DISCOVERY OF *LOXOSOMELLA VIVIPARA* (ENTOPROCTA: LOXOSOMATIDAE) IN THE MARINE SPONGE *HIPPOSPONGIA* CF. *GOSSYPINA* (PORIFERA: SPONGIIDAE) IN THE FLORIDA KEYS

by

Rachel Plunkett

A Thesis Submitted to the Faculty of

The Charles E. Schmidt College of Science

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

Florida Atlantic University

Boca Raton, FL

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DISCOVERY OF *LOXOSOMELLA VIVIPARA* (ENTOPROCTA: LOXOSOMATIDAE)

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Shirley A. Pomponi, Department of Biological Sciences, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

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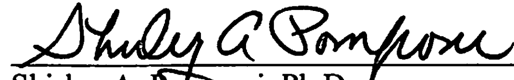

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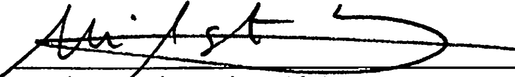

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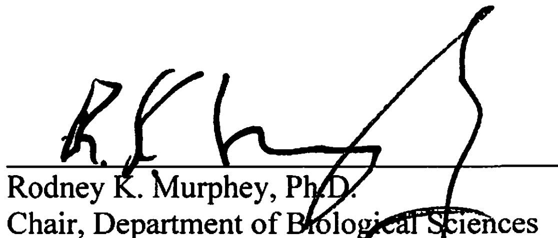

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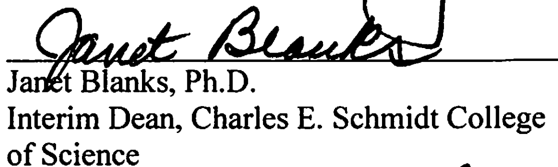

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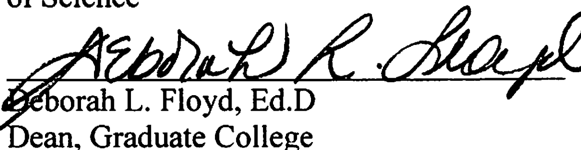

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ABSTRACT

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Title: Discovery of *Loxosomella vivipara* (Entoprocta: Loxosomatidae) in the Marine Sponge *Hippospongia* cf. *gossypina* (Porifera: Spongiidae) in the Florida Keys

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Populations of a marine invertebrate symbiont were found on the outer surface and internal spaces of a keratose sponge from a shallow bay in the Florida Keys in May 2014. A total of 24 specimens of the seagrass and reef-dwelling sponge were collected between May 2014 and August 2015 to provide material to identify both host and symbiont, and elucidate information on the nature of the association. Based on a morphological analysis via light microscopy, histology, and scanning electron microscopy (SEM), and 99% similarity in aligned partial sequences from 28S and 18S nuclear ribosomal genes (rDNA), the symbiont was identified as the solitary entoproct *Loxosomella vivipara* Nielsen, 1966 (Entoprocta: Loxosomatidae). A partial sequence from the Internal Transcribed Spacer Region 2 (ITS2) of *L. vivipara* was registered to GenBank for the first time. The identity of the host sponge, based on a morphological investigation, is resolved as "velvet sponge" *Hippospongia* cf. *gossypina* Duchassing and Michelotti, 1864 (Demospongiae: Spongiidae). This is the first report of an entoproct commensal

from *Hippospongia* cf. *gossypina*, a sponge that formerly had great commercial value when it was abundant throughout the Bahamas, Florida Keys and Gulf of Mexico. Other common sponge species at the study site were collected to investigate the host specificity of *L. vivipara*. Evidence that *L. vivipara* favorably selects the sponges *Hippospongia* cf. *gossypina* and *Chondrilla nucula* over other potential host sponges at the study site is provided. Commensalism is the most plausible justification for this relationship: *L. vivipara* is dependent on sponges for protection and food particles, while the sponges are unaffected by its presence. Further evidence of host-specific inquilinism is provided for *L. vivipara* associated with *Hippospongia* cf. *gossypina*, but not for *L. vivipara* associated with *C. nucula*. An inquilinistic association between an entoproct and sponge is a rare discovery only mentioned in one previous study. Sponge aquiferous qualities such as aperture and canal size, canal and choanocyte chamber arrangement, seawater pumping rate, and food particle size selection are likely factors that inhibit or enable inquilinism in sponge-entoproct associations – a potential topic for future researc

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LIST OF TABLES ix

LIST OF FIGURES x

1 PREFACE 1

2 INTRODUCTION 2

2.1 Entoprocts 2

2.2 The Solitary Entoproct *Loxosomella* Keferstein, 1862 3

2.3 Distinctive Features of *Loxosomella vivipara* Nielsen, 1966 4

3 RESEARCH OBJECTIVES 7

3.1 Specific Aims 7

3.2 Research Questions 7

4 MATERIALS AND METHODS 7

4.1 Sponge Collections 8

4.2 Molecular Studies 8

4.3 Morphological Studies 9

4.3.1 Light Microscopy 10

4.3.2 Scanning Electron Microscopy 10

4.4 Sponge Species Comparisons 11

5 RESULTS 12

5.1 Molecular Results 12

5.2 Morphological Results 12

5.3 Comparison of Host Sponge Species 22

6 DISCUSSION 23

6.1 Comments on Morphology and Reproduction 23

6.2 Host Specificity 26
6.3 Location of *Loxosomella vivipara* 27
6.4 Remarks on the Nature of the Symbiosis 29
6.5 Conclusions 31
7 APPENDIXES 36
8 REFERENCES 43

LIST OF TABLES

Table 1. Results of evaluating for the presence of entoprocts in different sponge species (two of each) collected from Summerland Key in August 2016 (n=14). Symbols in table indicate if *Loxosomella vivipara* was present (+) or absent (-) in each sponge sample. 23

Table 2. Sequence alignments of 28S rDNA between 5 query replicates and *L. vivipara* from GenBank (Accession no. GU125730). Black shading indicates a match, light or no shading indicates mismatch. 37

Table 3. Sequence alignments of 18S rDNA between two query replicates and *L. vivipara* from GenBank (Accession no. GU125745). Black shading indicates match, light or no shading indicates mismatch. 38

LIST OF FIGURES

Figure 1. Basic body plan of the solitary entoproct genus *Loxosomella*. Lophophore (1); tentacle (2); mouth (3); pharynx (4); esophagus (5); stomach (6); intestine (7); rectum (8); anal cone (9); anus (10); attachment organ (11). 3

Figure 2. Distribution of *Loxosomella vivipara* based on published reports. 5

Figure 3. SEM micrograph of an adult *Loxosomella vivipara* specimen (left view) attached to the sponge *Hippospongia* cf. *gossypina* (s). Dashed lines show three body regions: calyx (ca), stalk (st), & foot (ft). Note the lateral epithelial wing of the calyx (bracket) and an early-stage bud (arrowhead) developing in the left atrial pocket. 14

Figure 4. SEM micrograph of an adult specimen of *L. vivipara* with a lophophore containing 16 contracted tentacles (te). 14

Figure 5. Light micrograph of an adult specimen of *L. vivipara* (frontal view) with 14 tentacles. 15

Figure 6. Light micrograph of an adult *L. vivipara* specimen with 15 tentacles. Three of the tentacles are shown extended (te). The transparent lateral wing (wi) and two different sized buds (arrowheads) are visible. 15

Figure 7. SEM micrograph of the tentacles (te) of *L. vivipara* containing many long cilia (ci). 16

Figure 8. SEM micrograph of the lateral cilia (ci) on the tentacle (te) of a specimen of *L. vivipara*. 16

Figure 9. High magnification SEM micrograph of the lateral cilia (ci) on the tentacle (te) from a specimen of *L. vivipara*. 17

Figure 10. SEM micrograph of a bud (b) protruding from the parent (p) *L. vivipara* specimen, with 12 developing tentacles (te). 17

Figure 11. Light micrograph of an adult specimen of *L. vivipara* (left view) with tentacles extended (black arrowhead) and a full-grown bud (double black arrowhead). The foot of the adult (white arrowhead) is easily distinguished (dashed line) from the stalk. A large heel gland (double white arrow) is shown extending into the stalk. 18

Figure 12. Light micrograph of the stalk and foot of an adult specimen of L. vivipara (frontal view) stained with toluidine blue. (Left) Deep groove along underside of the foot (asterisk); heel (h). (Right) Enlarged frontal view of foot 18

Figure 13. Longitudinal section stained with hematoxylin and...................................... 19

Figure 14. SEM micrograph of the foot (ft) (left view). The deep groove (asterisk) at the center of the foot is grasping part of the sponge (s). Secretions (arrowheads) produced by secretory granules inside the heel gland are visible. 19

Figure 15. Light micrograph of adult *L. vivipara* specimen (left-abfrontal view) with “neck-gland” (arrow); Full-grown bud (b). 20

Figure 16. SEM micrograph of an adult specimen (abfrontal view) with an opening (arrow) that leads to the neck-gland. Eight protozoans are attached to the surface of this specimen (arrowhead). A sensory structure (circle) can be seen on the right side... 20

Figure 17. (Left to right) Stages of the budding cycle viewed through light microscopy.

Arrows indicate the bud in each image. 21
Figure 18. Light micrographs of *L. vivipara* specimens showing bud with dark pigmented spot (arrow). 21
Figure 19. A small *L. vivipara* specimen with embryos in the brood chamber (arrow) 21
Figure 20. (Left) High density aggregations (~ 50 individuals) of *Loxosomella vivipara* attached on the outer surface of a 1 cm3 piece of *Hippospongia* cf. *gossypina*. (Center) Lower density aggregations of *Loxosomella vivipara* inside the internal canal system of *Hippospongia* cf. *gossypina*. (Right) Low density aggregation of *Loxosomella vivipara* inside of *Hippospongia* cf. *gossypina* stained with toluidine blue. 22
Figure 21. Massive form of *Hippospongia* cf. *gossypina* viewed from underwater......... 39
Figure 22. SEM of the inhalant pores of *Hippospongia* cf. *gossypina.* 40
Figure 23 (a) Ligh micrograph of fiber mesh network of *Hippospongia* cf. *gossypina* (b) SEM of mesh network showing diameter of some fibers. 40
Figure 24. SEM cross section of a fiber of *Hippospongia* cf. *gossypina.* 41
Figure 25. (a) Lectotype and (b) paralectotype for *Hippospongia gossypina* Duchassing & Michelotti, 1864.. 42

1 PREFACE

The initial direction of this thesis project was to determine if amphipods and snapping shrimp serve as ‘pollinators’ for broadcast spawning sponges by transferring gametes as they move from sponge to sponge. While observing sponges under a dissecting microscope, small (<1mm) organisms were discovered and became the focus of this research project. The initial hypothesis (based on morphological observations) was that the organism was a cercaria, a larval stage in the trematode life cycle, and that the sponge was an intermediate host in a trematode life cycle. A few months following the thesis proposal seminar for this research project in August 2015, rDNA sequences revealed that the organisms in question were actually a species of entoproct (Entoprocta). Upon more detailed re-examination of the morphological characteristics, it became clear that despite superficial similarities, these organisms were not larval trematodes because they lacked some key anatomical features that trematodes typically possess (e.g., no oral sucker or syncytium). The research questions were revised to address this new research pathway.

