THE GALL-FORMER *SABINELLA TROGLODYTES* (CAENOGASTROPODA: EULIMIDAE) AND ITS ASSOCIATION WITH *EUCIDARIS TRIBULOIDES* (ECHINODERMATA: ECHINOIDEA)

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Abstract *Eulimids snails are renowned for their parasitic habits on Echinodermata. These gastropods can be ectoparasites, endoparasites and some groups are able to make gall in their host. This is the case of the Caribbean eulimid* Sabinella troglodytes*, which lives in association with the cidaroid sea urchin* Eucidaris tribuloides*. The present work describes the lifecycle of* S. troglodytes*, studied* in situ *over four years and reports on unprecedented data on the species' reproductive behaviour. During the dry season, from September to March, most galls are occupied by couples of snails and newly laid egg masses. In observations during the wet period of the year, May to August, adult specimens are rarely found and juvenile stages can be observed whilst settling and crawling the spines. The feeding mechanism can also be observed in live specimens maintained in the laboratory indicating that the snails erodes the epithelium and the calcareous matrix as well.*

Finally this work also records for the first time the occurrence of S. troglodytes *in the South Atlantic Ocean, extending its range of distribution, previously restricted to Florida and Caribbean, to Brazil.*

Key words *Eulimidae, gall, sea urchin, symbiosis.*

INTRODUCTION

Parasitism among gastropods has been studied since the mid-19th century and several marine families can be recognized as parasites, e.g., Epitonidae, Coralliophilidae, Pyramidellidae and Eulimidae (Vaney, 1913). Eulimid gastropods represent an extensively studied taxonomic group, containing, approximately, 4,000 valid species worldwide (from tropics to poles) (Warén & Gittenberger, 1993). Although considered one of the most diverse and abundant families of tropical marine mollusks (Warén, 1983, Bouchet *et al*. 2002, Dgebuadze *et al*. 2012), Eulimidae is majorly known by its parasitic interactions with very diverse echinoderm species (Ponder & Gooding, 1978; Cantera & Neira, 1987; Hales, 2006; Queiroz *et al*., 2011a; 2013a).

Eulimids can occur as either endo or ectoparasites (Sasaki *et al*., 2007; Kochzius *et al*., 2009) but some eulimids have a unique parasitic strategy, forming structures called galls (Warén, 1983). Galls can occur as tumors or neoplasms and can be defined as the abnormal growth of tissue as a result of disorderly cell proliferation (Robert, 2010). Typically, they are the result of the continued presence of a parasitic organism (White, 1965).

Only six (*Prostilifer*, *Scalaribalcis*, *Tropiometricola*, *Stilifer, Trochostilifer* and *Sabinella*) of the 101 genera in the family (Bouchet, 2012), are known as gall-formers (Warén, 1983). *Sabinella* Monterosato, 1890 is one of the more well-known genera because it makes unusual galls distally on sea urchin spines. Among the eleven species of *Sabinella*, six of them are recognized as associates with cidaroid sea urchins (Warén, 1981; 1983; Warén & Mifsud, 1990; Warén, 1992; Rodriguez *et al*., 2001), and are found in Europe, North America (Atlantic and Pacific Oceans), Caribbean, Australia and New Zealand.

Despite the taxonomical studies dealing with the genus (Warén, 1981; 1983; Warén & Mifsud, 1990; Warén, 1992; Rodriguez *et al*., 2001), knowledge of biological aspects of the association is scarce. Therefore the main goals of the present study are to provide unique data on the life cycle of *Sabinella troglodytes* and report on the relationship between the two organisms.

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Furthermore there is no record of the relation between sea urchins and the genus *Sabinella* in the South Atlantic Ocean. Thus, the present study provides the first report of the gall-former eulimid *Sabinella troglodytes* (Thiele, 1925) and its association with the cidaroid sea urchin *Eucidaris tribuloides* (Lamarck, 1816) for the region.

