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Reproductive Biology, Taxonomy, and Aspects of Chemical Ecology of Latrunculiidae (Porifera)

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Abstract. Sexual reproduction has been observed for the first time within the widely distributed sponge family, the Latrunculiidae. *Latrunculia magnifica* Keller 1889 was studied mainly in the northern Red Sea in the Gulf of Eilat and the Gulf of Suez. The sponge is hermaphroditic and viviparous. The embryo develops to a large (mean 868 ± 144 μm, max. 1200 μm) parenchymella larva. The period of reproduction lasts for several months, ceasing only during the winter. Like oocytes, sperm appear to develop from archeocytes, which is uncommon among sponges. The presence of brooded larvae in *L. magnifica* supports the position of Latrunculiidae within the order Poecilosclerida, subclass Ceractinomorpha, rather than within the Tetractinomorpha. The absence of a periflagellar sleeve from around the base of the choanocyte's flagellum lends further support to this idea. Nuclear magnetic resonance (NMR) analysis of secondary metabolites within the sponge and its nudibranch predator (*Chromodoris quadricolor*) confirms that both species contain the same latrunculin homologue (either A or B). The latter finding indicates the presence of a compound derived from the diet (*i.e.*, sponge) within the nudibranch.

Introduction

Sponge species belonging to the family Latrunculiidae are distributed from the polar region (*e.g., Latrunculia apicalis*) to the tropics (*e.g., L. corticata*) and from shallow to deep (2460 m) water (Hartman, 1982). Despite the wide distribution of this family, the biology of its members has rarely been studied, and their mode of sexual reproduction is virtually unknown. The sole evidence about latrunculiid development is that on some occasions young sponges were observed developing directly within the parental body without first going through a free larval stage (Bergquist, 1978).

The Latrunculiidae is characterized by small spicules (microscleres) of the discorhabd type, which are usually densely packed in the dermal crust (Fig. 5). The large spicules (macroscleres) are monoactinal, diactinal, or both, and are arranged either radially at the surface with an additional axial orientation or in a confused disposition at the inner parts of massive forms (Bergquist, 1978; Hartman, 1982; Soest, 1984). Latrunculiidae is one of three sponge families that do not possess chelae microscleres, but which Lévi (1973) (following Topsent, 1928) assigned to the order Poecilosclerida (subclass Ceractinomorpha). His arguments are based on the family's affinities with two other well-established Poecilosclerid families (Mycalidae and Esperiopsidae). The taxonomic position of the Latrunculiidae is, however, still not clear. In fact, some researchers believe this family should be assigned to the order Hadromerida, subclass Tetractinomorpha (Dendy, 1922; Burton, 1930; Bergquist, 1978; Hartman, 1982). Their association of the Latrunculiidae to this order relies on the type of the megascleres, their arrangement within the sponge, and the surface position of the microscleres. Soest (1984) states that the arguments are weak for either choice of association of Latrunculiidae with any of these orders.

A representative species within this family, *Latrunculia magnifica* Keller 1889, is a bright red branching sponge, prominent in the coral reefs of the Red Sea. Its geographical distribution stretches from Djibouti (Gulf of Aden, Indian Ocean) to Eilat (Northern Red Sea) (Keller, 1889; Row, 1911; and Ilan, unpub.).

The major aim of the present study was to determine the mode of reproduction of this species. But in addition to the ecological implications of revealing the previously unknown mode of reproduction in the Latrunculiidae, such an investigation would also help clarify the taxo-

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nomic position of the Latrunculiidae. This is because the mode of embryonic development is a major criterion for distinguishing between the two subclasses Tetractinomorpha and Ceractinomorpha. An additional criterion distinguishing Hadromerida from other sponge orders is the presence of a periflagellar sleeve around the base of the choanocytes' flagella (Vos *et al.,* 1991). Therefore, a study of the choanocyte chambers was also carried out.