2 INTRODUCTION

2.1 Entoprocts

Entoprocts (Entoprocta = Kamptozoa) are a small phylum of benthic aquatic (primarily marine) invertebrates that suspension feed on phytoplankton and other small organic particles using a crown of ciliated tentacles (the lophophore) (Iseto 2003; Nielsen 2016). The different types of cilia serve to trap and transport food to the food groove, and further to the mouth (Riisgard et al. 2000; Nielsen 2016). The tentacle crown surrounds the atrium; a depression on the ventral side of the body. The mouth is located on the perimeter of the atrium, and the anus sits anterior to the mouth atop a small anal cone within the concavity of the atrium (Nielsen 2002, 2016).

Entoprocts can be solitary (family Loxosomatidae) or colonial (families Loxokalypodidae, Pedicellinidae and Barentsiidae) (Nielsen 2010, 2016). Solitary entoprocts often form associations with filter feeding animals such as polychaetes, sipunculids, bryozoans, sponges, and ascidians; taking advantage of the ventilating currents they produce (e.g. Nielsen 1964; Williams, 2000; Yakovis 2002; Iseto 2003; Nielsen 2008, 2016; Tamberg et al. 2013). This study focuses on *Loxosomella vivipara*, a solitary entoproct that has previously been documented to form ectosymbiotic associations with marine sponges, clustered on the outer surface (ectosome) of the sponge around the osculum (Nielsen 1966a; Fuchs et al. 2006; Fuchs et al. 2010; Emschermann 2011).

2.2 The Solitary Entoproct *Loxosomella* Keferstein, 1862

Solitary entoprocts (family Loxosomatidae) present the typical body plan shown in *Figure 1*. Species belonging to the genus *Loxosomella* Keferstein, 1862 are characterized as having a differentiated foot that extends posteriorly, contains a bean-shaped gland at the frontal end, and has a groove along the underside (Nielsen 1964, 1989; Iseto and Hirose 2010). The foot structure allows the animal to grip to the surface of the animal host, and also glide over surfaces, similar to the locomotion of a slug (Iseto 2002; Iseto and Hirose 2010) or grab the body surface of the host animal (Iseto et al. 2008). In some species, the foot is highly specialized for attachment at a specific location on the host, and may even degenerate with age so that the organism eventually becomes permanently fixed to the substratum (Nielsen 1964, 1989; Williams 2000; Iseto 2001).

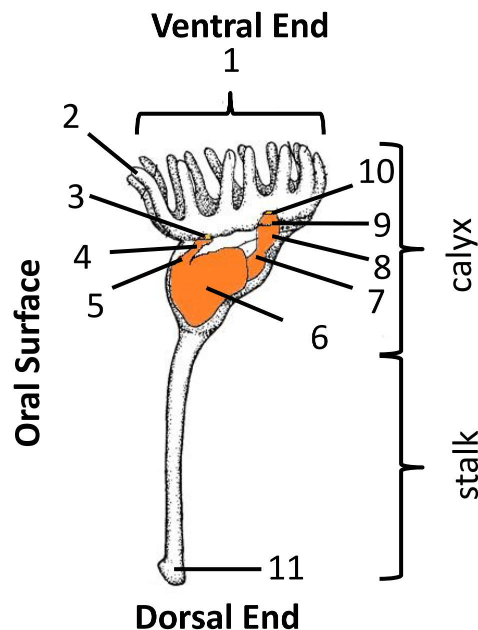

Figure 1. Basic body plan of the solitary entoproct genus *Loxosomella*. Lophophore (1); tentacle (2); mouth (3); pharynx (4); esophagus (5); stomach (6); intestine (7); rectum (8); anal cone (9); anus (10); attachment organ (11).

Loxosomatids have a short lifespan of 6–10 weeks (Emschermann 1993), and maintain their populations through both sexual and asexual reproduction (Nielsen 1966a, 1966b; 1971; Fuchs et al. 2010). Most species are protandric hermaphrodites that can phase change from male to female (Nielsen 2016). The male releases sperm into the water, which then fertilizes eggs within the oviduct of the female (Nielsen 2016). Eventually a stalk forms within the female's brood pouch (in the atrium) and embryos become cemented to the stalk (Nielsen 2016) as they develop into trochophore-like larvae (Nielsen 1966a, 1971, 2016; Iseto 2003, Iseto et al. 2007). The larvae are released from the parent animal for a short free period, and eventually settle and undergo metamorphosis (Nielsen 2016). In asexual reproduction, solitary entoprocts produce buds at the laterofrontal region of the calyx (Iseto 2003, Iseto et al. 2007), which later detach from the parent. Newly liberated buds can swim using a ciliary current produced by the tentacles (Ryland and Austin 1960), and/or crawl across the substrata using the foot for approximately one day to find a suitable attachment site (Iseto et al. 2007). The locomotor capabilities of asexual buds are thought to be the primary mode of dispersal and founder colony establishment for solitary entoprocts (Iseto et al. 2007).

2.3 Distinctive Features of *Loxosomella vivipara* Nielsen, 1966

Loxosomella vivipara Nielsen, 1966 was first discovered as a commensal on the sponge *Sarcotragus fasciculatus* (*Ircinia fasiculata,* Pallas,1766), attached to a piling at Crandon Marina in Key Biscayne, Miami, Florida. The holotype is in the Zoological Museum, University of Copenhagen: ZMUCENT-0015, and additional samples are in the author's possession (C. Nielsen, Natural History Museum of Denmark, personal communication). Nielsen (1966a) also found *L. vivipara* on *Chondrosia collectrix*,

Tedania ignis, and *Chondrilla nucula*. *Loxomella vivipara* is described as a large species (310-1260 μm) with an orally-aborally flattened calyx (Nielsen 1966a). The lophophore is directed forward with 12-16 long cylindrical ciliated tentacles. From a frontal view, the stomach is in the shape of a rounded triangle and there is a pair of lateral pockets on either side of the stomach (paired atrial pockets). *Loxosomella vivipara* closely resembles some other species found primarily on sponges from the Mediterranean (Nielsen 1966a; 2008) such as *L. alata* from *Spongelia* (Barrois 1877); *L. raja* from *Euspongia nitens, Cacospongia scalaris* and *C. cavernosa* (Schmidt 1875; 1878); and *L. tethyae* from

Figure 2. Distribution of *Loxosomella vivipara* based on published reports.

Tethya sp., *Stylatella* sp., and *Microciona prolifera* (Harmer 1885; Bobin and Prenant 1953). Through careful examination, *L. vivipara* can be distinguished from similar species due to the lack of wing-like extensions on the foot, a large foot gland that extends into the stalk, and the presence of lateral wing extensions of the calyx (Nielsen 1966a; Fuchs et al. 2006). From an abfrontal view, the wings contain a few scattered sensory

bristles, and there is also a pair of sensory papillae at the upper ends of the wings (Nielsen 1966a). In some specimens, there is a pluricellular organ (neck-gland) located on the aboral surface of the calyx just above the anus, that has an opening which leads into a hollow ball-like structure (Nielsen 1966a). Nielsen (1966a) deemed this organ to be an important trait for species-level identification because it is unique to *L. vivipara*. The neck organ in this species is unique among the Loxosomatidae (Emschermann 2011).

The life cycle of *L. vivipara* is unique; sexual reproduction leads to the formation of a larva (second generation), from which a juvenile entoproct emerges (third generation) and then the larva degenerates (as opposed to the larva metamorphosing into a juvenile) (Nielsen 1966a). Following the discovery in Miami, Florida by Nielsen (1966a), samples of *L. vivipara* have only been collected from a few other sites (Figure 2): Luymes' Saba Bank in 1972 (Emschermann 2011), Twin Cays, Belize in 2004 from the sponge *Tedania ignis* (Fuchs et al. 2006; Fuchs et al. 2010), and also Bimini, the Bahamas from the sponge *Chondrosia collectrix* (Nielsen 1966a). *Loxosomella vivipara* specimens collected from Saba Bank in May and June of 1972 were later detailed by Emschermann (2011) and compared to Nielsen's type material. The specimens were all detached from their original settling substrates, but the high abundance of sponge spicules with these samples led the authors to conclude that the Loxosomatid entoprocts were detached from the surfaces of sponges (of unknown taxa) (Emschermann 2011).

3 RESEARCH OBJECTIVES

3.1 Specific Aims

The goals of this project were to: (1) confirm the identity of sponges and symbionts (entoprocts) found in to associate with one another in the Florida Keys, (2) determine if these symbionts show signs of specificity to certain species or types of sponges.

3.2 Research Questions

Question 1: What is the best available species match for the symbionts based on alignments with registered 18S, 28S and ITS2 GenBank sequences?

Question 2: How does the morphology of *L. vivipara* from the Florida Keys compare to descriptions of the type specimens from Key Biscayne, Miami, Florida?

Question 3: What is the best species match for the sponge based on a morphological analysis?

Question 4: Do other sponges abundant at this study site contain entoprocts, and what traits do the sponges that these entoprocts associate with have in common?

4 MATERIALS AND METHODS

4.1 Sponge Collections

Sponge samples were collected from the study site off Mote Marine Laboratory, Tropical Research Laboratory, Summerland Key, FL (24˚39’41.4”N 81˚27’16.5”W) (Appendix II, Fig. 1). Forty (40) whole or partial sponges were collected from the study site during 10 collection events between May 2014 and August 2015 (24 *Hippospongia* cf. *gossypina*; 4 *Chondrilla nucula*; 4 *Geodia gibberosa*; 2 *Tethya* sp., 2 *Ircinia* spp., 2 *Cliona sp.*, and 2 *Spheciospongia vesparium*). A sample voucher of each of the 8 sponges containing entoprocts collected for the molecular study was placed in a Whirl-Pak® bag and frozen at -80˚C for subsequent identification. Samples were collected either by scuba diving or snorkeling under the auspices of Florida Atlantic University’s Scientific Diver Program in accordance with daily permit restrictions using a personal Florida saltwater fishing license.

4.2 Molecular Studies

To identify the taxonomic group of the commensal organisms, partial 28S and 18S nuclear ribosomal (rDNA) sequences and a partial sequence from the Internal Transcribed Spacer Region 2 (ITS2; located between 5.8S and 28S rRNA genes) were used. Molecular studies were performed in collaboration with Dr. Stephen A. Bullard and Raphael Orelis Ribeiro at the Aquatic Parasitology Lab at Auburn University. In May

2015, 80 entoprocts were removed from the sponges and kept frozen at -80˚C overnight for DNA extractions[1]. Total genomic DNA was extracted using DNeasy™ Blood and Tissue kit (QIAGEN). Partial sequences of rDNA were polymerase chain reaction (PCR) amplified in an Applied Biosystems Veriti 96-Well Thermal Cycler. For 18S PCR, the forward primer 18SE and reverse primer WORMB were used. For ITS2 PCR, the forward primer GA1 and reverse primer ITS2.2 were used. To amplify the D1 and D2 domain of 28S rDNA, the forward primer U178 (5' GCA CCC GCT GAA YTT AAG 3') and the reverse primer L1642 (5' CCA GCG CCA TCC ATT TTC A 3') were used. DNA sequencing was performed by GENEWIZ with ABI Prism 3730xl DNA analyzers (GENEWIZ, Inc., South Plainfield, NJ) using the same primers as used in the PCR, with addition of internal primers 388F, 1100F, and 1270R for 18S sequencing and the internal primer 1200R for 28S sequencing. To find the best available species match, the 18S (2 replicates), 28S (5 replicates), and ITS2 (1 replicate) sequences were aligned and compared to registered sequences in GenBank using BLAST (NCBI).

