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GONAD MATURATION OF TWO INTERTIDAL BLOOD CLAMS *Anadara granosa* (L.) AND *Anadara antiquata* (L.) (BIVALVIA: ARCIDAE) IN CENTRAL JAVA

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ABSTRACT

The reproductive cycles of male and female Anadara granosa and Anadara antiquata which have been studied and compared by histological techniques showed great anatomical similarity. Gametogenesis is associated with a system of follicle cells which break down as the gametes approach maturity. The arrangement of follicle cells is characteristics of the sex. In the female, gametogonia are peripheral to the follicle cells, whilst in the male they are interstitial. The process of spermatogenesis parallel the classical vertebrate pattern, i.e. successive layers of spermatogenic cells (spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa) occurring more or less regularly in succession toward the centre of the follicle. The diameter of the maximum size oocytes is 75μm for A. granosa and 65μm for A. antiquata. Spawning in both A. granosa and A. antiquata is progressing gradually throughout the year as indicated by the availability of various stages of oogonia and spermatogonia; for which the highest number of oogonia were those of 25-40μm diameter. The histological study indicated that both species are iteroparous with planktotrophic type of development, yet performing a short period of pelagic life (ca 1 month).

Keywords: *Anadara granosa*, *Anadara antiquata*, similar reproductive anatomy, spermatogenesis parallel the classical vertebrate pattern, continues spawning, planktotrophic iteroparous species.

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INTRODUCTION

Anadara granosa and *Anadara antiquata* are closely related marine bivalves. They both inhabit similar ranges of water depth, i.e. from intertidal to marginally sub-tidal zone, yet they prefer different localities, in which *A. granosa* is found in soft mudflats of sheltered estuaries, whereas *A. antiquata* thrives in a sandy-gravel sediments. These infaunal species either live freely unattached, e.g. *A. granosa* or posses a weak byssus to attach to rocks and sheltered crevices like those seen in *A. antiquata*. As a group they are poor burrowers, some living only partly buried.

This genus is commonly distributed as far south as southern Australia and as far north as the islands of Japan, India, and Mediterranean Sea. They often form the

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dominant species of shallow water benthic communities and many species of this genus are gathered for human consumption. However, literature on this wide zoogeographical species which makes them an ideal one for comparative study is inadequate.

Considering that both species are economically as well as ecologically important member in fisheries, study on qualitative changes in histological preparations of the reproductive tissues, and evidence of sequential protandric hermaphroditism on these congeneric species is clearly needed as a part of a wider ecological investigation.

MATERIALS AND METHODS

A. granosa were collected from two sites, *i.e.* Wedung in Demak Regency, and Tapak in the town of Semarang; whereas *A. antiquata* was taken from Bandengan in the Jepara Regency. Sampling of those blood clam populations was conducted at approximately monthly intervals at low water and daytime over 24 months within the period of August 1991 - August 1993.

Selected clams (20-25 individuals) ranging from 15-40mm in shell length were opened at the hinge by cutting the adductor muscles. Smallest blood clams were also included in order to determine the minimum size at which reproductive development first occurred. No spawning occurred between the time of collection and the subsequent processing of the tissues (6-8 hours). Following the routine protocols in Disbrey and Rack (1970), the whole tissue was fixed for 8 hours in saline (30%) Bouin's solution. A piece of tissue was excised transversely through the body mass comprising the digestive gland, reproductive tissue and muscular foot, dehydrated using increasing concentrations of ethanol, then transferred to xylene prior to embedding in paraffin wax (56°C MP). Sections were cut at 7μm, re-hydrated in descending ethanol series before staining in aqueous Haematoxylin-Eosin and mounted in Canada balsam (DPX). Following microscopic examination, stages in the reproductive cycle were photographed.

RESULTS AND DISCUSSION

a. Histology of Gonad Maturation

Examination of the histological preparations of both *A. granosa* and *A. antiquata* reveals no differences in the arrangement of the internal body organs. Also, no marked differences were noted between the two species regarding the colour of the gonad, final shape of the eggs or sperm, or the extent of the fully ripe gonad in relation to other organs. However, from the external appearance the relative sizes of the gonad are somewhat different. When ripe, the gonad of *A. granosa* always appeared to be less slender than that of *A. antiquata.* This is perhaps because *A. antiquata* has thicker blocks of muscular foot, so that the space left to accommodate the reproductive materials is less. The following descriptions thus apply to both species of *Anadara* in this study.