Material and Methods

Exemplars of the gall-former *Sabinella troglodytes* were collected between 2011 and 2015 from Barra and Porto da Barra beaches (13°00'S, 38°31'W). These places are located at the entrance of the Todos-os-Santos Bay (TSB) (13°00'S, 38°32'W). 28 specimens of *S. troglodytes* were collected together with their hosts in both sites, by free diving. At Barra beach, the sea urchins were collected at a depth of three meters in large, natural reef pools (Queiroz *et al*., 2011a). At Porto da Barra beach sampling was conducted in the subtidal zone (Queiroz *et al*., 2011b). The specimens of *S. troglodytes* and associated hosts were taken to the laboratory for the observations of biological characteristics. Each host and its parasite were then photographed and preserved in 70% ethanol. Specimens were deposited in the Mollusca Collection of the Museu de Zoologia da Universidade Federal da Bahia (UFBA) and in the Mollusca Collection of the Museu de Zoologia da Universidade de São Paulo (MZSP).

Systematics

Class Gastropoda Cuvier, 1795
Family Eulimidae Philip, 1853
Genus *Sabinella* Monterosato, 1890

Sabinella troglodytes (Thiele, 1925)
(Fig. 1A–F)

Eulima troglodytes Thiele, 1925: 112–113[146–147], pl. 13 [25], figure 4.
Mucronalia nidorum Pilsbry, 1956: 109–110, pl. 6, figures 4–6; Sarasúa and Espinosa, 1977:3.
Sabinella troglodytes Warén, 1983: 71; Warén & Moolenbeek, 1989: 172, figures 9–11; Redfern, 2001: 83, pl. 39.

Type locality South of Cape Verde.

Material examined BRAZIL. Bahia**;** Salvador, Barra beach, 13°00'S, 38°31'W, 1.5–2m depth, UFBA 0021, 1 specimen (male) (05/V/2011), UFBA 0034, 2 juvenile specimens (2/II/2013), UFBA 0035, 4 specimens (1 male, 1 female, 2 juveniles) (4/IX/2015), UFBA 0036, 1 specimen (male) (4/IX/2015); Porto da Barra Beach, 13°00'S, 38°32 W, 3–4m depth; UFBA 0022, 1 specimen (female) (05/V/2011); MZSP 112552, 1 specimen (2/II/2013) MZSP 131563 3 specimens (12/II/2014); MZSP 131562 4 specimens (4/IX/2015). Remaining 11 specimens (2 males, 2 female and 7 juveniles) in authors' collection. All specimens found on *Eucidaris tribuloides.*

Description Species relatively large (to 4mm), with a milky-white, pointed, glossy shell with pronounced sutures and a robust (non-brittle) appearance. Protoconch whorls are narrow, obtuse and slightly tilted (usually to the left) (Fig. 1C). Teleoconch whorls (particularly the body whorl) are very rounded (convex) (Fig. 1A–B, E–F). It is possible to see some incremental scars in the teleoconch whorls (Fig. 1B, E–F). The aperture is pear-shaped and flared at the base (Fig. 1B and D). The outer lip is prosocline, sinuous and expanded. In lateral view it is slightly leaned to left side, close to suture and becoming rounded just after it. The end of the outer lip is slightly leaned, forming a curved border (Fig. 1F). Operculum yellowish. In living specimens tentacles are white and showing an orange mark around each eyespot (Fig. 1A).

Geographic range Cape Verde**,** USA (Florida), Gulf of Mexico, Caribbean Sea, Bermuda, Aruba, Bonaire, Curaçao and Abaco islands, and Brazil (Bahia State) (Pilsbry, 1956; Sarasúa & Espinosa, 1977; Warén, 1983; 1992; Redfern, 2001; Turnell *et al*., 2009).