4.3 Morphological Studies

The general external and internal morphology of the entoproct specimens collected from the Florida Keys in this study was observed and documented. Light microscopy and scanning electron microscopy was used to compare diagnostic features (total length, foot shape, epithelial wings, sensory structures, etc.) of these entoprocts to previous descriptions of *Loxosomella vivipara* and other similar *Loxosomella* entoprocts collected from sponges. Sponges were identified in collaboration with Dr. Maria C. Diaz from Florida Atlantic University's Harbor Branch Oceanographic Institute. The

[1] Additional entoprocts were placed in RNA-later for potential future transcriptome analyses (n=20); this is not part of the current study.

reference materials of Hyatt (1894), Van Soest (1978), Van Soest et al. (1983) and the dichotomous key and terminology of Cook and Bergquist (2002) were used to complete the sponge species description.

4.3.1 Light Microscopy

To ensure that all specimens remained in a similar anatomical position, they were flash killed in hot tap water (65˚C), and fixed in a final dilution of 4% formaldehyde (10% formalin) (Dr. S.A. Bullard, Auburn University, personal communication). Whole mount specimens were observed unstained or stained with toluidine blue. For histology, several entoprocts were mounted in paraffin wax blocks, sectioned (5-7µm), and then stained with hematoxylin and eosin.

4.3.2 Scanning Electron Microscopy

The ultrastructure of the foot, atrial pocket, lateral epithelial wings, and the lophophore were documented using scanning electron microscopy (SEM). To make these observations, five small (3-5mm) sponge fragments were fixed in fresh 2% glutaraldehyde in sodium cacodylate buffered-sea water fixative (Dr. Patricia Blackwelder, University of Miami, personal communication). Post-fixation was conducted at the University of Miami Center for Advanced Microscopy (UMCAM) using the protocol from Miller et al. (2011) with minor adjustments: (1) 3 changes of buffer (10 min each); (2) 1% osmium tetroxide post-fixative (45 min); (3) 3 changes of buffer (10 min each); (4) dehydration through a graded series of ethanol (20, 40, 60, 70, 90, and 100; (5) dry samples with hexamethyldisilazane (HMDS); (7) sputter coat with gold. Samples were imaged in an FEI XL-30 Field Emission ESEM/SEM at UMCAM.

4.4 Sponge Species Comparisons

To investigate sponge host specificity, two fragments (approximately 3-4cm^3 each) from individuals of *Hippospongia* cf. *gossypina*, *Geodia gibberosa*, *Sphesiospongia vesparium*, *Ircinia campana*, *Tethya* sp., *Cliona varians.*, and *Chondrilla nucula* were collected over a two day period and examined for presence of entoprocts.

5 RESULTS

5.1 Molecular Results

All rDNA sequences (from both 18S and 28S regions) matched with 99% similarity to deposited sequences from *Loxosomella vivipara* specimens from the sponge *Tedania ignis* in Twin Cays, Belize (28S Accession no. GU125730, 18S Accession no. GU125745, Fuchs et al. 2010). In the 28S region, only one base pair difference was detected out of 374 positions (Appendix I, Table 1) and out of 1668 positions, only one base pair differed in the 18S region (Appendix I, Table 2). ITS sequences have not previously been deposited for *L. vivipara* in GenBank, however, the query ITS2 sequence (59 bp) matched with 95% similarity to an ITS2 sequence for *Loxosomella plakorticola* from the sponge *Plakortis* sp. (Homosclerophorida, Plakinidae) from Japan (Accession no. AB560867, Sugiyama et al. 2010). This is the first time that rDNA of *L. vivipara* from Florida has been sequenced, and it is the first time that the ITS2 region of *L. vivipara* has been sequenced from any locality.

5.2 Morphological Results

Traits of the Florida Keys specimens match those described for *L. vivipara* (Nielsen 1966a, Fuchs et al. 2006, Emschermann 2011). The total length (calyx + stalk + foot) of 10 measured specimens ranges between 240 -1,040 µm. In most specimens, the calyx length is proportional to the stalk + foot length (Figure 3). When observed in lateral view, the lower end of the stalk (the foot) superficially resembles a human foot (hence, the name). Adult specimens have between 12-16 long ciliated tentacles (Figure 4, Figure 9).

and mature buds typically have 12 tentacles (Figure 10, Figure 11). The calyx is orally-aborally flattened, and the lophophore is directed forward. The characteristic lateral epithelial wings are apparent in most specimens (e.g., Figure 3, Figure 6). Smaller individuals did not always appear to have the wings. All specimens have a foot that is easily demarcated from the stalk (Figure 3, Figure 11). The foot does not possess lateral wings. The characteristic large, bean-shaped heel gland can be seen in most specimens using light microscopy, and it often extends into the lower portion of the stalk (Figure 11, Figure 12). The heel gland has several gland cells that contain secretory granules (Figure 13). (*See also* Iseto and Hirose 2010).

In the Florida Keys specimens, the neck-gland that was described by Nielsen (1966a) to be a key trait to species level identification for *L. vivipara*, was observed in many, but not all of the adult specimens (Figure 15, Figure 16). Sensory structures were observed in several specimens from the Florida Keys (Figure 16), that match the location and descriptions of sensory structures found on *L. vivipara* specimens from Key Biscayne (Nielsen, 1966a), Belize (Fuchs et al., 2006), and some of the Saba Bank specimens (Emschermann, 2011).

Evidence of asexual reproduction through budding was extremely common and several stages of the bud development process were captured (Figure 17). SEM reveals that as the bud develops, a long groove forms on the part of the bud facing away from the parent, and at this groove the tentacles develop (Figure 10). Several buds had dark pigmented spots on the calyx (Figure 18), similar to descriptions by Rützler (1963) for the buds of *L. bimaculate*. Larvae with developing buds were not observed in the FL

keys; however a few specimens appeared to be brooding embryos on the brood stalks (Figure 19).

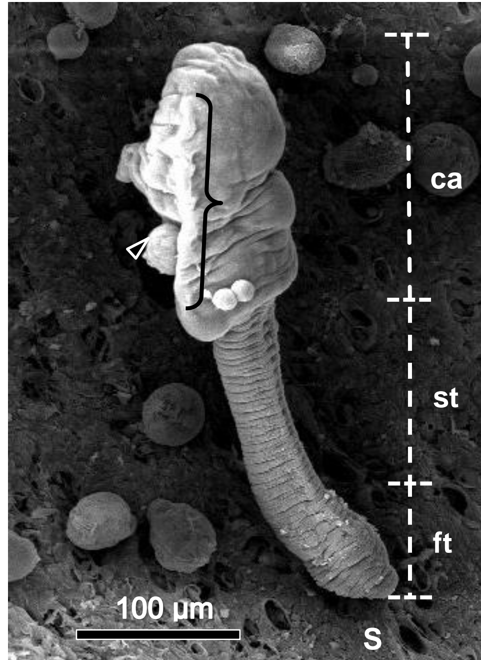

Figure 3. SEM micrograph of an adult *Loxosomella vivipara* specimen (left view) attached to the sponge *Hippospongia* cf. *gossypina* (s). Dashed lines show three body regions: calyx (ca), stalk (st), & foot (ft). Note the lateral epithelial wing of the calyx (bracket) and an early-stage bud (arrowhead) developing in the left atrial pocket.

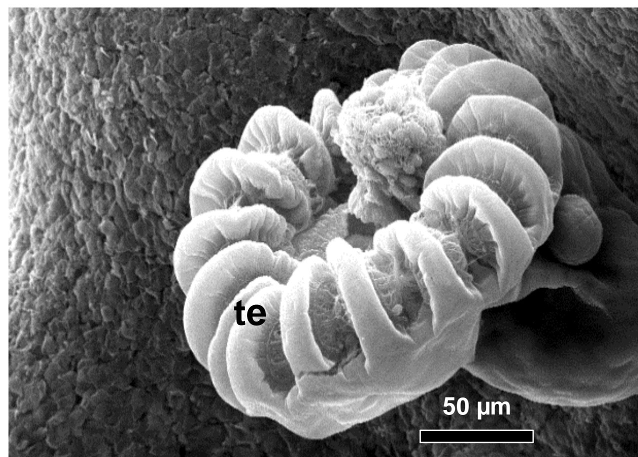

Figure 4. SEM micrograph of an adult specimen of *L. vivipara* with a lophophore containing 16 contracted tentacles (te).

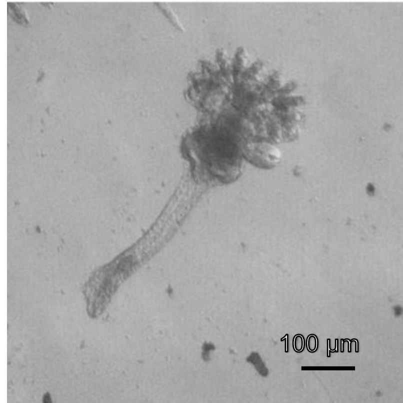

Figure 5. Light micrograph of an adult specimen of *L. vivipara* (frontal view) with 14 tentacles.

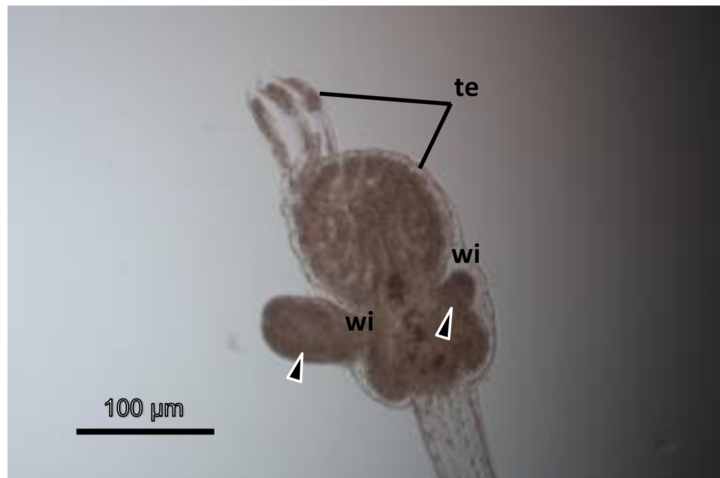

Figure 6. Light micrograph of an adult *L. vivipara* specimen with 15 tentacles. Three of the tentacles are shown extended (te). The transparent lateral wing (wi) and two different sized buds (arrowheads) are visible.

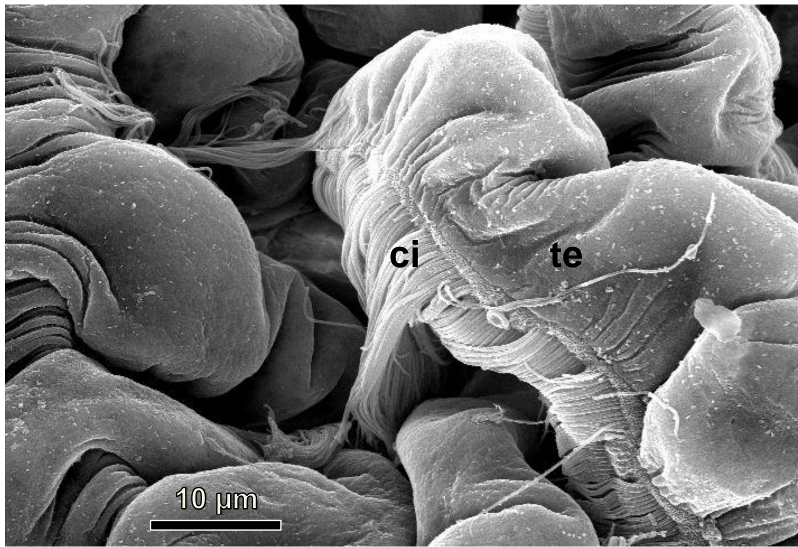

Figure 7. SEM micrograph of the tentacles (te) of *L. vivipara* containing many long cilia (ci).

