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MICROSCOPIC STRUCTURE OF THE EGG CAPSULE OF *ATLANTORAJA CYCLOPHORA* (ELASMOBRANCHII: RAJIDAE: ARHYNCHOBATINAE)

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Abstract

The microscopic structure of the egg capsules of *Atlantoraja cyclophora*, a species endemic to the Atlantic region of South America, is presented in this paper. In a transverse section, the dorsal face of the egg case is 70 μ in width, and is subdivided into four layers. Ventrally, the egg capsule wall is 60 μ wide and composed of three layers. Both faces are covered by prickles which render the egg capsule surface striated to the naked eye.

Key words: rajoids, egg capsules, *Atlantoraja cyclophora*, oviparity.

Resumo

A estrutura microscópica da cápsula ovífera de *Atlantoraja cyclophora*, endêmica na região sul-americana do Atlântico, é apresentada neste artigo. Em seção transversal, a face dorsal da cápsula ovífera tem uma largura de 70 μ , e está subdividida em quatro camadas. Ventralmente, a parede da cápsula ovífera tem uma largura de 60 μ e é conformada por três camadas. Ambas as faces acham-se cobertas por protuberâncias, que fazem com que a superfície da cápsula seja estriada ao olho nu.

Palavras-chave: rajideos, cápsulas ovíferas, *Atlantoraja cyclophora*, oviparidade.

Introduction

Skates (Rajidae) are unique among the Rajiformes in being oviparous. Females are internally fertilised and secrete a capsule around the fertilised egg, consisting of a multilaminar, proteinaceous matrix, which is subsequently shed in the environment. The females select oviposition sites and egg capsules are attached to marine debris of diverse origin in advantageous locations, which may be visited yearly by females (Hamlett & Koob, 1999). Egg capsules are structurally variable and may be species-specific but cannot be identified until they are first correlated with particular species (obtained within pregnant females of a species). They protect the embryo throughout its development, so that they may withstand prolonged exposure to the corrosive action of sea water and predation (Hamlett & Koob, 1999).

In the western South Atlantic, skate egg capsules have been described for *Sympterygia*, *Atlantoraja* and *Psammobatis* (Oddone & Vooren 2002; Braccini & Chiaramonte 2002; Mabragaña et al. 2002; Oddone & Vooren 2004; Oddone et al. 2004), even though for *Sympterygia bonapartii* (Mabragaña et al. 2002) and *Psammobatis extenta* (Braccini & Chiaramonte 2002) the descriptions may be somewhat rudimentary and incomplete. However, all these descriptions have dealt with the gross morphology of the egg capsules and not with its microscopic structure.

Ishiyama (1958) demonstrated that the microscopic structure of Japanese skates' egg capsules (genera *Dipturus*, *Okamejei*, *Bathyraja* and *Rhinoraja*) is systematically informative. However, there have been no further studies on this topic. The purpose of this study is to present the microscopic structure of the egg capsule of *Atlantoraja cyclophora* (Regan 1903), a species endemic to the Atlantic region of South America.

Materials & Methods

Egg capsules of *Atlantoraja cyclophora* were extracted from gravid females caught between Chuí and Cape Santa Marta Grande (South Brazil) between the latitudes 30°40'S and 34°30'S, in depths between 100 and 300 m, on board of the R/V *Atlântico Sul*. Egg capsules were fixed with formalin 4% for 12 hours, then preserved with ethanol 70% and deposited in the Laboratório de Elasmobrânquios e Aves Marinhas (FURG - Fundação Universidade Federal do Rio Grande).

Transverse sections of approximately 0.5 mm in width where manually done on the egg capsules (Figure 1) by using a scalpel, on both ventral and dorsal faces, following Ishiyama (1958). The sections were placed on histological slides temporarily covered with distilled water for observation, and then sketched using a stereomicroscope Jenamed II, at the Laboratório de Morfologia Funcional from FURG.