Germ cell development for these two anadarinids is associated with a follicular system similar to that described for *Teredo navalis, Mya,* and *Petricola* (Coe, 1943) and for *Macoma balthica* (Caddy, 1967). Gonad development was found to begin in the basal region of the visceral mass, where the pedal musculature meets the digestive gland. Examination of the fresh visceral mass revealed that to a varying extent the gonad envelops the dark green digestive gland. The male gonad is smooth, white to semi opaque; whilst the female gonad is more granular and red-orange in colour.

The histological study showed that these *Anadara* species are iteroparous for they may reproduce successfully over several seasons. Their reproductive tissue

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consists of many ramifying tubules, in which the primordial cells gave rise to spermatogonia and oogonia in the male and female respectively, as well as to accessory follicle cells in both sexes. The arrangement of these follicle cells enables the two sexes to be differentiated in section, even at this early stage. From this common point of origin, it is convenient to describe the histological changes that take place in the ovary and testis separately.

b. Testis

The process of gametogenesis within the lamellibranch testis has received little attention in the literature compared with that for the ovary. However, Coe & Turner, (1938) and Quayle (1943) account that process of spermatogenesis parallel the classical vertebrate pattern, i.e. successive layers of spermatogenic cells (spermatogonia, primary and secondary spermatocytes, spermatids, and spermatozoa) occurring more or less regularly in succession toward the centre of the follicle. Coe & Turner (1938) also states that for *Mya arenaria* sperm are proliferated from spermatogonia on the follicle wall. Despite the fact that no plates of the earlier stages are presented by any of these authors, the sequential arrangement of the germinal cells in male reproductive tissues of *A. granosa* and *A. antiquata* studied here appeared to be the same as their description for *Mya arenaria*.

The stages of spermatogenesis and oogenesis for both *A. granosa* and *A. antiquata* are similar to the usual pattern described for bivalves, included follicle (nutritive) cells of the sort observed in *Paphia staminea, Macoma balthica, Abra alba, Abra tenuis* or *Tapes philippinarum* (Quayle, 1943; Caddy, 1967; Nott, 1980; Sbrenna and Campioni, 1994) in either sex of both species. Male individuals appeared to have a faster rate of gametogenic activity than females. Meanwhile, head of spermatozoa measured only 3μm with an average tail length 50μm. immature sperm cannot move properly. Early stages of male reproductive system exhibits a similar division into follicle cells and primary germ cells observed in females. However, instead of peripheral arrangement of gonia, these are arranged interstitially to the follicle cells through the whole space of the developing follicle (Plate 1A). As in the female, the follicle cells break down (Plate 1A: empty rounded spots), so that sperm developed from the spermatogonia in the middle of the follicle are released into suspension. Even when the follicle cells have disappeared, the spaces left by them are quite evident (Plate 1A). At this point, mature sperm are arranged with their acrosomes in a centripetal position and their tails occupying the central position of the lumen (Plate 1B). In this way, the classical conformation is established with the earliest stages lying near to the follicle wall (Plates 1C, 1D). Even in the most mature males some early spermatogenic stages are always found lining the follicle wall as a narrow band (Plate 1E); these also persist after spawning (Plate 1F) and it seems probable that they act as a reservoir of germ cells for follicle redevelopment (Plates 1G, 1H).