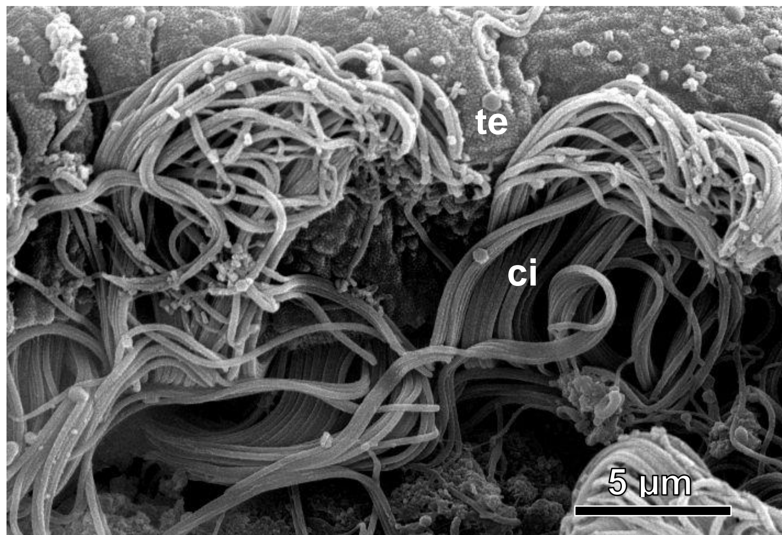

Figure 8. SEM micrograph of the lateral cilia (ci) on the tentacle (te) of a specimen of *L. vivipara*.

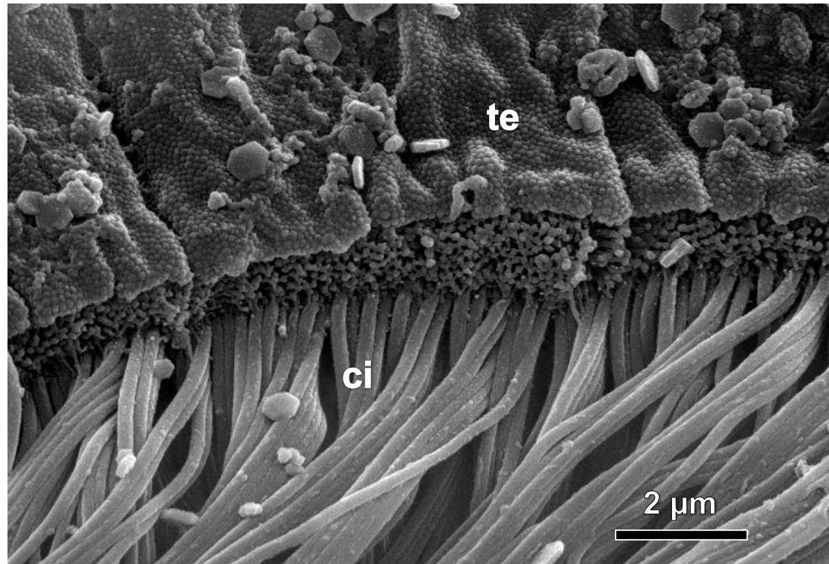

Figure 9. High magnification SEM micrograph of the lateral cilia (ci) on the tentacle (te) from a specimen of *L. vivipara*.

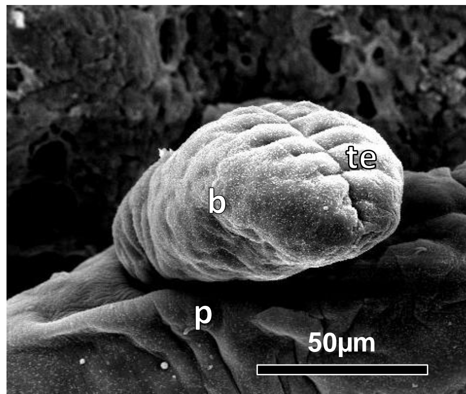

Figure 10. SEM micrograph of a bud (b) protruding from the parent (p) *L. vivipara* specimen, with 12 developing tentacles (te).

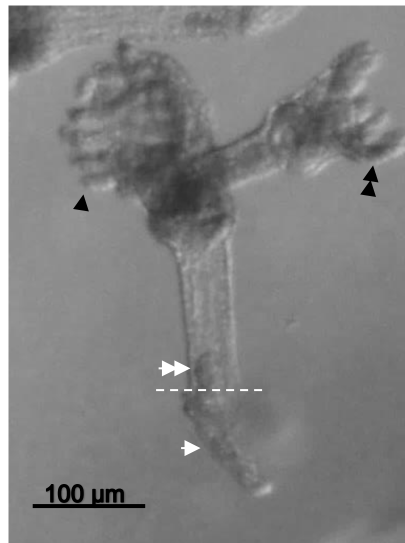

Figure 11. Light micrograph of an adult specimen of *L. vivipara* (left view) with tentacles extended (black arrowhead) and a full-grown bud (double black arrowhead). The foot of the adult (white arrowhead) is easily distinguished (dashed line) from the stalk. A large heel gland (double white arrow) is shown extending into the stalk.

Figure 12. Light micrograph of the stalk and foot of an adult specimen of L. vivipara (frontal view) stained with toluidine blue. (Left) Deep groove along underside of the foot (asterisk); heel (h). (Right) Enlarged frontal view of foot .

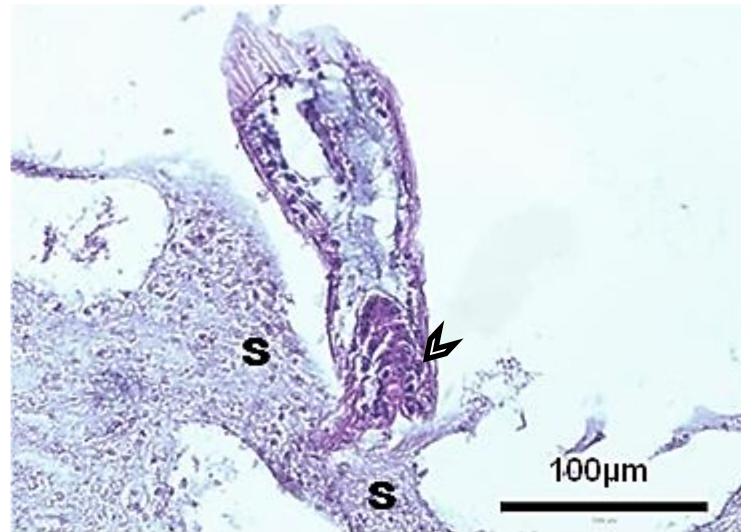

Figure 13. Longitudinal section stained with hematoxylin and eosin (frontal view) of the lower stalk and foot of a specimen attached to the sponge (s) *H*. cf. *gossypina*; heel with dark-stained gland cells containing secretory granules (arrowhead).

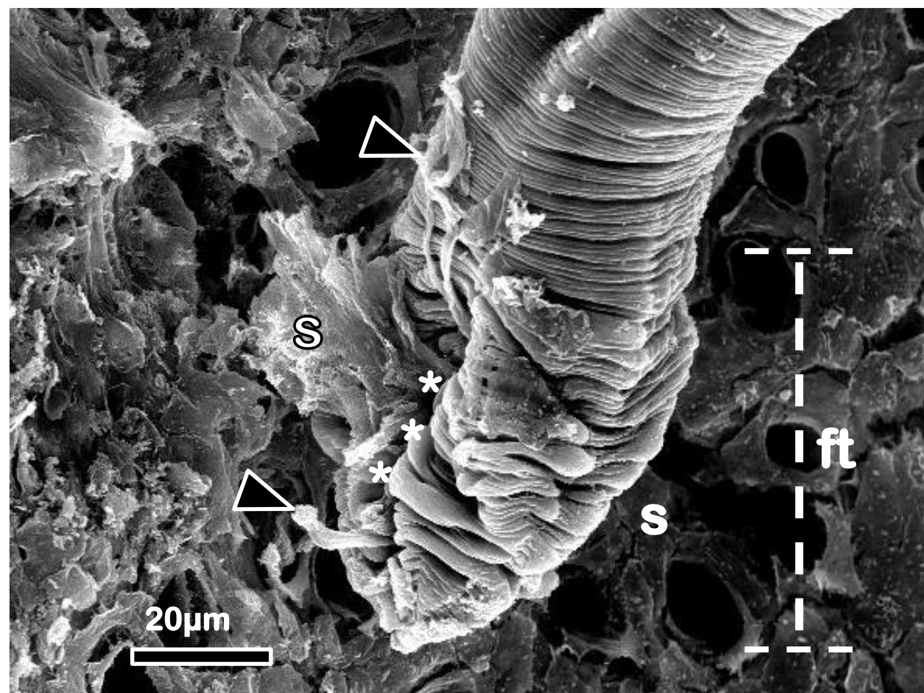

Figure 14. SEM micrograph of the foot (ft) (left view). The deep groove (asterisk) at the center of the foot is grasping part of the sponge (s). Secretions (arrowheads) produced by secretory granules inside the heel gland are visible.

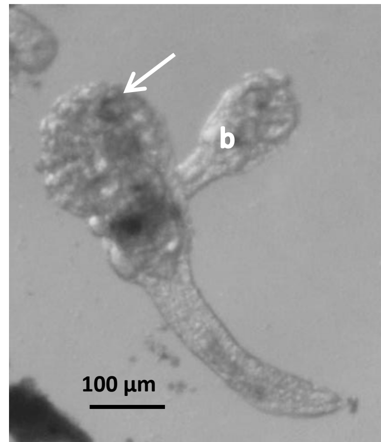

Figure 15. Light micrograph of adult *L. vivipara* specimen (left-abfrontal view) with "neck-gland" (arrow); Full-grown bud (b).

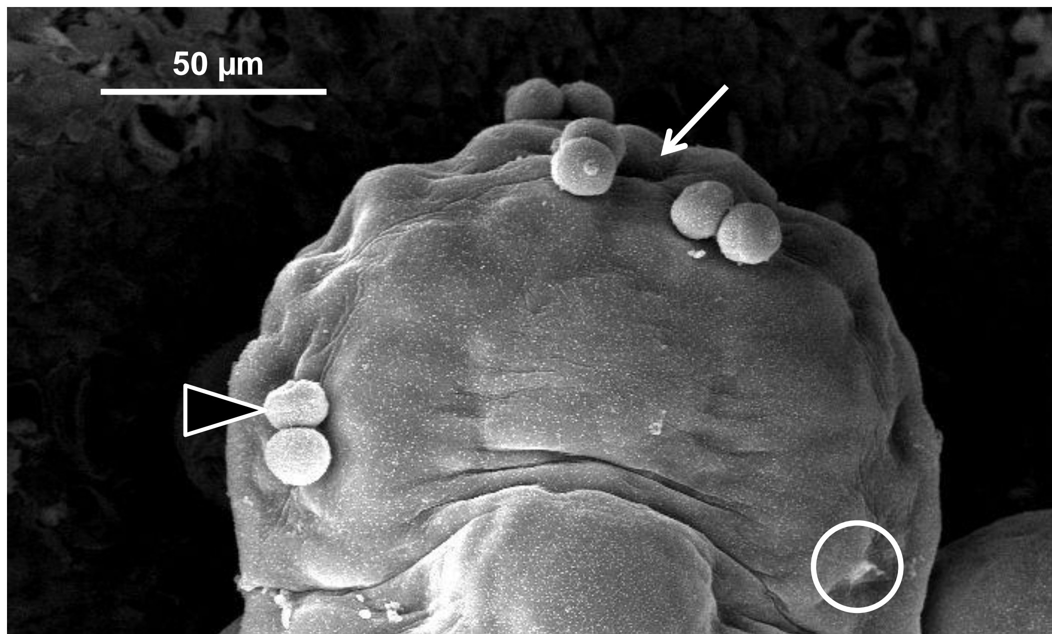

Figure 16. SEM micrograph of an adult specimen (abfrontal view) with an opening (arrow) that leads to the neck-gland. Eight protozoans are attached to the surface of this specimen (arrowhead). A sensory structure (circle) can be seen on the right side.