Chemical studies have established that *L. magnifica* contains macrolides, termed latrunculins, that are ichthyotoxic. These compounds interfere with actin rather than tubulin organization and thus alter cell shape and disrupt microfilament organization (Neeman *et al.,* 1975; Spector *et al.,* 1983, 1989; Schatten *et al.,* 1986). Several closely related macrolides were isolated from *L. magnifica,* with the two homologues latrunculin A and B constituting the major components (Groweiss *et al.,* 1983). Of 20 different animal collections, all those collected from the Gulf of Eilat contained latrunculin B, the two from the Gulf of Suez contained latrunculin A, and those from the Tiran straits contained a mixture of both macrolides (Groweiss *et al.,* 1983). The chemical content of different sponge populations from throughout the range of the species distribution has not yet been carefully examined. So the validity and significance of this geographical trend as well as the possibility that it extends to larger regions remain open.

The nudibranch *Chromodris quadricolor* preys upon *L. magnifica.* Mebs (1985) raised the possibility that latrunculin derived from the sponge might protect its nudibranch predator from predation by fish. He demonstrated that spots of extracts made from *L. magnifica, C. quadricolor,* and nudibranch mucus appear in thin layer chromatography (TLC) to have the same retention time (Rf) as latrunculin B (although with TLC one cannot differentiate latrunculin A from B). More conclusive evidence has not yet been provided. Other studies have also exhibited the presence of the same secondary metabolites in various sponge species and their nudibranch predators (*e.g.,* Faulkner and Ghiselin, 1983; Rogers and Paul, 1991; Gavagnin *et al.,* 1992). Because *L. magnifica* contains both latrunculin A and B, we may ask: (1) Are these compounds distributed among sponges in geographically distinct populations, and can the compounds be used as chemotaxonomical markers to distinguish between populations? (2) What kind of compounds are unambiguously present within the nudibranchs, and do they follow the geographical distribution pattern exhibited by the sponges?

Materials and Methods

Sponge collection

The study area was in the northern Red Sea. Most of the samples were collected in the vicinity of the Interuniversity Institute, Marine Biological Laboratory, Eilat, located at the northern end of the Gulf of Eilat. Additional samples were collected along the Sinai Peninsula: along the Gulf of Eilat, in the vicinity of the Tiran straits, and at several locations in the Gulf of Suez. One collection is from the Central Red Sea (27°44′N, 34°07′E), one from the South Red Sea (Dahlak Island), and one from the Gulf of Aden—Indian Ocean (Djibouti).

On average three sponges were collected each month in the northern Red Sea, but occasionally (once or twice a year) oocytes and embryos were sought in cuts made in tens to hundreds of sponges during their collection for chemical studies in other locations in the Red Sea.

Anatomical studies

Samples of tissue were fixed each month either in preparation for electron (EM) or light (LM) microscopy. The fixative was 2.5% glutaraldehyde for EM and 4% formalin for LM. Both solutions were buffered in seawater. Preparation for transmission EM and LM included dissolving the siliceous spicules by treatment with hydrofluoric acid for 4–6 h. For scanning EM the procedure included washing, dehydration, critical-point-drying in liquid CO_2 and coating with gold palladium. A JEOL JSM-840A SEM was used. For transmission EM the samples were postfixed with 1% OsO_4, dehydrated, embedded, sectioned and stained with acetate and lead citrate. A JEOL 1200-EX TEM was used. Samples for LM were rinsed in 70% ethanol, dehydrated, embedded, sectioned, and stained with hematoxylin and eosin.

Every sponge was examined for reproductive elements. If present, these were measured and the developmental stages determined. The aim was to establish the timing and duration of the reproductive season, whether the species is gonochoric (separate sexes) or a simultaneous hermaphrodite, whether it broods its embryos or sheds gametes to the water, what kind of embryos develop in this species, and what type of cells give rise to the gametes.

Chemical analysis

For this study, sponges were collected from different sites in the North Red Sea (Eilat, Tiran straits, Gulf of Suez), the South Red Sea (Dahalak Archipelago), and the Indian Ocean (Djibouti). Nudibranchs were collected only in Eilat and the Gulf of Suez. The content of latrunculin within the sponges (*L. magnifica*) and nudibranchs (*C. quadricolor*) was evaluated as follows. Sponges and nudibranchs were freeze-dried and then extracted three times in a mixture of CH_2Cl_2-MeOH (9:1, v:v). The solvent was then removed under vacuum, and the residue of each extract was separated into three fractions by vacuum chromatography through a short silica gel column, with CH_2Cl_2, 2% MeOH-CH_2Cl_2, and 10% MeOH-CH_2Cl_2, as