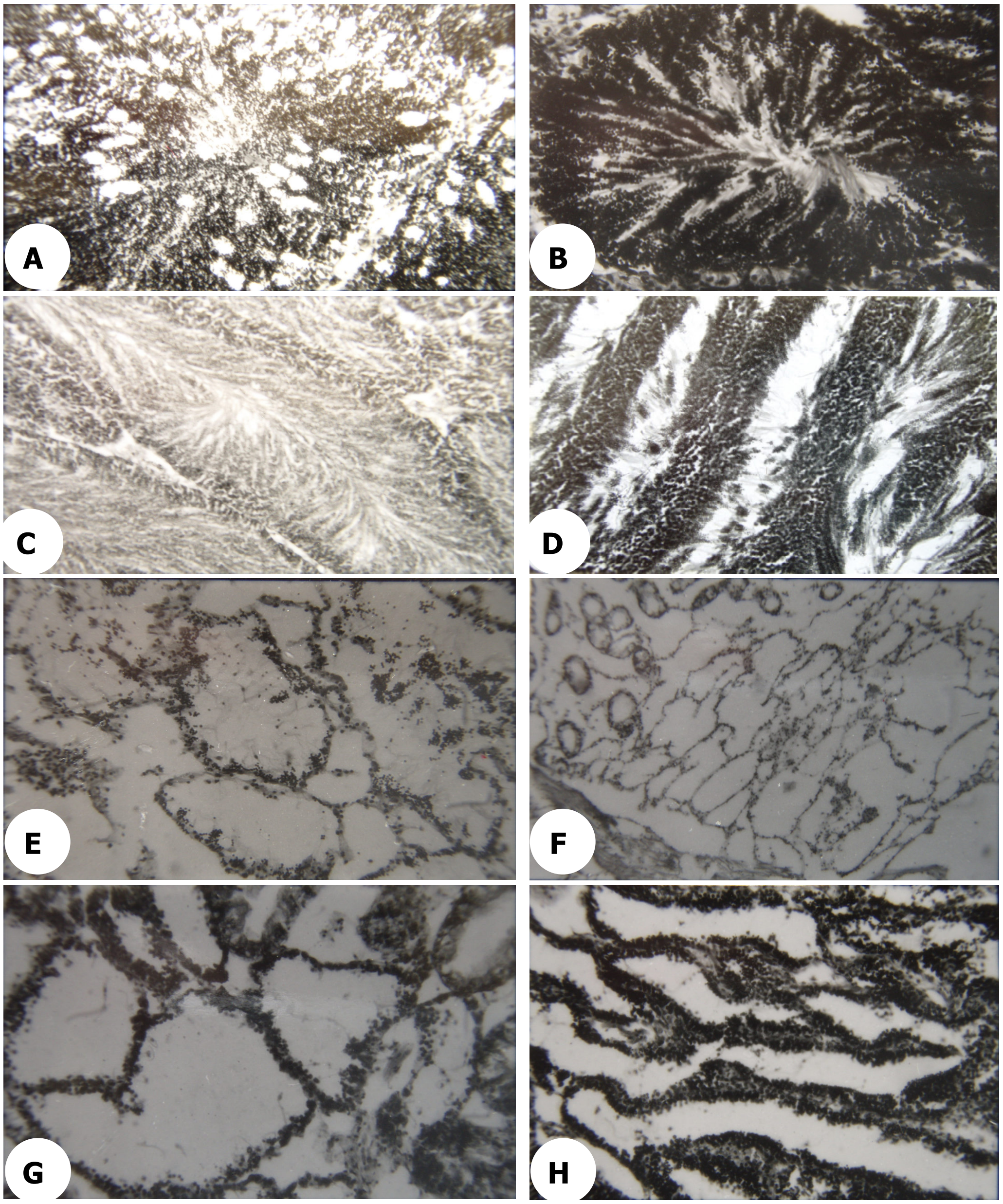

Plate 1. Photomicrographs of sectioned male gonads of *A. granosa* and *A. antiquata* at various stages of development. A) Early male follicle with densely staining spermatogonia in the interstices of the follicle cells, spaces left by disintegrated follicle cells still evident, x250. B) Ripe male stage 3, sperm are arranged with their acrosomes in a centripetal position where their tails occupying the central position of the lumen, x250. C) Ripe male stage 3, sperm streaming towards centre of the follicle, x100. D) Spawning male stage 2, with layers of ready-to-develop spermatogonia lining the follicle wall, x250. E) Spawning male stage 1, some lumen contains residual sperm, x250. F) Empty follicles at spent stage, x100. G) Redeveloping male stage 1, a layer of undifferentiated early stage spermatogonia line the empty follicle, x250. H) Redeveloping male stage 2, a layer of several cells deep of developing spermatogonia. The spermatids can be differentiated under higher magnification, x250.

