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Ultrastructure of oogenesis of two oviparous demosponges: *Axinella damicornis* and *Raspaciona aculeata* (Porifera)

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A B S T R A C T

We investigated the cytology of the oogenic cycle in two oviparous demosponges, *Axinella damicornis* and *Raspaciona aculeata*, during 2 consecutive years both by light and electron microscopy. Oocytes of both species were similar in their basic morphological features but differences were noticed in time required to complete oocyte maturation and mechanisms of acquisition of nutritional reserves. The oogenic cycle of *A. damicornis* extended for 7–8 months in autumn-spring, while that of *R. aculeata* did it for 3–5 months in summer-autumn. Yolk of *A. damicornis* was predominantly formed by autosynthesis. Oocytes endocytosed bacteria individually and stored them in groups in large vesicles. Bacteria were digested and lipidic material was added to the vesicles to produce a peculiar granular yolk hitherto unknown in sponges. Scarce cells carrying heterogeneous inclusions were observed in the perioocytic space, and were interpreted as putative nurse cells. Such cells were presumably releasing lipid granules to the perioocytic space. In contrast, large numbers of nurse cells were found surrounding the oocytes of *R. aculeata*. They transported both lipid granules and heterogeneous yolk bodies to the oocytes. *R. aculeata* also produced some of their yolk by autosynthesis. The involvement of nurse cells in the vitellogenesis of *R. aculeata* shortened the oocyte maturation, whereas a largely autosynthetic vitellogenesis in *A. damicornis* prolonged the duration of oogenesis.

1. Introduction

Sexual reproduction in demosponges exhibits profuse variation in terms of mode (oviparism/viviparism and gonochorism/hermaphroditism), dynamics (from very short gametogenic cycles to nearly continuous gametogenesis) and morphology of gametes (Fell, 1974; Reiswig, 1983; Simpson, 1984; Boury-Esnault and Jamieson, 1999; Riesgo et al., 2007a,b; Riesgo and Maldonado, 2008a). Origin of gametes in demosponges is also diverse. To date there is no evidence of a predetermined germline, and demosponge gametes are known to derive from at least three types of somatic cells (archaeocytes, choanocytes, or storage cells), depending on the species (Fell, 1974, 1983; Reiswig, 1983; Simpson, 1984; Willenz and Hartman, 2004).

Oocytes usually derive from archaeocytes (see Fell, 1983 and Simpson, 1984 for reviews), although in few cases, choanocytes have been suggested as the oocyte anlagen (Diaz, 1973a,b; Gaino et al., 1986). Oocytes usually develop relatively scattered through the sponge mesohyl (Fell, 1983; Simpson, 1984), though in some species appear clustered or aggregated (e.g., Lévi, 1956; Diaz, 1973a,b; Fell and Jacob, 1979; Fromont, 1994; Riesgo et al., 2007b). Demosponge oocytes usually differ morphologically in aspects such as oocyte size, type and abundance of yolk, presence of enveloping nurse or follicular cells, and collagenous covers (see Fell, 1974, 1983; Simpson, 1984 for reviews).

Yolk formation (i.e., vitellogenesis) in poriferans, has been reported to take place by: 1) autosynthesis, with or without using pino- or endocytosed basic precursors (proteins, lipids, etc); 2) heterosynthesis, with yolk and/or yolk precursors supplied by somatic cells (nurse cells), or 3) both types simultaneously (Fell, 1974, 1983; Simpson, 1984; Sciscioli et al., 1991). All these three types of yok formation patterns have been also reported for most marine invertebrates (Nørrevang, 1968; Anderson, 1974; Eckelbarger, 1994; Ramírez Llodra, 2002). It is postulated that basal invertebrates predominantly form their yolk by the most “primitive” mechanism: autosynthesis (Eckelbarger, 1994). The diverse populations of inclusions traditionally called yolk can be roughly divided into fatty yolk -lipid droplets- and proteid yolk -composed of protein and carbohydrates (Nørrevang, 1968; Anderson, 1974). Proteid yolk shows a remarkable uniformity throughout the animal kingdom, occurring as membrane-bound electron-dense bodies with a homogeneous structure that, in some cases, possess a finely outlined dense core