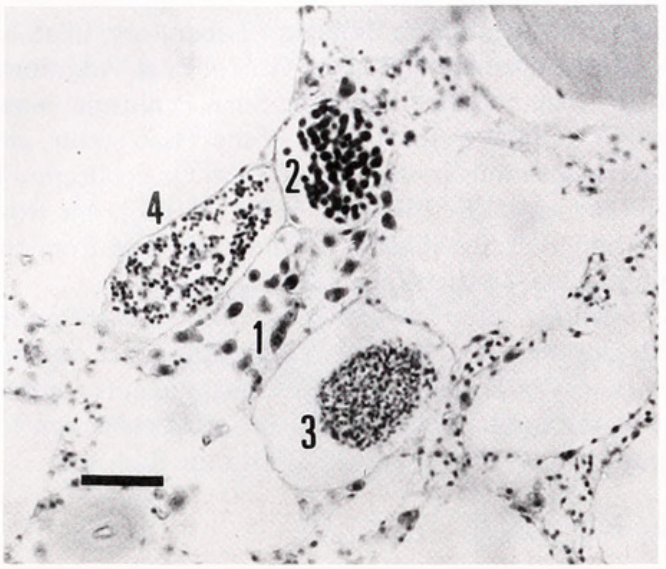

Figure 1. *Latrunculia magnifica* spermatogenesis. Four developmental stages of spermatozoa (1–4), demonstrating an increase in number coinciding with decrease in size of the spermatids in each spermatic cyst. The development appears to start with a few large archeocytes (1) which diminish in size and proliferate to become many small sperm (4). Scale bar size = 25 μm.

eluant. The solvent was removed from each fraction under vacuum. The fractions obtained from nudibranch extracts were compared by TLC and ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) with authentic samples of latrunculins and with extracts of the sponge.

Results

Latrunculia magnifica was usually found in water deeper than 3 m, but also in shallower water near the Tiran straits or in other locations with strong water currents. In shallow water, the encrusting form was more common, while among deeper sponge populations the usual branching form was dominant (data not shown). The sponge prefers walls with a steep to negative slope, or protruding bodies (*sensu* Abelson *et al.*, 1994). Frequently the sponge harbored epi- and endosymbionts, such as brittle stars, barnacles, starfishes, amphipods, polychaetes, and the associated nudibranch *Chromodoris quadricolor*.

L. magnifica individuals contained reproductive products during most months of the year. The small number of sponges collected during some months hampered quantitative determination of the proportion of sponges within the population that were reproducing. Individuals were devoid of reproductive elements only during winter (November to January). Primary oocytes were noticed (in some, but not all sponges) starting from February until September. Incubated larvae were detected between June and October.

