mature sperms (600x); f) immature testicle with the absence of spermatocysts (2400x). Spermatogonia (sg), spermatocyte (sc), spermatid (sd), mature sperm (ms), pre-vitellogenic oocyte (po).

DISCUSSION

The external structure of the testicles of *Holacanthus ciliaris* on the coast of Ceará State is similar to that one described by Feddern (1968) for *H. Ciliaris* and *H. bermudensis*, in Florida (USA), being bilobed, elongated and ribbon-like. The asymmetry of the testicular lobes found in the species under this study,

has been observed in other teleostean fish, e.g. Serranidae (Ferreira, 1993 and 1995) and in the pomacanthids species *Centropyge multispinis* and *Geniacanthus caudovittatus* (Bruce, 1980). The testicles of *H. ciliaris* differed from the ones of *C. Multispinis* and *G. caudovittatus*, that are white in color.

In relation to the internal structure, the testicles of *H. ciliaris* showed to be similar to those of *H. tricolor*,

which contained germ cells in all spermatogenesis stages, forming a continuous solid mass filling out the interior of the lobes (Hourigan & Kelley, 1985).. The *vas deferens*, as observed in teleostean fish (Lagler *et al.*, 1977), was posterior to the merging point between the two asymmetric lobes.

The research works that address the morphology and histology aspects of *Centropyge interruptus* (Moyer & Nakazono, 1978), *C. multispinis* and *Geniakanthus caudovittatus* (Bruce, 1980), *Holacanthus tricolor* (Hourigan & Kelley, 1985) and *H. passer* (Arellano-Martínez *et al.*, 1999), do not describe the male germ cells. However, the descriptions of the male germ cells in *H. ciliaris*, are similar to the ones of the fresh water fish *Hoplias malabaricus* (Erythrinidae Family) (Marques *et al.*, 2000) in their several maturity stages.

The observation of male germ cells in the several maturity stages in angelfish, *Holacanthus ciliaris*, also presenting mature sperms in the light of spermatogenic tubules, indicates that males are active and ready to eliminate their gametes any time of the year, assuming a probable batch-spawning strategy. Moreover, the presence of previtellogenic oocytes, secondary sperm sinuses, remnant lumen, and lamellar organization in the testicles of *H. ciliaris*, is a characteristic of protogynous species (Aldenhoven, 1984; Hourigan & Kelley, 1985; Sadovi & Shapiro, 1987), as the results indicated a possible protogeny in the species, what should be further investigated.

CONCLUSION

As a roundup of the gathered information on reproduction of angelfish, *Holacanthus ciliaris*, one can conclude that its testicles are formed by two longitudinal and asymmetric lobes below the swimming bladder, and that they merge in the posterior region to form the main gonadal duct, being in agreement with the patterns observed in teleosts. Still, the lobes were ribbon-like, with firm texture and coloration between translucent and amber. The testicles of all the collected mature individuals, with germ cells in all development phases, supported the conclusion that this species reproduces in a batch-like fashion along the year on the coast of Ceará State, Brazil.

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