Remarks *Sabinella troglodytes* was always found in association with the sea urchin *E. tribuloides* (Fig. 2A). A total amount of 28 snails were collected in nine galls. Seven of these galls were inhabited by couples of snails and their brood, composed of juvenile individuals and/or egg masses (composed of lathery capsules (Fig. 2B–C)). In these cases it was noted that the female is larger than the male (Fig. 2A). Egg masses were laid inside the gall cavity by the female (Fig. 2B), each one consisting of approximately 150 to 200 capsules, organized in a

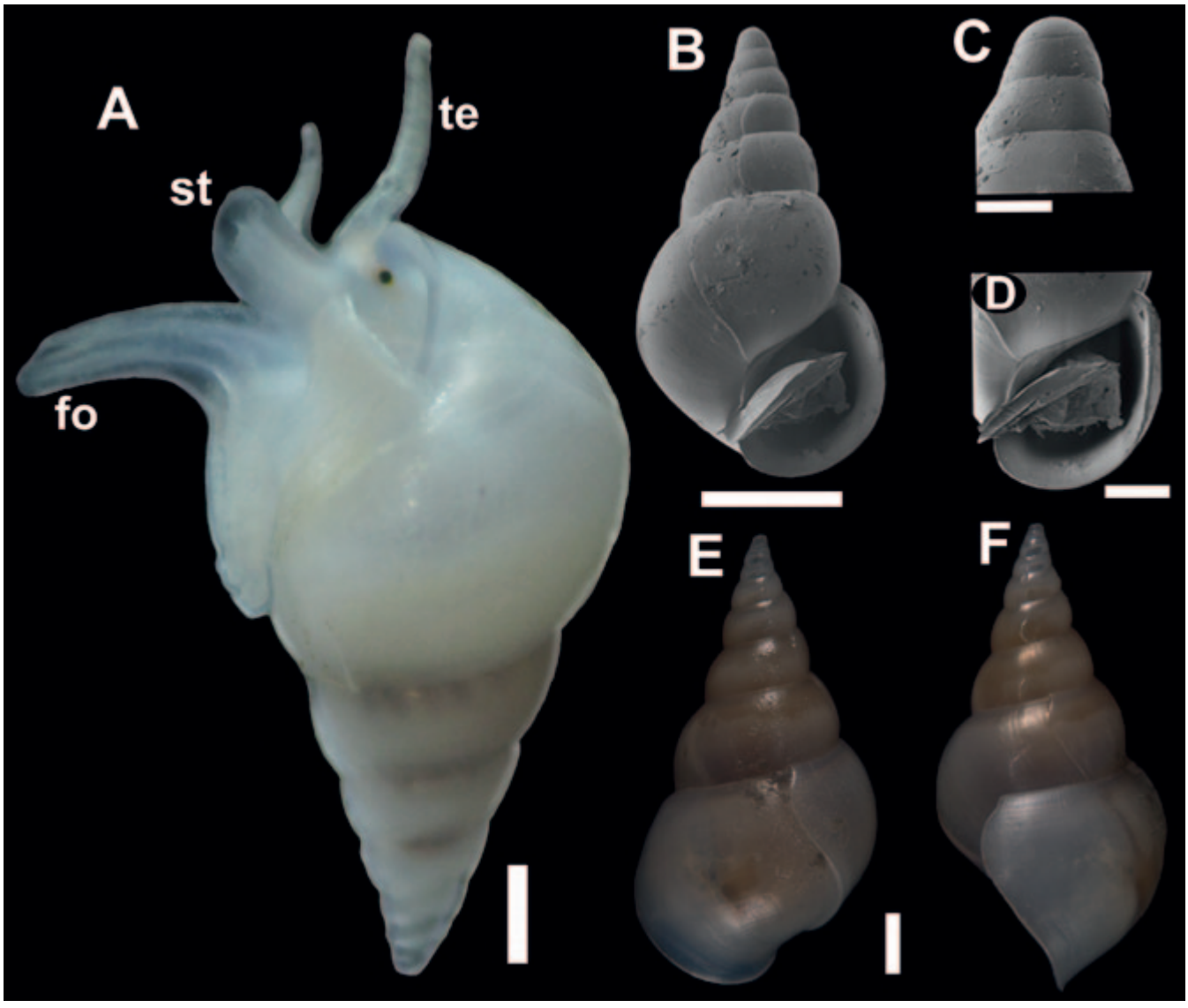

Figure 1 Shell of *Sabinella troglodytes*. A – Live specimen; B–D – SEM; E and F – Newly fixed specimen. A – Live female showing the foot (fo), snout (st) and tentacles (te); B – Ventral view; C – Protoconch; D – Aperture and operculum in detail; E – Dorsal view; F – Outer lip in lateral view. Scale bars: A, B, E and F=500µm; C=100µm; D=200µm.

spherical arrangement similar to a floral bouquet. A maximum of three egg masses were observed in a single cavity (Fig. 2B). Only in two galls a single large snail, most likely a female, was recorded. Field observations conducted from 2011 to 2015, spotted two adults specimens (male and female) with some egg masses (Fig. 2A–B) generally from December to February. However, on the remaining months of the year, from February to December, most galls had only juvenile snails, with very few galls containing couples with egg masses. During the specific period of June to July, only juveniles were found. No empty galls were observed, and the occurrence of pairs of snails without egg masses was not observed either. Observations of live snails in the laboratory demonstrated that the species feeds on the sea urchin spines, eroding the epithelium and the calcareous matrix as well. The eulimids were seen with the snout attached to the gall-spine (Fig. 2C). During detailed observation of the specimens (Fig. 2D), it was possible to verify *Sabinella troglodites* static and with the snout completely attached to the spine. After five minutes of non-stop feeding, the eulimid started the detachment process from the spine (Fig. 2E–F). Firstly, the snail, with the snout still attached, began to