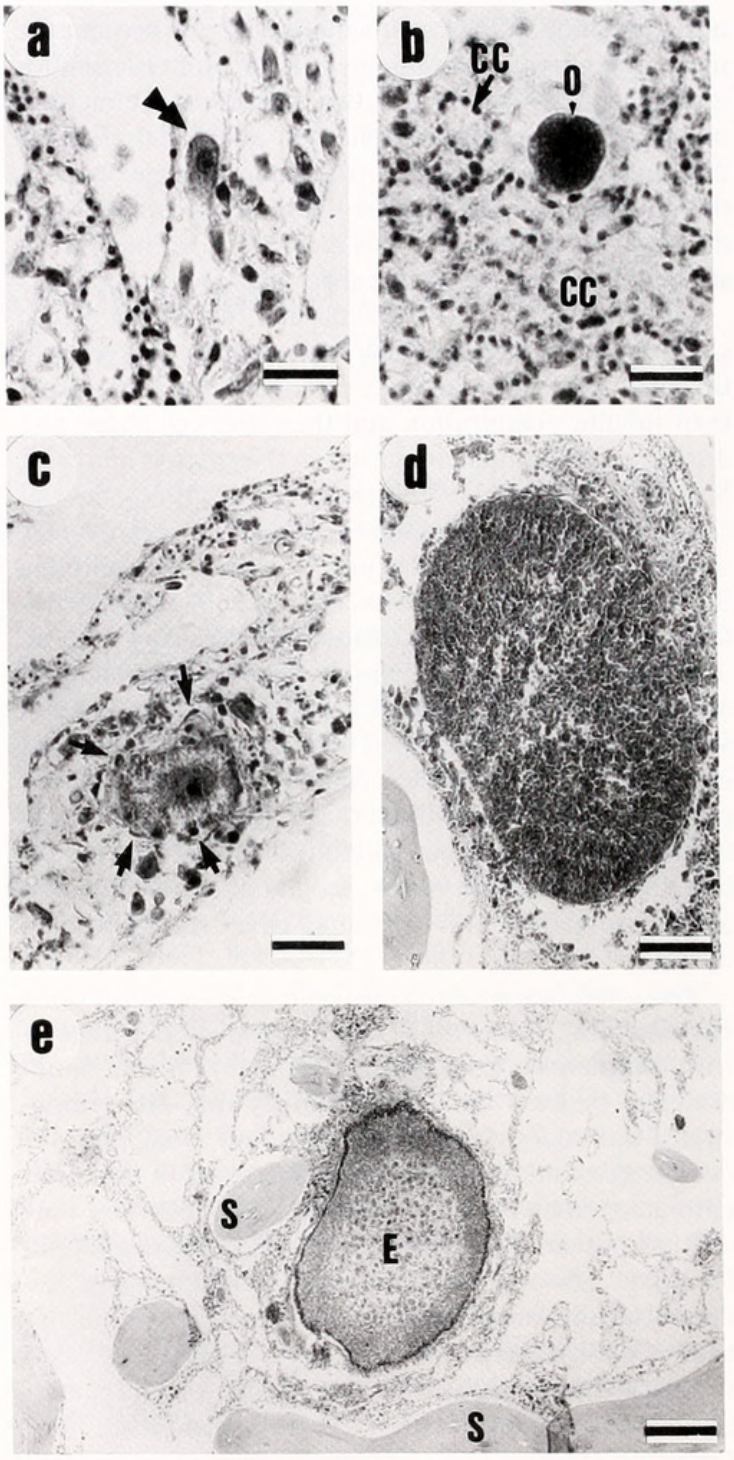

Figure 2. *Latrunculia magnifica* oocyte and embryonic development. (a) Primary oocytes (arrowhead) are slightly larger than nearby amebocytes. Scale bar = 25 μm. (b) Primary oocytes (o) larger than in 2a. For comparison see choanocyte chamber (cc) adjacent to the developing oocyte. Scale bar = 25 μm. (c) The growing oocyte, still with a prominent nucleus and nucleolus, is surrounded by nurse cells (arrows). Scale bar-50 μm. (d) A mature oocyte that has reached 570 μm. The nucleus is no longer evident. Scale bar = 100 μm. (e) A developing embryo (E) contains several types of cells. Those that will differentiate into ciliated cells are at the periphery of the embryo. Spongin fibers (s) can also be seen. Scale bar = 100 μm.