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c. Ovary

During oogenesis, the oogonia are initially flattened and attached to the follicle wall by the broad micropylar surface (Plate 2A). As they grow, they become more elongated and basally constricted (Plate 2B). When the follicles approached maturity, the accessory cells break down and the rapidly growing oocytes increase in volume. Finally the oocytes detach from the follicle wall and round off in the lumen. Nuclei in these unfertilised oocytes were clearly visible (Plate 2C). At this stage, the animal can be considered mature. In this condition the visceral mass is distended with gametes which are readily visible through the thin body wall for *A. granosa*. In *A. antiquata* however, this ripe stage is less visible macroscopically due to their thicker body wall.

After spawning (stage 2), follicles still contain few mature ova (Plate 2D). In both sexes, gametogenic activity which takes place from the undifferentiated cells lining the old follicles may proceed simultaneously at this stage thus making a quick transition to the active stage of redeveloping. At a later stage of spawning (stage 1, Plate 2E), re-sorption of un-spawned oocytes progresses with the development of the next generation of oocytes. However, it is not clear how the old follicles in redeveloping stages redevelop and nurture the new sets of oogonia, and a similar uncertainty exists concerning the mechanism of re-sorption of un-spawned oocytes.

Table 1. The average density and the overall mean oocytes size for *A. granosa* in Wedung (n= 423), and Tapak (n = 270), and *A. antiquata* in Bandengan (n = 403) throughout 25 months study period (± 1 Standard Deviation).

Parameters	Populations		
	A. granosa		*A. antiquata*
	Wedung	**Tapak**	**Bandengan**
Mean Overall Density of Oocytes, mm^{-2}	471 ±75	440 ± 155	272 ± 176
Average Density of Various Size of Oocytes, mm^{-2}			
Small (5-20µm)	119 ± 35	171 ± 65	71 ± 44
Medium (25-40µm)	269 ± 44	224 ± 52	178 ± 51
Large (45-65µm)	82 ± 34	45 ± 37	25 ± 20