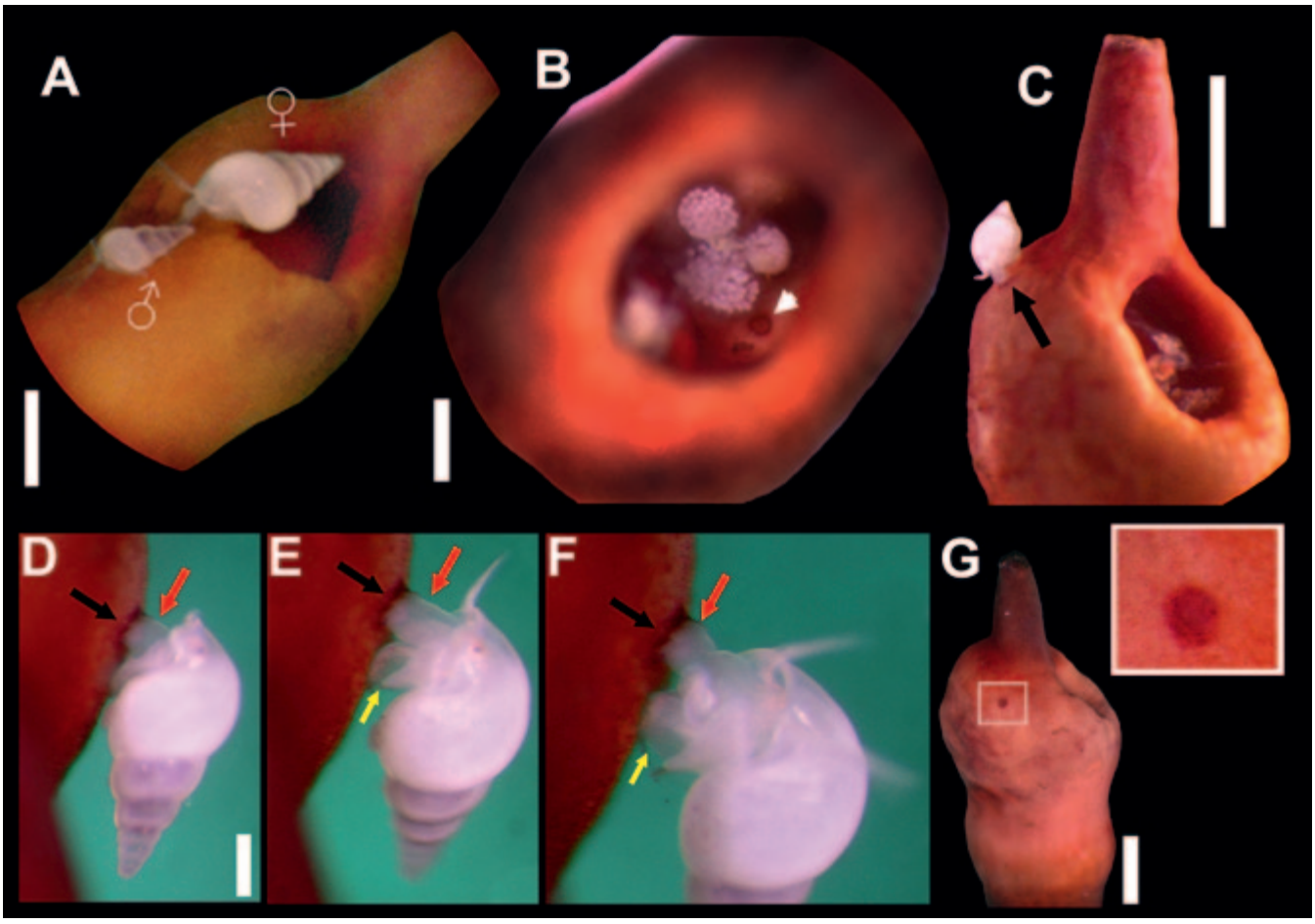

Figure 2 *Sabinella troglodytes* parasitizing *Eucidaris tribuloides* (laboratory observations). A–B – Biological observations; C–G – Feeding process. A – Male and female individuals on urchin spine; B – Egg capsules and feed scar (arrow) inside the gall; C – Female starting feeding process on the exterior region of the gall; D – Snail attached and feeding on spine calcareous matrix; E–F – Detachment process showing the foot position for detachment; G – Feeding scar provided by snail. Scale bars: A=10mm; B=2mm; C and G=5mm, D=300µm. Legend: black arrow – feeding scar; red arrow – snout; yellow arrow – foot.

move its foot and consequently moving itself constantly (Fig. 2F). Then, in a vigorous way, the snail detached most of the foot and removed the snout. After detaching the snout the snail remained attached to spine only by the posterior section of the foot. Finally, *S. troglodytes* turns its anterior region to spine, attaching the anterior portion of the foot and moving away from the feeding area, leaving behind a feeding mark process similar to a dark spot, where the snout was adhered (Fig. 2G). Such patterns can occur close to the aperture or inside the cavity itself (Fig. 2B).

DISCUSSION