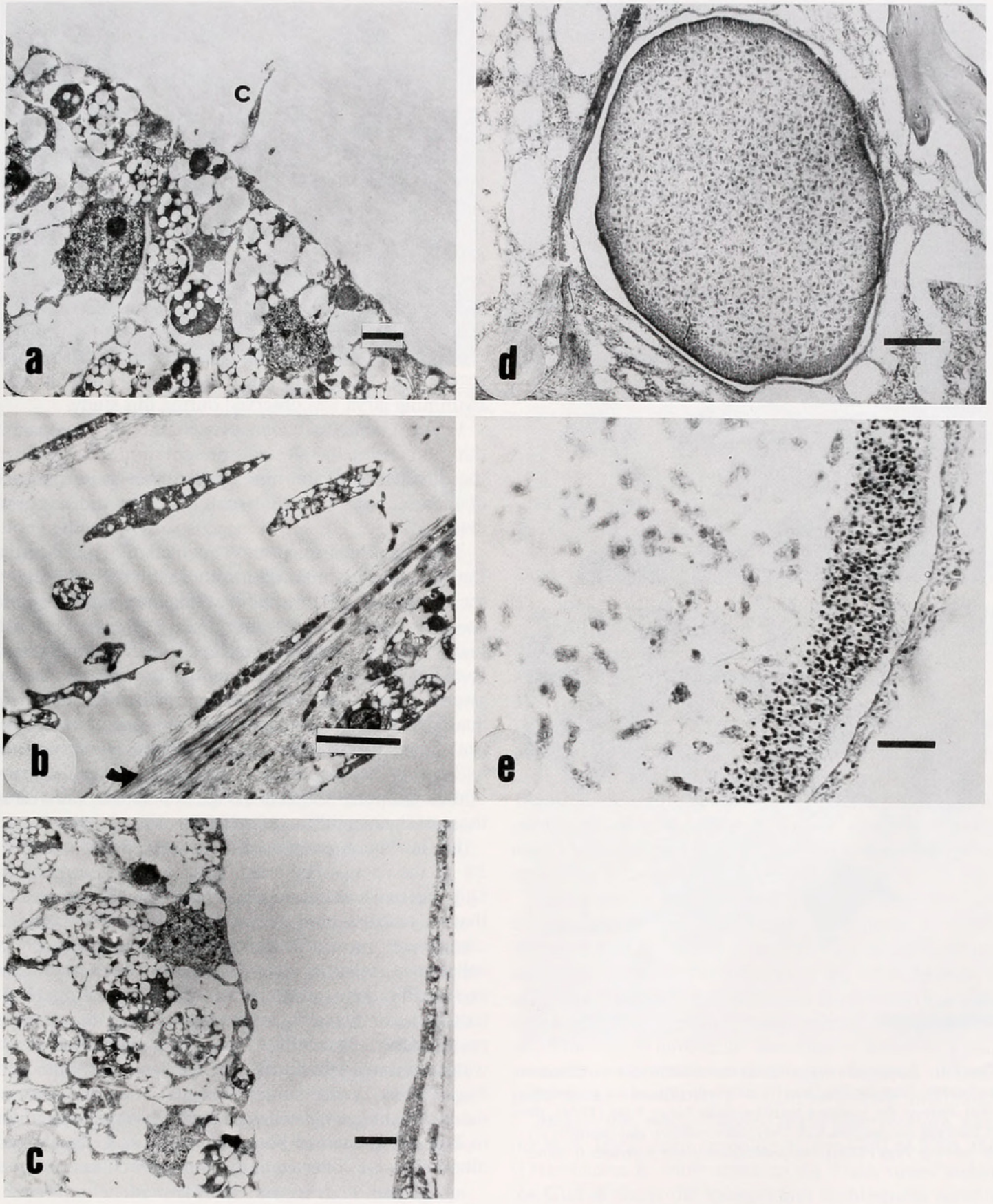

Figure 3. *Latrunculia magnifica* larval development. (a) Surface of the embryo. Elongated cells, with only a few having a cilium (c). Scale bar = 2 μm (TEM). (b) The area surrounding the developing embryo. Collagen deposition (arrow). Scale bar = 5 μm (TEM) (c) Embryo (left) and its cyst envelope (right). Scale bar = 2 μm (TEM). (d) Cross section through an incubating larva. Several cell types are evident, and some collagenous deposition inside the larva occurs. Scale bar = 100 μm (LM). (e) The surface of a developing larva is densely beset with ciliated cells. Inside the larva various cell types are evident. Scale bar = 25 μm (LM).