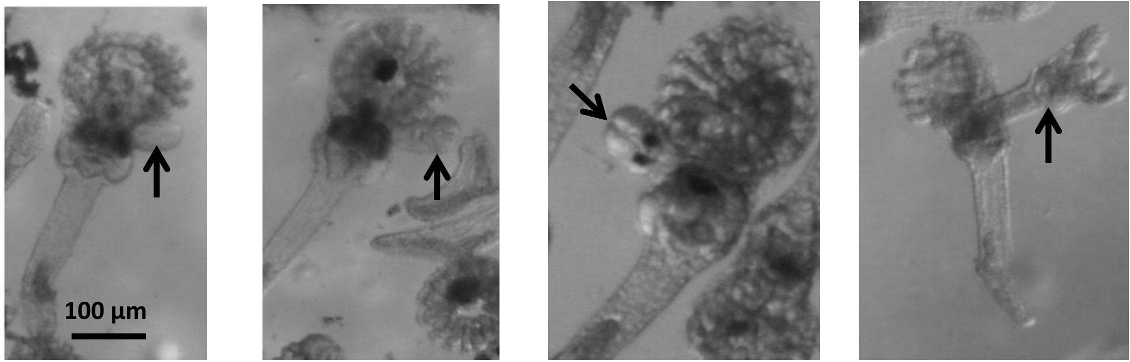

Figure 17. (Left to right) Stages of the budding cycle viewed through light microscopy. Arrows indicate the bud in each image.

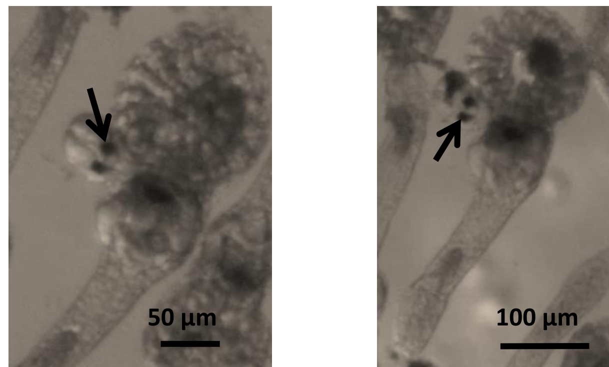

Figure 18. Light micrographs of *L. vivipara* specimens showing bud with dark pigmented spot (arrow).

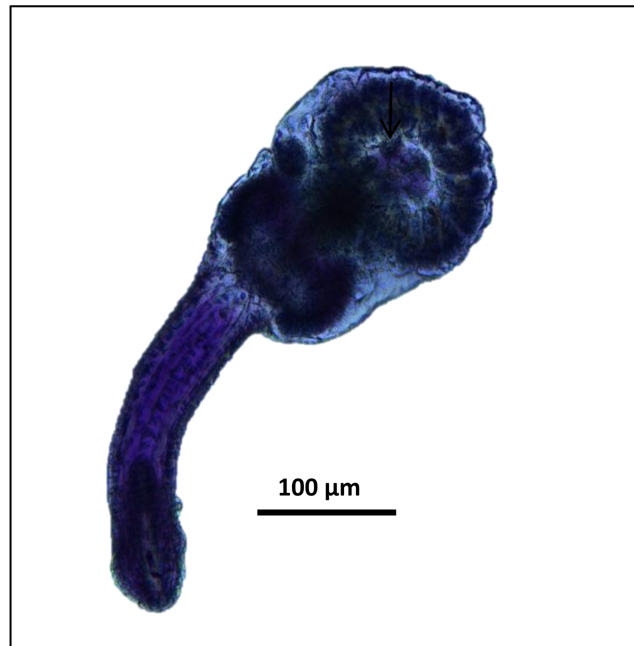

Figure 19. A small *L. vivipara* specimen with embryos in the brood chamber (arrow).

5.3 Comparison of Host Sponge Species

Of the 14 sponges collected for this comparative study, only four contained entoprocts. *Hippospongia* cf. *gossypina* and *C. nucula* were the only sponge taxa that contained entoprocts, while the other five sponge taxa did not contain entoprocts (

Table 1. Results of evaluating for the presence of entoprocts in different sponge species (two of each) collected from Summerland Key in August 2016 (n=14).). The *Hippospongia cf. gossypina*. samples had more entoprocts than the *C. nucula* samples. Each of the *C. nucula* fragments had fewer than 20 entoprocts, while the *Hippospongia.* cf. *gossypina* fragments contained about 50 entoprocts per cm^3. Entoprocts were located in the highest densities on the outer surface of *Hippospongia* cf. *gossypina*, and were also found in several lower

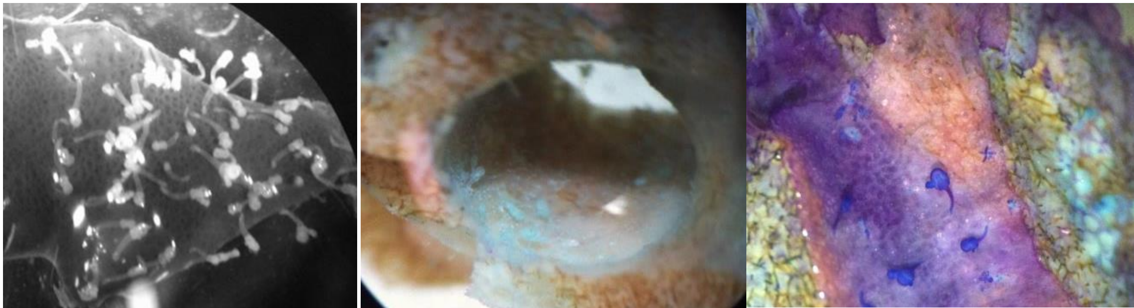

Figure 20. (Left) High density aggregations (~ 50 individuals) of *Loxosomella vivipara* attached on the outer surface of a 1 cm3 piece of *Hippospongia* cf. *gossypina*. (Center) Lower density aggregations of *Loxosomella vivipara* inside the internal canal system of *Hippospongia* cf. *gossypina*. (Right) Low density aggregation of *Loxosomella vivipara* inside of *Hippospongia* cf. *gossypina* stained with toluidine blue.

C. nucula.

Table 1. Results of evaluating for the presence of entoprocts in different sponge species (two of each) collected from Summerland Key in August 2016 (n=14). Symbols in table indicate if *Loxosomella vivipara* was present (+) or absent (-) in each sponge sample.

Sponge	*H.* cf. *gossypina*	*C. nucula*	*I.* campana	*C.* varians	*G. gibberosa*	*S. vesparium*	*Tethya sp.*
***L. vivipara* present (+) or absent (-)**	(+) (+)	(+) (+)	(-) (-)	(-) (-)	(-) (-)	(-) (-)	(-) (-)

6 DISCUSSION

6.1 Comments on Morphology and Reproduction

The Florida Keys entoproct specimens contain key morphological characters used to distinguish *L. vivipara* from other similar entoprocts. There are a few interesting points to note about the "neck gland", bud development and reproductive features of the Florida Keys *L. vivipara* specimens. In the Florida Keys specimens, the neck-gland was observed in many, but not all of the adult specimens (Figure 15, Figure 16). This organ is difficult to find through light microscopy from most angles, but is most easily noticed from a lateral-abfrontal view. The function of this organ is still unclear (Nielsen 1966a; Emschermann 2011; C. Nielsen, Natural History Museum of Denmark, personal communication), therefore it may also be worthy to document that all of the specimens containing a neck-gland were also carrying buds (but not all specimens with buds had a neck-gland). This observation is similar to findings by Emschermann (2011) who stated that about 10% of all *L. vivipara* specimens (types 1-4) from Saba Bank had a developed neck-gland that matched Nielsen's description for this species.

In the Florida Keys specimens, evidence of asexual reproduction through budding was extremely common and several stages of the bud development process were captured (Figure 17). SEM reveals that as the bud develops, a long groove forms on the part of the bud facing away from the parent, and at this groove the tentacles develop (Figure 10).

Several buds were seen to have dark pigmented spots on the calyx (Figure 18), similar to what has been described by Rützler (1963) for the buds of *L. bimaculata*. Rützler (1963)

mentions that the parent *L vivipara* specimens contain swellings near the atrial pockets with granular cells that stain in acidified toluidine blue, and that the material from the parent animal appears to become transferred from the parent to the buds as they develop in the atrial pockets. The single spot located on the bud stretches into a dumbbell shape and divides into two symmetrical dark pigmented spots (Rützler 1963). The only mention of dark pigment spots previously on *L. vivipara* was by Nielsen (1966a) when he mentions that the larvae produced through sexual reproduction contain a pair of pigmented eyes.

Nielsen (1966a) describes a unique form of sexual reproduction for *L. vivipara* that involves three separate generations (parent, larvae, juvenile). In Saba Bank, only about 4% of all specimens were reported to have the unique form of sexual reproduction and larval development that Nielsen had described (Emschermann 2011). In this study of *L. vivipara* in the FL keys, no larvae were identified, but some female individuals were observed to have embryos in the brood chamber of the atrium. The females with embryos were collected in September 2014 and August 2015. Perhaps this is indicative of a very short breeding period for this species where only a small percentage of the population transitions to a female phase for larval production sometime after August It is also important to mention here that in late January 2015 (water temperature 21˚C) no entoprocts were found on collected *H.* cf. *gossypina*. This was the only month during the study in which collected specimens of *H.* cf. *gossypina* did not contain entoprocts. These entoproct populations appear to fluctuate in size with regard to season or temperature, which is perhaps attributable to a seasonally influenced sexually reproductive timeframe.

6.2 Host Specificity

The solitary entoprocts are known commensals of a wide range of benthic organisms that create strong feeding currents, such as ascidians, sponges, polychaetes, sipunculids, and bryozoans (Nielsen 1964); and only a handful of loxosomatids have no association with specific organisms or substrata (Iseto 2005). Of the loxosomatids that associate with filter-feeding hosts, certain species seem to display a higher degree of host specificity than others (Iseto 2005). In some cases the affinity is to the extent that specialized organs can be observed which allow for effective attachment to a specific location on a particular host animal (Williams 2000).