In Brazilian waters, there are few recorded interactions between Eulimidae and their hosts, the Echinodermata (*e.g.* Simone & Martins, 1995; Queiroz *et al*., 2011a; 2013a; Bispo *et al*., 2015). The only species that has been documented in association with Echinoidea is *Scalenostoma subulatum* (Broderip, 1832); however, its specific host was not identified (Rios, 2009) and there are few reports recording unique species-specific associations. The main examples that can be cited are *Melanella frielei* with *Mesothuria intestinalis* (Warén, 1983) and *Annulobalcis aurisflamma* with *Tropiometra carinata* (Simone & Martins, 1995; Queiroz *et al*., 2011a; Bispo *et al*., 2015). Dgebuadze *et al*. (2012) recorded possible specific associations between *A. maculatus, A. wareni* and *A. albus* with *Comatella nigra, Comaster nobilis* and *Oxycomanthus benetti*, respectively, but further study is required to considerer these

eulimids as specific parasites. According to Warén (1983), *Sabinella troglodytes* seems to be exclusively associated with *E. tribuloides* and this is backed up by Pilsbry (1956); Warén (1992); Warén & Moolenbeek (1989) and Redfern (2001), as well as the biological data obtained by the present study which suggests that *S. troglodytes* is very adapted to its host.