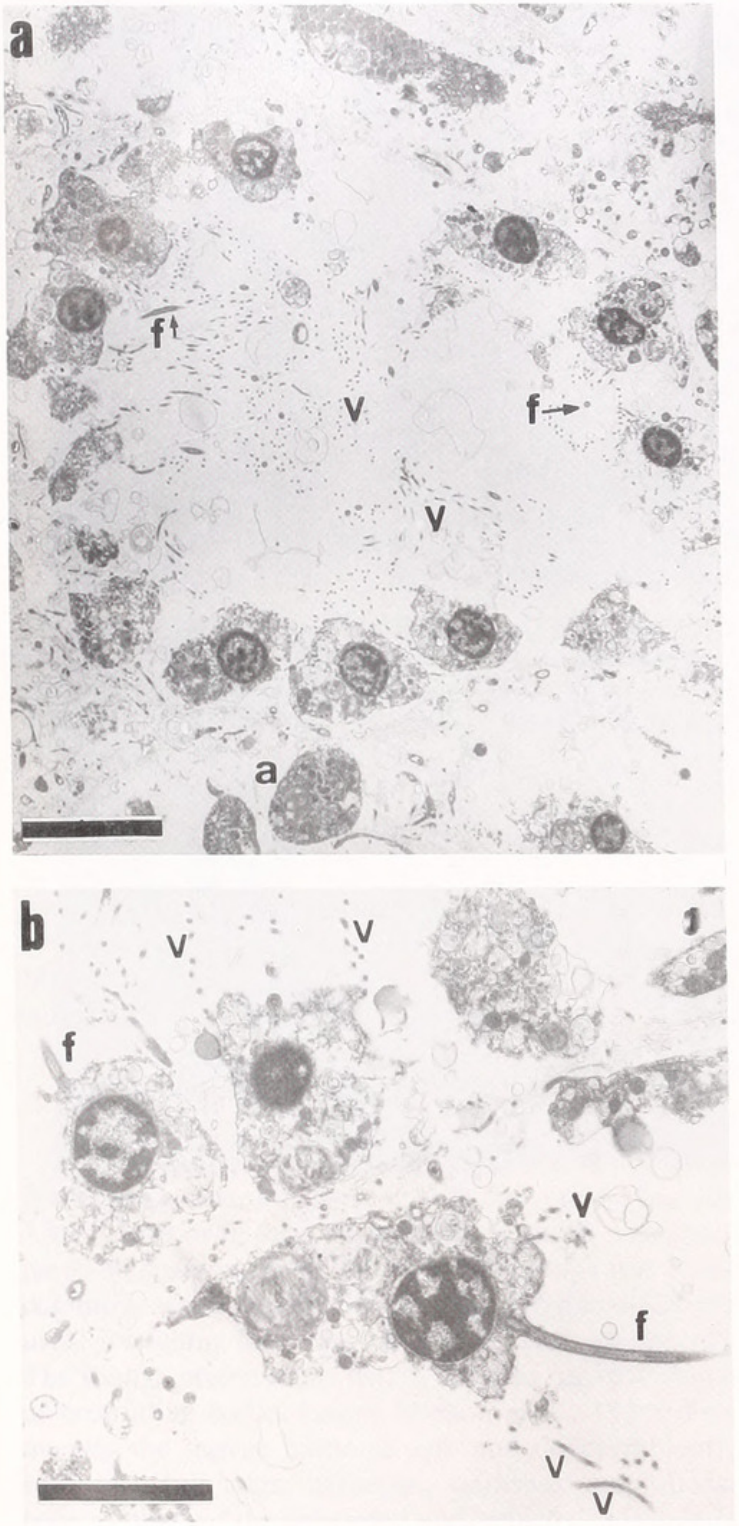

Figure 4. *Latrunculia magnifica* choanocyte chamber. (a) Choanocyte chamber. Note the flagellum (f), microvilli (v), and the amebocyte (a) that destroys the captured particles. Scale bar = 5 μm (TEM). (b) Two enlarged choanocytes. Cell body, microvilli (v), and flagellum (f). Scale bar = 2.5 μm (TEM). No periflagellar sleeve is present in either picture.

L. magnifica proved to be a simultaneously hermaphroditic species and the gametes of both genders were present in some of the reproducing sponges. The spermatozoa appear to develop from archeocytes (Fig. 1). The spermatic cysts are encircled by surrounding cells, and the sperm grow in number while decreasing in size (Fig. 1). The diameter of the cyst is 47 ± 13 μm, and they are present in different developmental stages (Fig. 1). Oocytes develop in size by engulfing surrounding nurse cells (Fig. 2). Large oocytes (393 ± 91 μm) are fertilized internally and are incubated until they develop into mature parenchymella larvae (Fig. 3d). The embryos are bright orange to red in color. They are distributed throughout the mesohyl (never in the cortex) and are very distinct against the pale red color of the inner parts of *L. magnifica*. The developing embryos are encircled with a layer of cells, some of which secrete a collageneous coat of fibrils around the embryo (Fig. 3b). The inner cells of the embryo are of several types (Fig. 3a–e). The fully ciliated larva is large and reaches up to 1200 μm (mean 868 ± 144 μm). No free-swimming larva was observed during this study.