Defining the host specificity of an organism requires knowledge of whether the organism occupies only a few (specialism) or many (generalism) types of hosts or host species. To date, *L. vivipara* has only been reported from sponges and from no other taxonomic group of host organisms; which means that it is likely a sponge specialist (Bruce, 1976). Not only is *L. vivipara* thought to be found exclusively in sponges, but to date it has only been found in the following species: *Sarcotragus fasciculatus (Ircinia fasciculata,* Pallas, 1766)*, Chondrosia collectrix*, *Tedania ignis*, *Chondrilla nucula*, and *Hippospongia* cf. *gossypina*. Nielsen (1966a) searched several other sponges for *L. vivipara* at the study site in Biscayne, including *Ircinia strobilina, Dysidea etheria, Callyspongia vaginalis, Neopetrosia longleyi, Sphesiospongia vesparium, Tethya sp., and Terpios jugax*, but did not find the loxosomatids inhabiting those sponges. The results of the sponge-species comparisons in this study showed that *L. vivipara* does not live in the sponges *Ircinia campana, Geodia gibberosa, Cliona varians, Tethya* sp., or *Sphesiospongia vesparium*, which all occupy the same habitat in the study area as *C.*

nucula, and *H.* cf. *gossypina* in the Florida Keys. These results indicate that *L. vivipara* has at least a moderate degree of specificity towards certain sponges.

To determine the precise qualities that make these sponges good hosts for *L. vivipara,* one would need to make comparisons of corrosion cast analyses of the aquiferous systems, measurements of seawater pumping rates, and food particle size selection. In the absense of such a study, some generalizations can still be made about similar qualities between *Sarcotragus fasciculatus, Chondrosia collectrix*, *Tedania ignis*, *Chondrilla nucula*, and *Hippospongia* cf. *gossypina*. Three different structural forms of aquiferous system are recognized for sponges: ascon, sycon, and leucon (Bergquist 1978; Boury-Esnault and Rützler 1997). All of the sponges that *L. vivipara* has been reported from are of the leucon-type. These sponges contain a high number of distinct chonaocyte chambers distributed throughout the mesohyl, which connect the complex and often long and meandering incurrent and excurrent canal systems (Bergquist 1978; Boury-Esnault and Rützler 1997). *Hippospongia* cf. *gossypina* is a very cavernous sponge with large subdermal cavities and internal lacuna ranging from 3-7mm in diameter, and contains from 2-6 collared membrane oscula (from 0.5-1.5cm in diameter) widespread over the upper portion of the sponge body (Appendix II).

6.3 Location of *Loxosomella vivipara*

Both *H.* cf. *gossypina* and *C. nucula* contained entoprocts in clusters on the outer surface of the sponge; however, most of the *H.* cf. *gossypina* samples also showed clusters of entoprocts inside of the canals. This is only one of a few instances in which entoprocts have been reported to inhabit the internal spaces of a sponge. Nielsen (1964) when discussing the possible benefit from the large masses of water produced by

sponges, states that "*Loxosomella* found on sponges, whether situated on the outer side of the sponge or in its canal system, may benefit from these water currents". Varela et al. (2011) reported to find *L. cubana* inside of the canals and on the outer surface of the sponge *Aiolochroia crassa* in Cuba. Nielsen (1966a) reported *L. vivipara* from only the outer surface around the osculum of sponges, and published reports of other loxosomatid species from sponges only mention observing them attached to the outer surfaces as well (e.g. Rützler 1968; Nielsen 2008; Sánchez-Tocino & Tierno de Figueroa 2009; Sugiyama et al. 2010; Giorgi et al. 2016).

It is possible that some entoprocts occupy the canals as a strategy to avoid molluscan and platyhelminth predators (Canning and Carlton 2000; Sánchez-Tocino & Cervera 2006) that would more easily find and consume entoprocts living on the outer surface. Another possible explanation is that certain sponges such as *H.* cf. *gossypina* and *A. crassa* have unique features of the aquiferous system that either make internal colonization easier or offer ideal feeding conditions for the loxosomatid inhabitants. It is also arguable that internal colonization is actually more common in other sponge species, but is rarely observed and reported because it requires slicing the sponge into 5-10mm cross-sections and thoroughly examining each section from different angles under a dissecting microscope.

Nielsen (1964) states that it would be interesting to look at exactly where in the canal systems of the sponges the loxosomatids are attaching to, and that no one has observed or made detailed notes of this phenomenon. The author also hypothesizes that it would be difficult for the entoprocts to secure any food particles once it has passed the ciliated chambers of the sponge. Interestingly, recent studies on the aquiferous system of

Spongia officinalis (a commercial sponge that is very similar in morphology and ecology to *H. gossypina*) using corrosion cast analysis revealed that these sponges possess what are known as "bypass canals" which are large ducts that actually bypass the choanocyte chambers and provide a direct connection between some of the incurrent and excurrent canals (Burlando et al. 2009). This same "bypass canal" system was also documented in *Chondrosia reneformis* by Bavestrello et al. (1988). Presumably, water flowing through these bypass canals that connect the incurrent canals to the excurrent canals would contain a wide range of particles available for consumption by entoprocts, since along this pathway fewer particles are taken up by the sponge compared to within the pathways that pass through the choanocyte chambers. Clusters of *L. vivipara* were usually closer to the ectoderm than the center of the sponge body. The canals that *L. vivipara* occupies inside of *H.* cf. *gossypina* were relatively large in diameter (~3-7mm), so were most likely a part of the excurrent pathways of the sponge. Perhaps *H.* cf. *gossypina* contains bypass canals that empty into these excurrent canals, which could explain why *L. vivipara* is located within these areas.

6.4 Remarks on the Nature of the Symbiosis

Entoprocts and sponges are both filter feeders, and the presence of another filtering organism nearby may either facilitate or diminish the feeding success of a filter feeding animal (Okamura 1984; 1985). Studies on the particle size overlap between food sources of *L. vivipara* and its sponge hosts would be necessary to discern the exact nature of the symbiosis in terms of the mutualism-parasitism spectrum. A study on *Loxosomella nordgaardi* and its bryozoan hosts revealed that the loxosomatids rely directly on the ciliary activity of the host bryozoans' lophophores for food, and that both organisms may

actually benefit from this interaction (Yakovis et al. 2002). Further studies on the particle size selection of *L. nordgaardi* and its most preferred bryozoan host species, *Tegella armifera*, revealed that while both species feed on diatoms, notable overlap exists only for the consumption of smaller food particles (less than 15µm) (Tamberg et al. 2013). Despite the overlap in food selection, an analysis of the gut contents revealed that the presence of *L. nordgaardi* had no effect on the size spectra and number of ingested diatoms of *T. armifera*; and therefore *L. nordgaardi* can be considered a commensal of *T. armifera* (Tamberg et al. 2013). Commensalism is defined as a relationship where one organism benefits from the association without seriously inconveniencing or harming the other organism (Dales 1957).

The nature of the symbiosis between *L. vivipara* and its sponge hosts is most likely of a commensalistic nature as well. In order for the entoprocts to be parasitic on these sponges, there would have to be a significant overlap in the particles that both organisms consume and a resultant negative disruption in the ability of the sponges to meet their normal dietary needs. Studies on *Spongia officinalis* (similar in morphology and ecology to *H. gossypina*) revealed that the sponges primarily take up picoplankton particles < 3µm (Stabili et al. 2006; 2008; Topku et al. 2010), and some nanoplankton (2– 20 µm) is taken up closer to the epidermis (Reiswig 1971; Schmidt 1970). Very few studies exist on food selection in entoprocts, however existing literature states that entoprocts mainly consume phytoplankton or other organic particles (Iseto 2005; Nielsen 2016), especially small rounded cells between 0-15µm (Tamberg et al. 2013). It is therefore possible that some overlap exists between the food sources consumed by these demosponge hosts and their *L. vivipara* symbionts; however, it seems unlikely that the entoprocts would

seriously disrupt the dietary needs of the much larger sponges. For the association to be mutualistic, the sponge would have to show some sort of benefit due to the presence of *L. vivipara*. For example, a filter feeding mutualism has been proven between the commercial sponge *Spongia* sp. and an endosymbiotic bivalve *Vulsella vulsella* in which the bivalve gains protection and food, while the sponge benefits through a dramatically increased pumping rate and higher absolute particle retention due to the additional flow from the bivalve's excurrent (Tsubaki and Kato 2014). Although laboratory experiments would be necessary to calculate specific pumping rates, it is not very likely that the sponges are benefiting from the ciliary currents of *L. vivipara*, which are much weaker than the currents produced by these sponges. The reported pumping rate for *S. officinalis* is 0.384 ml ml^{-1} s^{-1} (*converted from* Stabili et al. 2006), and *C. nucula* has been reported to reach a pumping rate of 0.023ml ml^{-1} s^{-1} (*converted from* Milanese et al. 2003). Commensalism is therefore the most likely category for this association. Again, laboratory experiments would clarify these speculations about the nature of the symbiosis.

6.5 Conclusions

There have been few published studies on entoprocts, especially in the Western Atlantic. These organisms are often overlooked due to their microscopic size (< 5mm), transparent bodies, and their meiobenthic lifestyle on biotic and abiotic substrata (Sugiyama et al. 2010). Little is known about the distributions and ecosystem functions of entoprocts. It is possible that these fast-reproducing filter-feeders, although small, may play an important role in the cycling of organic matter in shallow coastal lagoons, bays,

inlets and marinas. They may also provide advantageous services to various benthic sessile marine invertebrates through mutualistic symbioses.

In this study, two demosponges in the Florida Keys, *H.* cf. *gossypina* and *C. nucula,* were found to contain populations of solitary entoprocts. Entoprocts were not found in other common sponges collected from the same location (*I. campana, G. gibberosa, C. varians, Tethya* sp., and *S. vesparium*). Based on a match to morphological descriptions of *L. vivipara* from Key Biscayne (from *S. fasciculatus*) and Belize (from *T. ignis*) and a 99% match of similarity to aligned partial sequences from 28S and 18S nuclear ribosomal genes (rDNA) from *L. vivipara* in Belize (from *T. ignis*), the entoproct specimens collected from the FL Keys (from *H. cf. gossypina* and C. nucula) in this study have been confirmed as *L. vivipara*. *Loxosomella vivipara* was first described from the shallow water sponge *S. fasciculatus* in 1965 (Nielsen 1966a). It has also been found in *T. ignis* and *C. nucula* from Key Biscayne, *C. collectrix* from Bimini, Bahamas (Nielsen 1966a), and *T. Ignis* from Belize (Fuchs et al. 2006). This is the first report of *L. vivipara* from the FL Keys, the first report of an association between *L. vivipara* and *H.* cf. *gossypina*, the second report of an association between *L. vivipara* and *C. nucula*, and is the first time the ITS2 region of *L. vivipara* has been sequenced. This study therefore expands knowledge of the biogeographic distribution, molecular genetics, and host selection of *L. vivipara.*

Hippospongia gossypina (velvet sponge) was highly valued by the sponge fishing industry throughout the 19th and early 20th century, and made up a large portion of the take from the FL Keys the Bahamas. The velvet sponge, like other commercial sponges, was reported to have disappeared from these waters due to a fungal epidemic in 1938 (De

Laubenfels 1952; Storr 1964), and was even further affected by an epizootic event that occurred in the late 1980s, as well as subsequent over-fishing (DiResta et al. 1995). *Hippospongia gossypina* has rarely been mentioned in literature over the last 5 decades or so, other than a report that one velvet sponge that was taken from the Northeastern Gulf of Mexico (Lat. 29° 39' N, Long. 83° 56' W) during a sponge survey in 1947 (De Laubenfels 1952), and a statement by Storr (1964) that "all the velvet sponges were destroyed in the Bahamas, and none has been observed since". A report by Stevely et al. (2010) documented and quantified the contribution of commercial sponges to the total sponge community biomass in the middle and upper Keys; however, this study only mentions the commercial species (wool sponge *Hippospongia lachne,* yellow sponge *Spongia barbara,* and glove sponge *Spongia graminea*) and the most common large species (*Spheciospongia vesparia, Ircinia campana, Ircinia strobilina,* and *Ircinia spp.*), with no mention of *H. gossypina*. A later study by Stevely et al. (2011) documenting the abundance of marine sponges following mortality events also does not provide any mention of *H. gossypina*, but does report on the other similar commercial sponges (*H. lachne, S. barbara,* and *S. graminea*).