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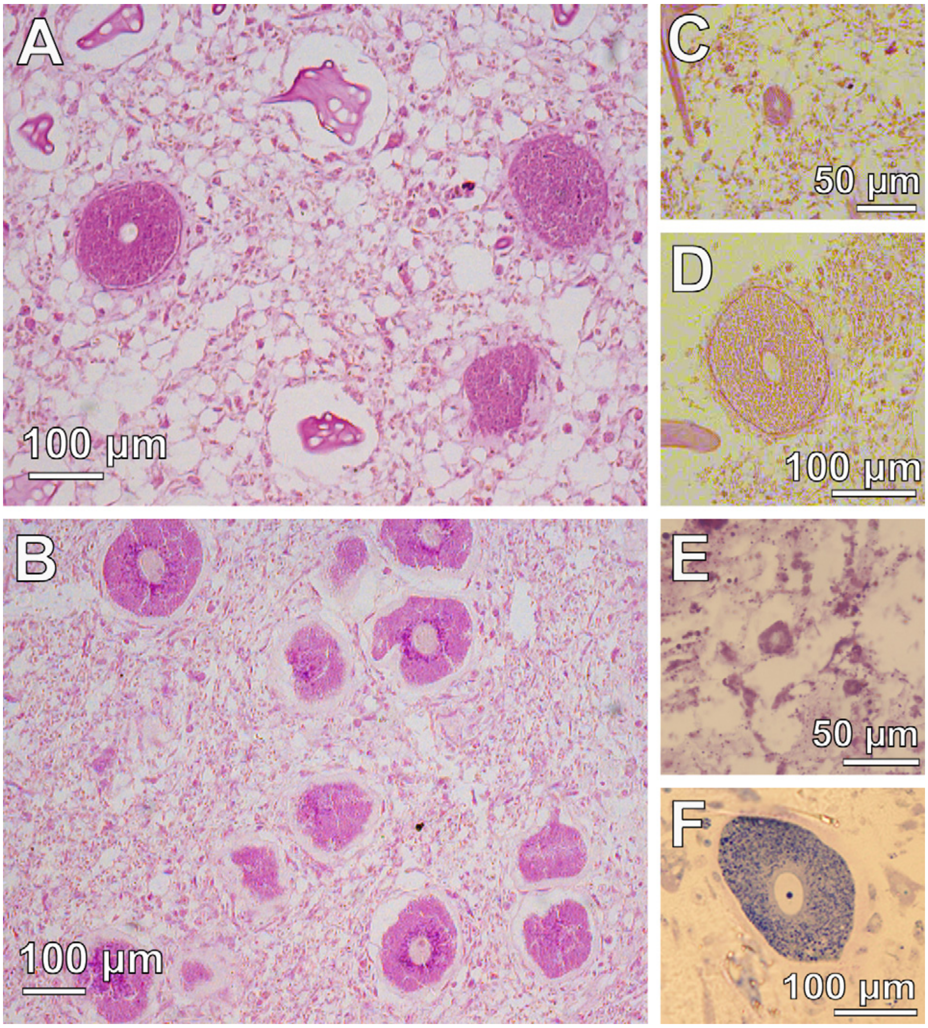

Fig. 1. Morphology and location of oocytes of *Axinella damicornis* and *Raspaciona aculeata* studied by light microscopy. Mature oocytes spread homogeneously within the mesohyl of (A) *Axinella damicornis* and (B) *Raspaciona aculeata*. Young (C) and mature (D) oocytes of *Axinella damicornis*. Young (E) and mature (F) oocytes of *Raspaciona aculeata*.

embedded in a less dense matrix (Nørrevang, 1968; Anderson, 1974; Adiyodi and Adiyodi, 1983). Only in some invertebrates, such as some molluscs, polychaetes, and echinoderms, yolk looks heterogeneous (e.g., ringed or vesiculated) (Anderson, 1974). In demosponges, yolk has been reported to have both homogeneous (e.g., Lévi and Lévi, 1976; Gallissian and Vacelet, 1976; Sciscioli et al., 1989, 1991, 2002; Riesgo et al., 2007a) and heterogeneous appearance (e.g., Diaz et al., 1975; Watanabe, 1978; Gaino et al., 1986; Gaino and Sarà, 1994; Lepore et al., 1995), but the latter is more common and abundant.

Since vitellogenesis remains poorly documented in oviparous demosponges, we selected two species belonging to different orders: *Raspaciona aculeata* (Johnston, 1842) (order Poecilosclerida) and *Axinella damicornis* Esper, 1794 (order Halichondrida). The reproductive cycle of *Raspaciona aculeata* has been studied by Riesgo and Maldonado (2008b), who reported the oogenesis to occur from July to November and described the gametes using light microscopy only. Similarly, little is known about the gametogenesis of *A. damicornis*. The duration of the gametogenic cycle was studied by Siribelli (1962) in the western coast of Italy and by Riesgo and Maldonado (2008b) in the northeastern coast of Spain. Whereas Siribelli (1962) reported the oogenesis to extend from February to July, Riesgo and Maldonado (2008b) documented it from October to May. In contrast, in the western coast of France, Lévi (1950) documented spawning of mature eggs of *A. damicornis* in September. Thus, it appears that timing and duration of oogenesis in *A. damicornis* depend on the particular environmental characteristics of each location. Again, all these studies described only the basic morphology of gametes using light microscopy. Therefore, since ultrastructural features of female gametes of *A. damicornis* and *R. aculeata* are largely unknown, we decided to investigate these oogeneses using light and electron microscopy to describe the process of oocyte maturation, with focus on the mechanisms of yolk production and storage.

2. Materials and methods

2.1. Sampling

We studied two populations of the oviparous demosponges *Axinella damicornis* and *Raspaciona aculeata*, in the sublittoral rocky communities of the North-eastern Mediterranean coast of Spain, between the localities of Blanes and Tossa de Mar (41° 11′ 18″ N, 2° 45′ 2″ W). A previous 2-year study based on repetitive monthly