Regarding the biological aspects of the association between *S. troglodytes* and *E. tribuloides,* there is little information (Warén, 1983). We observed that the lifecycle of this eulimid appears to occur in complete dependence of the host, as opposed to other species such as *Melanella eburnea* that can be found in the free-living state (see Queiroz *et al*., 2013a). Based on our field observations of the amount of adult couples in galls with egg masses, we consider that the period ranging from December to February, which is part of the dry season, marks the end of the reproductive period of *S. troglodytes*, which culminates in the egg masses being laid. However, the recruitment period appears to be between May to August, once, during these months, adult specimens are rarely found and juveniles are observed crawling the spines, although no galls are visible. Therefore, this reflects that the egg masses spotted in the beginning of the year have hatched and the juveniles are settling, in order to start a new reproductive period. This process consequently occurs during the whole dry season, ranging from August/September to February/March.

We also report for the first time the feeding process of *S. troglodytes*. Since the species has no radula (Pilsbry, 1956) it possibly uses some kind of acid secretion, similar to those observed in the cassid gastropods, to erode the spine. Cassid snails are able to drill holes in echinoid test using its radula associated with acid secretion (Fänge & Lidman, 1976; Hughes & Hughes, 1981). According to our laboratory observations *S. troglodytes* feeds on the host spines, mainly on the gall (Fig. 2C–F), using its acrembolic proboscis to erode and suck the calcareous matrix to access the scarce spine tissue, probably using some gland with a corrosive content. Other Echinoid species from the same sampling site (*e.g. Lytechinus variegatus*, *Echinometra lucunter* and *Tripneustes ventricosus*) have been observed but no associated eulimid was found, reinforcing the apparent specific association between *S. troglodites* and *E. tribuloides*. Therefore *S. troglodites* seems to be extremely adapted to its host *E. tribuloides*, with its complete lifecycle occurring in close interaction and depending on it.

The only species of *Sabinella* recorded in the Western Atlantic Ocean so far is *S. troglodytes*, found in North America (Florida) and the Caribbean (Pilsbry, 1956; Warén, 1983; 1992; Redfern, 2001; Turnell *et al*., 2009). To date, only one record of the genus has been reported in the South Atlantic, along the Uruguayan coast (Figueiras & Sicardi, 1980); however, it was not identified to species level. The present study extends the known distribution of *S. troglodytes* to the South Atlantic Ocean, specifically to Bahia State, in Brazil.

The marine invertebrate fauna of the Caribbean and Brazil were considered quite distinct from each other, however recent studies report similarities, in a wide range of phyla such as Cnidaria (Neves *et al*., include, 2006; 2008; 2010), Platyhelminthes (Bahia & Padula, 2009; Queiroz *et al*., 2013b), Mollusca (Sales *et al*., 2011; Queiroz *et al*., 2013a) and Crustacea (Queiroz *et al*., 2011b, Lima *et al*., 2014). *Sabinella troglodytes* provides another example of an invertebrate occurring in both Caribbean and Brazilian waters. However, relative to other locations, the Brazilian eulimid fauna is comparatively poorly known: 34 Brazilian eulimids; similar to the Bahamas (29 species) and fewer than that of the Canary Islands (36 species) and the Mediterranean Sea (47 species) (Redfern, 2001; Abad *et al*., 2003; Ramazoti *et al*., 2006; Rios, 2009; Queiroz *et al.*, include, 2013b; Souza & Pimenta, 2014; 2015). Despite the large extension of the Brazilian coast (8000km) (Tesler & Goya, 2005), other regions appear to have greater species richness than the Brazilian littoral. However, this apparently low richness in Brazil may reflect a lack of knowledge of eulimids in the region. The present work contributes to the knowledge of Eulimidae fauna providing relevant data on *S. troglodytes'* biology and to a better understanding of its relationship with the cidaroid sea urchin *E. tribuloides.*

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