Results & Discussion

To the naked eye, the egg capsule of *A. cyclophora* is dorsally and ventrally longitudinally striated, with this striation being evidently more pronounced dorsally than ventrally (Oddone et al. 2004). Microscopically, striations on the dorsal face resemble a regular prickly surface. Each prickle measures 30 µm in height and width, and is uniformly distributed and separated approximately 90 µm from one another (Figure 2), presenting a lighter lumen.

The dorsal face of the egg case is 70 µm in width and consisted of four layers, distinctive in colour and texture. The layers are: i) the *dorsal external layer*; dark brown in colour, characterised by its homogeneous aspect, and 18 µm wide; ii) the *dorsal first middle layer*; shiny yellow and markedly laminar, and 24 µm wide; iii) the *dorsal second middle layer*; which is subdivided in two layers, the most external being light brown and the internal yellow, and 20 µm wide (both sub-layers); and iv) the *dorsal interior layer*; also subdivided in two layers, the most external being dark brown continued by a yellow and laminar layer, and 8 µm wide (both sub-layers, Figure 2).

The ventral surface possesses randomly arranged prickles averaging 18 µm in width and 10 µm in height, and separated by about 96 µm from adjacent prickles. The same pattern of a light, uncoloured lumen is present, which could suggest the presence of an empty duct. In transverse section, the ventral face of the egg capsule is 60 µm in width, and comprises three well-delimited layers: i) the *ventral external layer*, dark-brown, and 24 µm wide; ii) the *ventral middle layer*, light brown and laminar and =18 µm wide; iii) the *ventral interior layer*, reddish-brown, and 18 µm wide (Figure 2,3).

Ishiyama (1958) observed that the main portion of the rajoid egg capsule is made of two or more kinds of tissue, which he called an inner, "pulpy layer" which corresponds with the central, light coloured layer on both faces of *A. cyclophora*, and an outer layer (Ishiyama 1950, *apud* Ishiyama 1958). Ishiyama (1958) failed in not specifying whether his observations had been done using the ventral or the dorsal wall of the egg capsule. Therefore, comparisons between our study and his are not warranted. Microscopic structures described as "tubercles" by this author would correspond with the prickles described in the present study for *A. cyclophora* (i.e., the striations of the egg capsules). Also the dimensions of such "tubercles" observed by Ishiyama remain unknown. He distinguished the egg capsules into two types: the "northern" forms (*Bathyraja* and *Rhinoraja*), characterised by a much thickened outer layer with either tubercles or prickles on the surface of a horny, brown substance, and a rather thin inner layer without a noticeable difference in histological structure among different species. Ishiyama attributed the latter form to species belonging to cold-water areas. On the other hand, in the "southern" members (*Dipturus* and *Okamejei*), a specialisation was found not only in the outer layer but in the inner one as well.



Figure 1. Transverse section of the egg capsule of *Atlantoraja cyclophora* (anterior end). d.f.=dorsal face; v.f.=ventral face.