In this study, many healthy individuals of *H.* cf. *gossypina* were observed in the waters of Summerland Key, FL. Not only were these sponges found in the Lower Keys during sponge collections for this thesis research, but several were also found while scuba diving in Biscayne National Park at shallow reef sites East of Elliot Key (Rachel Plunkett, Florida Atlantic University, personal observations). This study therefore provides the first evidence that populations of the velvet sponge are returning – at least in

the waters of Summerland Key and Biscayne National Park where they have been observed directly.

This study contributes an extensive amount of additional information on these loxosomatids. Previous studies relied on a single collection event from one location, whereas in this study *L. vivipara* was collected from 40 sponge hosts from the same location on multiple occasions (10 times) over the course of a year. The results of this study combined with previous studies on *L. vivipara* provide evidence that this species is likely specific to sponges as hosts. It is possible that *L. vivipara* could occupy other host organisms, but none other than sponges have been reported to date. This particular symbiosis is most likely of a commensalistic nature in which the entoprocts are dependent on the host sponge for protection and food particles. *Loxosomella vivipara* seems to prefer leuconoid sponge hosts with complex, cavernous aquriferous systems. Other qualities such as the seawater pumping rate and food particle size selection of sponges are also likely factors in host selection for sponge-dwelling entoprocts.

This study also provides new evidence for inquilinism. The presence of entoprocts in the canals of a sponge is a rare discovery. To date, inquilinism has only been reported in two entoproct-sponge associations: *L. cubana* in *A. crassa* and *L. vivipara* in *H.* cf. *gossypina.* These Inquilinistic associations could possibly be attributed to certain aquiferous qualities unique to *A. crassa* and *H.* cf. *gossypina*. Presumably, inquilinism is not the best option for most *Loxosomella* that associate with sponges because the sponges would compete with the entoprocts and consume most of the food particles. Adequate food particles must be available to the entoprocts residing in the canals of *A. crassa* and *H.* cf. *gossypina*. It is possible that these sponges have

arrangements similar to the choanocyte chamber bypass structures described for *S. officinalis* (Burlando et al. 2009) and C. reneformis (Bavestrello et al. 1988). Inquilinism can also be a response to an abundance of predators on the surface of these sponges.

Several research directions could advance the knowledge of sponge specialist loxosomatids. Studies comparing the various qualities related to sponge architecture could reveal if certain features facilitate inquilinism by entoprocts and other lophophorate symbionts of sponges. Further studies on the host-symbiont signaling pathways between sponges and entoprocts; the spatial distribution of entoprocts on sponge surfaces; laboratory experiments on food source overlap, and comparisons of respiration rates and energy fluctuations between symbiotic and aposymbiotic sponges are also possible future research pathways.

7 APPENDIXES

7.1 Appendix I

Table 2. Sequence alignments of 28S rDNA between 5 query replicates and *L. vivipara* from GenBank (Accession no. GU125730). Black shading indicates a match, light or no shading indicates mismatch.

```
Query28S_1    1 GACTATTGCATCAGCTCCATCCGCTTCAATCCAAGCGGTTTCACGTACTCTTGAACTCTCTCTTCAAAGT
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Query28S_4    1 GACTATTGCATCAGCTCCATCCGCTTCAATCCAAGCGGTTTCACGTACTCTTGAACTCTCTCTTCAAAGT
Query28S_5    1 GACTATTGCATCAGCTCCATCCGCTTCAATCCAAGCGGTTTCACGTACTCTTGAACTCTCTCTTCAAAGT
28S_L_vivi    1 ------------------------------------------------------------TCTTCAAAGT

Query28S_1   71 TCTTTTCAACTTTCCCTCACGGTACTTGTCGACTATCGGAAACGTGCAGGTATTTAGCCTTAGATGGAGT
Query28S_2   71 TCTTTTCAACTTTCCCTCACGGTACTTGTCGACTATCGGAAACGTGCAGGTATTTAGCCTTAGATGGAGT
Query28S_3   71 TCTTTTCAACTTTCCCTCACGGTACTTGTCGACTATCGGAAACGTGCAGGTATTTAGCCTTAGATGGAGT
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Query28S_5   71 TCTTTTCAACTTTCCCTCACGGTACTTGTCGACTATCGGAAACGTGCAGGTATTTAGCCTTAGATGGAGT
28S_L_vivi   11 TCTTTTCAACTTTCCCTCACGGTACTTGTCGACTATCGGAAACGTGCAGGTATTTAGCCTTAGATGGAGT

Query28S_1  141 TTACCACCCACTTTGGGCTGCATTCTCAAACAACCCGACTCCTCGGACACTCACAGCAGTGTGAGGCTAG
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Query28S_3  141 TTACCACCCACTTTGGGCTGCATTCTCAAACAACCCGACTCCTCGGACACTCACAGCAGTGTGAGGCTAG
Query28S_4  141 TTACCACCCACTTTGGGCTGCATTCTCAAACAACCCGACTCCTCGGACACTCACAGCAGTGTGAGGCTAG
Query28S_5  141 TTACCACCCACTTTGGGCTGCATTCTCAAACAACCCGACTCCTCGGACACTCACAGCAGTGTGAGGCTAG
28S_L_vivi   81 TTACCACCCACTTTGGGCTGCATTCTCAAACAACCCGACTCCTCGGACACTCACAGCAGTGTGAGGCTAG

Query28S_1  211 CGACGTAAGGGCCTAGCACCCGCTCTGGGAGAAAGCCCCGTTCAAGAGAACTTGGTCGCCCCTCAAAGCA
Query28S_2  211 CGACGTAAGGGCCTAGCACCCGCTCTGGGAGAAAGCCCCGTTCAAGAGAACTTGGTCGCCCCTCAAAGCA
Query28S_3  211 CGACGTAAGGGCCTAGCACCCGCTCTGGGAGAAAGCCCCGTTCAAGAGAACTTGGTCGCCCCTCAAAGCA
Query28S_4  211 CGACGTAAGGGCCTAGCACCCGCTCTGGGAGAAAGCCCCGTTCAAGAGAACTTGGTCGCCCCTCAAAGCA
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Query28S_3  281 CTACAGGATAGCGTCCTTAACGCTACATGTCCCACGGGCTGCAGTTTCCCGGGGATTC------------
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Query28S_1  351 TTCCCGCTTCACTCGCCGT---------------------------------------------------
Query28S_2  351 TTCCCGCTTCACTCGC------------------------------------------------------
Query28S_3      ----------------------------------------------------------------------
Query28S_4  351 TTCCCGCTTCACTCGCCGTTACTAG---------------------------------------------
Query28S_5  351 TTCCCGCTTCACTCGCCGTTACTAG---------------------------------------------
28S_L_vivi  291 TTCCCGCTTCACTCGCCGTTACTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTAGTGATATGCTTA

Query28S_1      --------------
Query28S_2      --------------
Query28S_3      --------------
Query28S_4      --------------
Query28S_5      --------------
28S_L_vivi  361 AATTCAGCGGGTAA
```

Table 3. Sequence alignments of 18S rDNA between two query replicates and *L. vivipara* from GenBank (Accession no. GU125745). Black shading indicates match, light or no shading indicates mismatch.

```
Query18S_1    1 AACTTTTATAAAGTGAAACCGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAGATCGTACAACAGTTA
Query18S_2    1 -------------------------------------TCAGTTATGGTTCCTTAGATCGTACAACAGTTA
18S_L_vivi    1 -----------AGTGAAACCGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAGATCGTACAACAGTTA

Query18S_1   71 CTTGGATAACTGTGGGAATTCTAGAGCTAATACATGCAGAAAAGCTCTGACCCTCTGGGAAAGAGCGCAG
Query18S_2   34 CTTGGATAACTGTGGGAATTCTAGAGCTAATACATGCAGAAAAGCTCTGACCCTCTGGGAAAGAGCGCAG
18S_L_vivi   60 CTTGGATAACTGTGGGAATTCTAGAGCTAATACATGCAGAAAAGCTCTGACCCTCTGGGAAAGAGCGCAG

Query18S_1  141 TTATTGGTTCAAGCCAACCGCAGCTCACGCTGCGACTCTTTGGTGACTCTGGATAACCTTGTGCGGATCG
Query18S_2  104 TTATTGGTTCAAGCCAACCGCAGCTCACGCTGCGACTCTTTGGTGACTCTGGATAACCTTGTGCGGATCG
18S_L_vivi  130 TTATTGGTTCAAGCCAACCGCAGCTCGCGCTGCGACTCTTTGGTGACTCTGGATAACCTTGTGCGGATCG

Query18S_1  211 CATGACCTTGTGTCGGCGACGTATCTATCGAATGTCTGACCTATCAACTTTCGATGGTAGGTGATATGCC
Query18S_2  174 CATGACCTTGTGTCGGCGACGTATCTATCGAATGTCTGACCTATCAACTTTCGATGGTAGGTGATATGCC
18S_L_vivi  200 CATGACCTTGTGTCGGCGACGTATCTATCGAATGTCTGACCTATCAACTTTCGATGGTAGGTGATATGCC

Query18S_1  281 TACCATGGTTGTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACC
Query18S_2  244 TACCATGGTTGTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACC
18S_L_vivi  270 TACCATGGTTGTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGAAACGGCTACC

Query18S_1  351 ACTTCTACGGAAGGCAGCAGGCGCGCAAATTACCCAATGTCGGCTCGACGAGGTAGTGACGAAAAATAAC
Query18S_2  314 ACTTCTACGGAAGGCAGCAGGCGCGCAAATTACCCAATGTCGGCTCGACGAGGTAGTGACGAAAAATAAC
18S_L_vivi  340 ACTTCTACGGAAGGCAGCAGGCGCGCAAATTACCCAATGTCGGCTCGACGAGGTAGTGACGAAAAATAAC

Query18S_1  421 AATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACATTTCAAATCCCTTAACGAGGATCTATTG
Query18S_2  384 AATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACATTTCAAATCCCTTAACGAGGATCTATTG
18S_L_vivi  410 AATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACATTTCAAATCCCTTAACGAGGATCTATTG

Query18S_1  491 GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTTCAATAGCGTATATTAAAGTTGTTGCAGTT
Query18S_2  454 GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTTCAATAGCGTATATTAAAGTTGTTGCAGTT
18S_L_vivi  480 GAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTTCAATAGCGTATATTAAAGTTGTTGCAGTT

Query18S_1  561 AAAAAGCTCGTAGTTGGATCTCAGGCTTAGGCAGGTGGTCCACCTAGCGGTGGTACTGCTTGACCCGGCC
Query18S_2  524 AAAAAGCTCGTAGTTGGATCTCAGGCTTAGGCAGGTGGTCCACCTAGCGGTGGTACTGCTTGACCCGGCC
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Query18S_1  631 TACCTCCCGGTACGCTCTTGGTGCCCTTAATTGGGTGTCTCGGGTGTCTGGAACGTTTACTTTGAAAAAA
Query18S_2  594 TACCTCCCGGTACGCTCTTGGTGCCCTTAATTGGGTGTCTCGGGTGTCTGGAACGTTTACTTTGAAAAAA
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Query18S_1  771 TTTTGTTGGTTTATGGGCTCGAGGTAATGATTAAGAGGGACTGACGGGGGCATTCGTATTACGGTGTTAG
Query18S_2  734 TTTTGTTGGTTTATGGGCTCGAGGTAATGATTAAGAGGGACTGACGGGGGCATTCGTATTACGGTGTTAG
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Query18S_2  804 AGGTGAAATTCTTAGATCATCGTAAGACGAACAACTGCGAAAGCATTTGCCAAGAATGTTTTCATTAATC
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Query18S_1  911 AAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGATGCCATCTA
Query18S_2  874 AAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGATGCCATCTA
18S_L_vivi  900 AAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCCTAGTTCTGACCATAAACGATGCCATCTA

Query18S_1  981 GCGATCCGCCAGTGTTGGCAATATGACATGGCGGGCAGCTCCCGGGAAACCAAAGTTTTTGGGTTCCGGG
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18S_L_vivi  970 GCGATCCGCCAGTGTTGGCAATATGACATGGCGGGCAGCTCCCGGGAAACCAAAGTTTTTGGGTTCCGGG

Query18S_1 1051 GGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGG
Query18S_2 1014 GGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGG
18S_L_vivi 1040 GGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGG

Query18S_1 1121 CTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACTAGGAGGATTGACAGATTGAGAGCTCT
Query18S_2 1084 CTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACTAGGAGGATTGACAGATTGAGAGCTCT
18S_L_vivi 1110 CTTAATTTGACTCAACACGGGAAAACTCACCTGGCCCGGACACTAGGAGGATTGACAGATTGAGAGCTCT
```