Figure 4 depicts a choanocyte chamber. It is apparent that no periflagellar sleeve is present around the base of the choanocyte's flagellum. The chamber has a diameter of about 25 μm and is constructed of 3-μm-wide choanocytes.

The discorhabd microscleres, which are typical of the Latrunculiidae, are found mostly at the dermal crust of the sponge (Fig. 5). In addition, the sponge contains some spongin fibers. The gross morphology of *L. magnifica* is highly variable, and specimens collected in the different localities were within the variation found in any of the given localities, and thus indistinguishable. The chemical analysis, however, confirmed the existence of different geographical distributions of latrunculins within sponge and nudibranch populations and thus provided a means of distinguishing between sponge populations (as well as those of their nudibranch predator).

The latrunculin content of *L. magnifica* amounts to 1–2% of the sponge dry weight (except for sponges from Djibouti, in which it is only 1%). NMR analysis established that the nudibranch *C. quadricolor* from the Gulf of Eilat contains latrunculin B, the same as its prey *L. magnifica* collected at the same locality. Samples taken from sponges and nudibranchs found in the Gulf of Suez contain latrunculin A. In the Tiran straits, however, the sponges possess either latrunculin A or latrunculin B (but not both within the same individual). Sponges from the South Red Sea (Dahlak Island) contain only latrunculin A. Specimens of *L. magnifica* collected from a sponge population in Djibouti contained both homologs (A & B). No nudibranchs were collected at the two latter collection sites.

Discussion

The presence of spermatic cysts in different stages suggests that more than one short sperm release event occurs during a reproductive season. The presence of larvae dur-

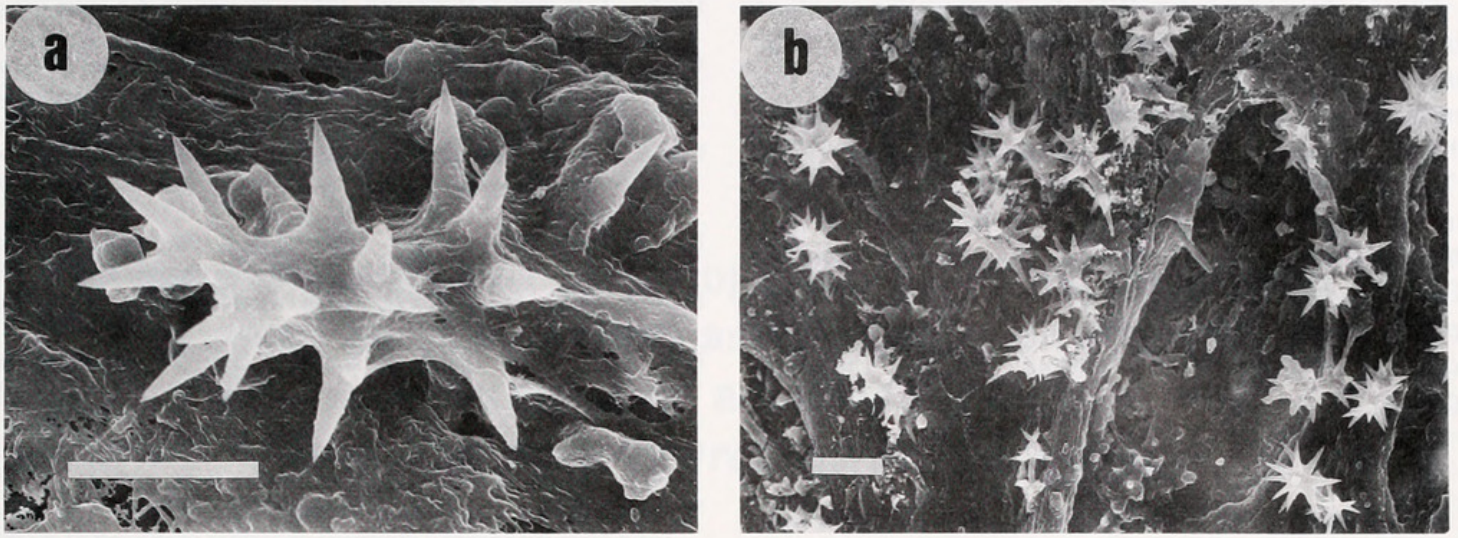

Figure 5. *Latrunculia magnifica* typical discorhabd microscleres. (a) A spicule embedded in spongin. Scale bar = 10 μm (SEM). (b) A layer of discorhabds near the sponge's surface. Scale bar-20 μm (SEM).