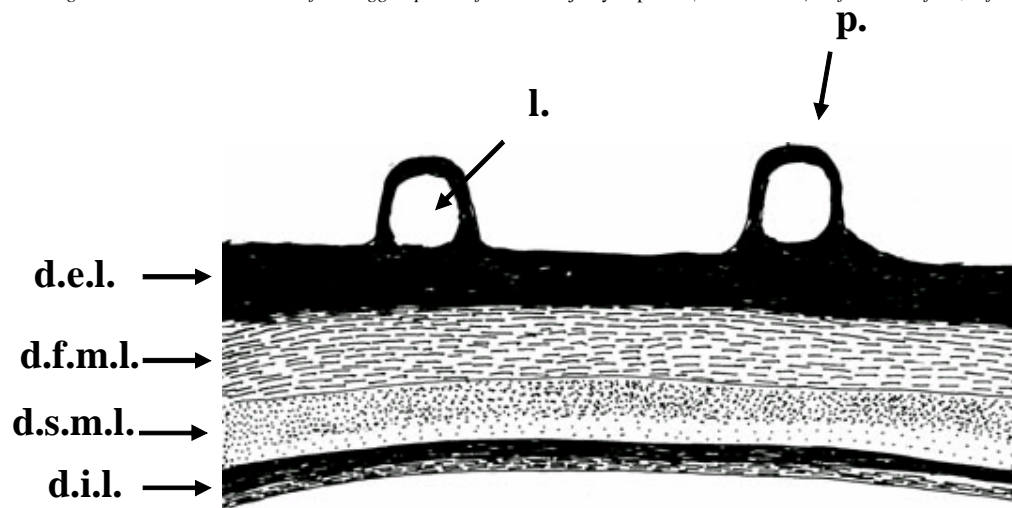


Figure 2. Transverse section of the dorsal face of the egg capsule of *Atlantoraja cyclophora* (200X); p=prickles; l.=lumen; d.e.l.=dorsal external layer; d.f.m.l.=dorsal first middle layer; d.s.m.l.=dorsal second middle layer; d.i.l.= dorsal interior layer.

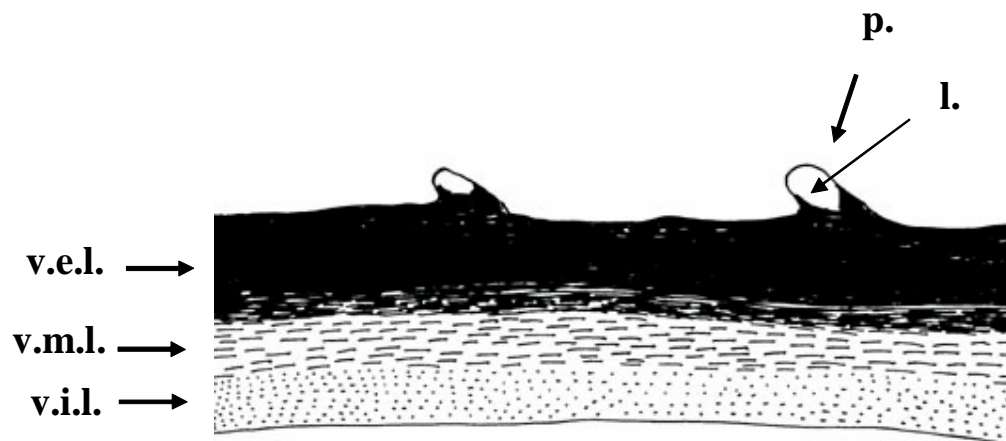


Figure 3. Transverse section of the ventral face of the egg capsule of *Atlantoraja cyclophora* (200X); p=prickles; l.=lumen; v.e.l.= ventral external layer; v.m.l.= ventral middle layer; v.i.l.= ventral interior layer

In Southern Brazil, specimens of *A. cyclophora* are found at up to 300 m in depth and temperatures ranging between 10.0°C and 17.6°C (Vooren 1997, Oddone & Vooren 2004). The pronounced striations in the egg capsule surface could serve as protection for the embryo throughout development, as it has to withstand a more prolonged exposure in colder waters than species that inhabit shallower waters, which are subjected to higher temperatures. Egg capsules of *Sympterygia acuta* and *Rioraja agassizi*, for instance, have smooth surfaces (C.M. Vooren, unpublished data) and these are species that complete their life cycle on the shallower areas of the continental shelf, up to 40-50 m in depth (Vooren 1997).

In *Scylorhinus canicula* the most internal part of the egg capsule is formed by piled *laminae* (Knight & Hunt, 1976) which would likewise correspond with the pulpy layer described by Ishiyama (1958). Krishnan (1959) described for the oviparous shark *Chiloscyllium griseum* the so-called "pulpy layer" as rather uncoloured too (in relation to the most external layers), and being characterised by horizontal lamination. Knight & Hunt (1976), proposed a 45° orientation between both lamina in vertical longitudinal sections for oviparous dogfishes. In both faces of *A. cyclophora*, the central, pulpy layer, was differentiated into two layers. This could reflect the presence of a single protein component but with fibres disposed in different directions.

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