7.2 Appendix II

Species Description Sheet for *Hippospongia* cf. *gossypina*

SAMPLE ID- 5-5-15-1-1; 5-5-15-1-2; 6-5-15-1-1; 6-5-15-1-2; 6-5-15-1-3; 7-5-15-1-1; 7-5-15-1-2; 7-5-15-1-3 (8 sponges)

LOCATION, GPS- 24°39'38.2"N 81°27'17.8"W DATE- 5/5/15 – 5/7/15

SUBSTRATE- Sand/scattered mixed algae DEPTH- 1.5-3m

SPECIES- *Hippospongia* cf. *gossypina* (Duchassing and Michelotti, 1864)

ORDER- Dictyoceratida FAMILY- Spongiidae

SHAPE AND SIZE- Massive globular to subglobular from 6-15cm in width and height (Figure 21). Sponge body profusely lacunose, with lacuna 3-7mm in diameter that may run all the way to the surface. Subdermal cavities present.

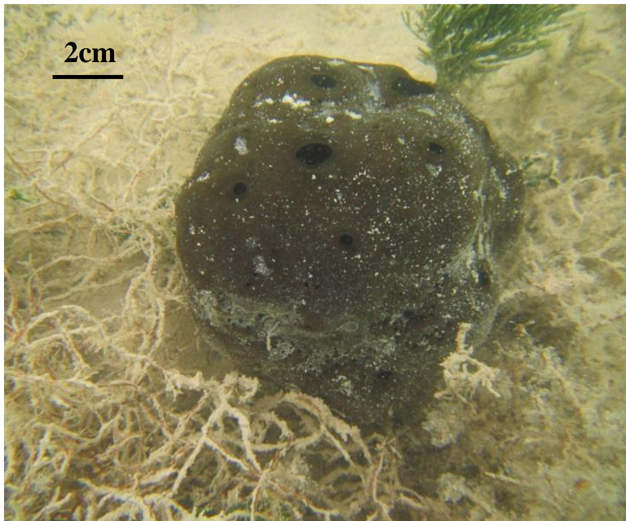


Figure 21. Massive form of *Hippospongia* cf. *gossypina* viewed from underwater.

COLOR (EXTERNAL/INTERNAL)-.Black externally, tan to rust-colored internally.

SURFACE- Surface unarmored. Appears smooth to the naked eye when viewed underwater alive. Once taken out of the water, the skin recedes and a meandering surface

is evident with meanders 2-3mm in width and several cm in length. Microconules less than 0.5mm in height and separation are evident when viewed under stereomicroscope.

CONSISTENCY- Very compressible.

APERTURES (OSCULES, AND OSTIA) - Collared membrane oscula (0.5-1.5cm in diameter), 2-6 in number, widespread over the upper portion of the sponge body. Smaller apertures densely arranged around the lateral sides of the sponge body (from 2-3mm in diameter), often arranged in clusters. Ostia (from 6-41µm in diameter) arranged in clusters observable through SEM (Figure 22).

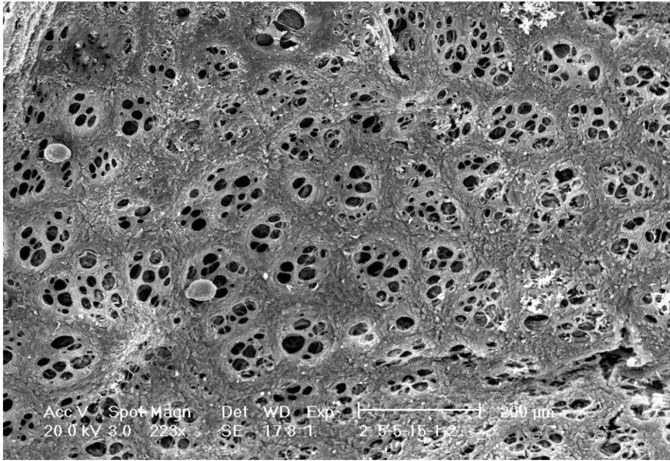


Figure 22. SEM of the inhalant pores of *Hippospongia* cf. *gossypina.*

ODOR, MUCUS, EXHUDATE, ETC - Odor similar to species of *Ircinia* (M.C. Diaz and S.A. Pomponi, personal communication).

SKELETAL ELEMENTS- Homogenous and clear spongin fibers (from 10-42µm in diameter) (Figure 23a,b).

SKELETAL ARRAGEMENT- Highly developed fiber network characterized by the complete absence of primary fibers. Specimens have a network of un-cored secondary fibers (Figure 24) with irregularly shaped meshes; some rectangular to polygonal meshes are distinguished.

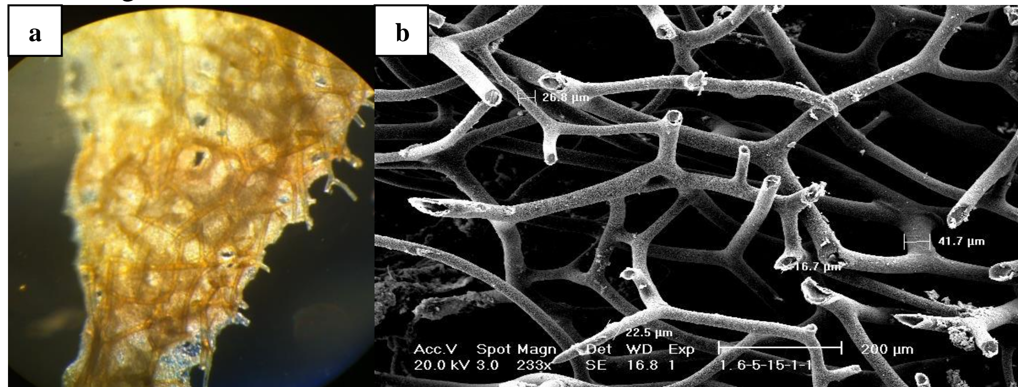


Figure 23 (a-b). (a) Ligh micrograph of fiber mesh network of *Hippospongia* cf. *gossypina* (b) SEM of mesh network showing diameter of some fibers.

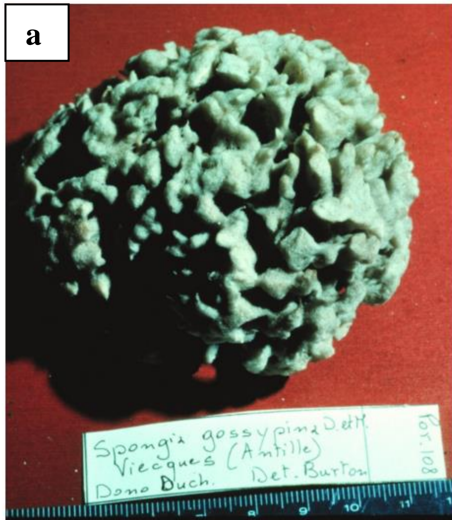
Figure 24. SEM cross section of a fiber of *Hippospongia* cf. *gossypina*.

HABITAT- Shallow lagoon habitat with a thick, loose sandy bottom dominated by sponges and mixed macroalgae.

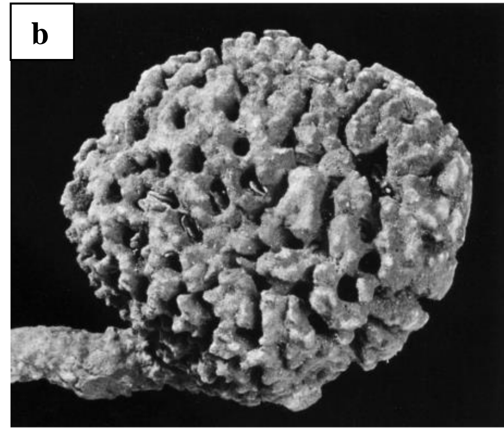
DISTRIBUTION- Greater Antilles, Bahamas, Florida, Northern Gulf of Mexico

REMARKS- Descriptions from Duchassing & Michelotti (1864) as well as the dichotomous key and terminology of Cook and Bergquist (2002) were used to complete this description.

The specimens studied resemble *Hippospongia gossypina* based on descriptions of the overall external and internal morphology; nevertheless, the most recent description of the species (VanSoest 1978) does not describe any large visible oscules, which are abundant and obvious in all of the specimens we studied. The external appearance of some of the types of this species (Lectotype MSNT Por. 108 and Paralectotype ZMA Por. 02087) (Figure 25 a,b) depicted by VanSoest et al. 2016 (World Porifera Database) do not appear to match these specimens. Therefore, we maintain our specimens are very closely related to *Hippospongia gossypina* and for now will use the terminology “cf.” (Latin for confer) to indicate that the specimen resembles the named species very closely, but has certain minor features not found on some of the type specimens. A comparative study of fresh *Hippospongia gossypina* material from different localities and museum type specimens could clarify the identity of our Florida specimens.



Spongia gossypina lectotype specimen
Description Lectotype MSNT Por. 108, Photo Klaus Rützler
Author van Soest, Rob
JPG file - 408.17 kB - 981 x 1 122 pixels
added on 2013-02-18
198 views
Porifera taxa
Spongia gossypina Duchassaing & Michelotti, 1864 accepted as *Hippospongia gossypina* (Duchassaing & Michelotti, 1864) checked van Soest, Rob 2013-02-18



ossypina paralectotype specimen
on Paralectotype ZMA Por. 02087, Photo L.A. van der Laan, from Van Soest, 1978: pl.IV fig. 4
n Soest, Rob
65.45 kB - 1 212 x 902 pixels
2013-02-18

axa
ossypina Duchassaing & Michelotti, 1864 accepted as *Hippospongia gossypina* (Duchassaing & Michelotti,

Figure 25 (a-b). (a) Lectotype and (b) paralectotype for *Hippospongia gossypina* Duchassing & Michelotti, 1864

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