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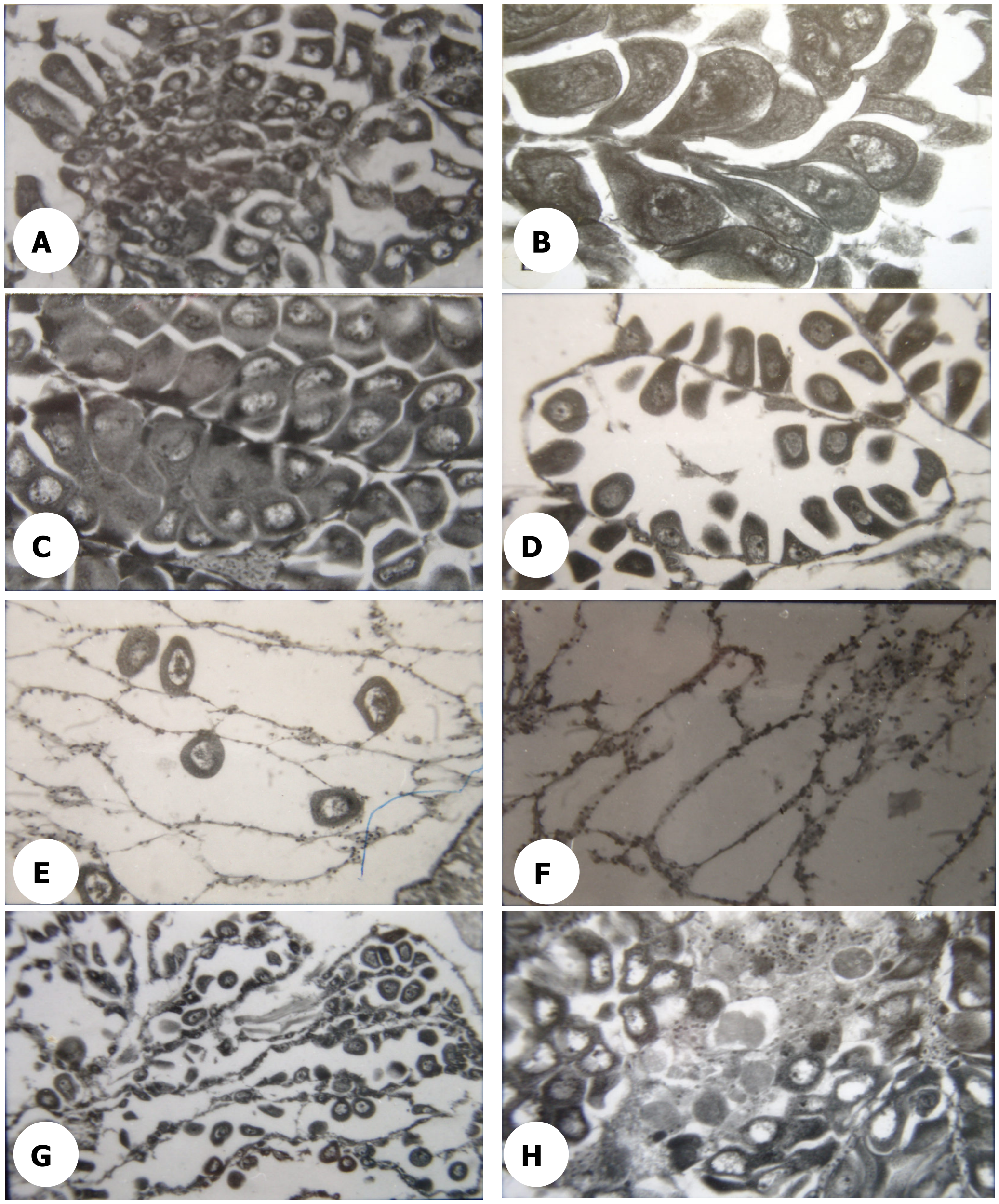

Plate 2. Photomicrographs of sectioned female gonads of *A. granosa* at various stages of development. A) Developing female stage 1, showing a number of early stage oogonia with some elongated oogonia, x250. B) Developing female stage 2, stalked oocytes attached to the germinal epithelium of the follicle, x400. C) Ripe female stage 3, mature oocytes at maximum density, x250. D) Spawning female stage 2, empty spaces left by the discharged oocytes appear in the centre of the lumen; as the results of the reduced pressure the oocytes appear rounded in shape, x250. E) Spawning female stage 1, empty lumen with thin follicle wall and some residual rounded oocytes, x250. F) Spent stage, completely empty follicle, sex is undetermined, x250. G) Redeveloping female stage 1, a number of early stage oogonia and some residual oocytes in large, elongated and relatively empty follicles, x100. H) Redeveloping female stage 2, follicles are approximately full of developing oogonia and mature oocytes, x100.

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Differences in the reproductive strategies of pairs of closely related species have been reported by Menge (1975) for *Pisaster ochraeus* and *Leptasterias hexactis*, and by Hartnoll (1976) for *Alcyonium digitatum* and *Alcyonium hibernicum*. Also Menge (1975) cites several other examples of such pairs of related species where the larger has pelagic larvae and the smaller broods its offspring. They concluded that size differences may be responsible for the different reproductive strategies adopted by two otherwise similar species. The diameter of the maximum size oocytes is 75μm for *A. granosa* and 65μm for *A. antiquata*. Mature eggs appear spherical and brownish with distinct nuclei, whilst immature eggs tend to be irregularly shaped and stalked at one end. Accordingly, the average size of oocytes throughout the study period was divided into three categories, i.e. small (5-20μm), medium (25-40μm) and large (45-65μm; **Table 1**).