ing many months of the year serves to confirm this conclusion. An extended reproductive season has been found among other brooding sponges in the Red Sea (Ilan and Loya, 1988, 1990; Ilan and Vacelet, 1993). This strategy could be a means of ensuring settlement on vacant spots as they become available on the reef. Like *Niphates* sp. (Ilan and Loya, 1988), *L. magnifica* also does not release larvae during winter, possibly avoiding the cover of post larvae by the seasonal algal bloom. As in the present study, the development of sperm from archeocytes has been documented among other sponges as well, although spermatozoids are generally thought to develop from choanocytes (Reiswig, 1983). The *L. magnifica* spermatic cyst is within the regular size found for sponges (Bergquist, 1978; Reiswig, 1983; Simpson, 1984), although the larva is larger than the average parenchymella (Bergquist, 1978; Fell, 1983; Simpson, 1984).

The large size of the larva may provide an advantage for rapid settlement and possession of a relatively large size compared with other invertebrate larvae. This initial large size is considered to elevate post-larval survivorship (*e.g.,* Hughes and Connell, 1987). The apparent difference in the size of oocytes and larvae can be attributed to the gain of nutrients by the embryo from the parental sponge, or, as Fell (1983) indicated, the mature oocyte is probably the size of the larvae, but what seems to be an initial stage of embryogenesis is actually a developing oocyte with nonhomogeneous cytoplasm that eventually reaches the size of a larva.

This study is the first to document the brooding embryonic mode of development within the Latrunculiidae. The finding that *L. magnifica* is a viviparous species is of taxonomic importance because it supports the view that the Latrunculiidae should be placed within the order Poecilosclerida, subclass Ceractinomorpha (Topsent, 1928; Lévi, 1973; Soest, 1984). An additional species from the same family (*Negombata* sp.) studied at the same locations was also found to brood embryos (Ilan, unpub. data), which further supports this idea. In addition, the absence of a periflagellar sleeve from around the base of the choanocyte's flagellum, which is typical of hadromerid sponges (Vos *et al.,* 1991), also strengthens this view. It should be noted that the presence of spongin fibers in this species (as well as in the *Negombata* species mentioned before), which gives the species elastic properties, also supports the non-Hadromerid positioning of the Latrunculiidae, since this order is characterized by the absence of spongin fibers and therefore is firm but non-elastic (Bergquist, 1978; Hartman, 1982). This example demonstrates that when skeletal morphology is not sufficient to distinguish between two taxa, other characters, including anatomical and developmental ones, may be used.

The chemical composition of sponges has been used to assist in their classification (*e.g.,* Bergquist *et al.,* 1984). But latrunculins occur within other sponge species such as *Spongia mycofijiensis* (Crews *et al.,* 1988; Quinoa *et al.,* 1988). Thus they cannot be used as chemotaxonomical characters to distinguish between species. Within *L. magnifica* the type of latrunculin homologs is, however, a solid character with which to distinguish between different populations. In addition, *L. magnifica* and the nudibranch *C. quadricolor,* when collected at the same location, have the same latrunculin homolog. In the Gulf of Eilat, this is latrunculin B, while south to the Tiran straits and in the Gulf of Suez, the sponges and nudibranchs contain latrunculin A. The identical geographic appearance of the latrunculin homologs in the sponge and the nudibranch populations provides strong circumstantial evidence to support the idea of dietary origin of the compounds found within the nudibranch tissues.

Some questions remain. Why does latrunculin, a potent disrupter of actin microfilament organization (Spector *et al.*, 1989), not affect *C. quadricolor?* Do the larvae of the nudibranchs use the different latrunculin homologs as chemical inducers for specific settlement on their prey? The occurrence of both homologs in Djibouti is not well understood. One explanation is that the cells producing the latrunculin within the sponge are symbiotic bacteria. If so, such bacteria could also be found in several other species—like the taxonomically unrelated *Spongia mycofijiensis*—and within different populations of the same species.

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