Irrespective of the time of collection and size of the individual clams, the average density of oocytes in *A. antiquata* is much lower than that of *A. granosa*, suggesting a lower fecundity (Table 1). On the basis of size composition and oocyte densities amongst the populations of *A. granosa*, it seems that the Tapak population maintained more continuous reproductive activity by having a relatively higher percentage of small oocytes in reserve, albeit that the overall average density of oocytes is lower than amongst female specimens in Wedung (Table 1). Moreover, the narrow range of confidence interval (standard deviation) for the oocyte densities of *A. granosa* from Wedung suggested a relatively uniform reproductive condition amongst member of the population. This confirms that they were approximately in the same stages of reproduction at any given time. Meanwhile, a wider range of standard deviation for oocyte densities was noted for Tapak and particularly for the Bandengan populations (Table 1), suggesting more variability amongst female individuals with respect to their reproductive condition. Physiological factor which may account for this difference is the pinnotherid crab infestation in the population of *A. antiquata* in Bandengan (Afiati, 2002). This pea crab was never found in *A. granosa* from Tapak and was observed only once (0.0013%) in an isolated Wedung specimen. During the two consecutive years of study, the level of occupancy of *A. antiquata* by the crabs varied. The highest percentage occurred was 44.3%, and indeed the condition of crab-infested clams was significantly poorer ($0.012<P<0.02$) than those of the un-infested ones. Moreover, histological preparation of reproductive tissues of the infested clams has never observed their peak of ripeness; whilst the 75.10% undifferentiated *A. antiquata* also suggesting reproductive injuries among those infested. Morphologically, *A. antiquata* has indeed thicker blocks of muscular foot, so that the space left to accommodate the reproductive materials is less.

Table 1 suggests that spawning in both *A. granosa* and *A. antiquata* is progressing gradually throughout the year as indicated by the availability of various stages of oogonia; for which the highest number were those of 25-40μm diameter. Progressive spawning would provide larvae in the plankton for a considerable length of time, at densities depending upon the intensity of spawning. It is also apparent that the reproductive strategies differ considerably for the two species (Table 1). On the basis of same size range of egg diameter (45-65μm), *A. granosa* produced 48 – 116 ripe eggs per mm^2, while *A. antiquata* produced only 25 – 45 ripe eggs per mm^2. In both cases, the size and number of the eggs indicates planktotrophic development but with a short period of pelagic life (*ca* 1 month; Wong *et al*, 1985; Afiati, 1994; 1999a; 1999b), as Sastry, (1979) stated that such planktotrophic larvae spend long periods in

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the plankton (>3 months) compared to lecitotrophic ones (<3 months).
The smallest size at which reproductive tissue was observed histologically in *A. antiquata* revealed that sections of 18.4-19.8mm animals had not yet become sexually active. Some juveniles measuring 20.7-22.1mm showed mature gonads and the smallest females with ripe ovaries were found at 23-25mm shell length. Meanwhile, for *A. granosa* it occurred when the animal had attained 15.6-15.7 mm shells length, whereas differentiated specimens less than 20mm long are mostly males (Afiati, 1994). It is thus clear that *A. antiquata* reached a size larger than that of *A. granosa* before they began to undergo gonad development.

So, even though fecundity increases with size in most marine bivalves (Nott, 1980), and therefore planktotrophic development in *A. antiquata* – in particular for this population frequently infested by pea crab (*Pinnotheres* sp) - would otherwise result in the production of less number of larvae than in *A. granosa* (Table 1) and to the survival of even less, the more stable environments inhabited by this species and its growth strategy to escape predation pressure (Afiati, 1994) seemed to insure sustainability of this species in Bandengan.

CONCLUSION

Following the findings of Pathansali & Soong, (1958); Pathansali, (1964); Broom, (1983); Wong *et al* (1985), the blood clam *A. granosa* and *A. antiquata* (Toral-Barza & Gomez, 1985) has separate sexes yet is not sexually dimorphic. *A. antiquata* produce less number and smaller size of oocytes than that of *A. granosa*. From the ecological point of view, the difference in size composition and oocyte densities among the populations, likewise in *A. granosa* from Tapak, might reflect environmental disturbance experienced by the population. Yet, for *A. antiquata*, some natural drawbacks in its reproduction strategy seemed to be poised by its unique somatic growth strategy and stable habitat to secure survivorship of this prototype Anadarinid species.

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