A New Sponge-Inhabiting Loxosomella (Entoprocta: Loxosomatidae) from Okinawa Island, Japan, with Special Focus on Foot Structure

Authors: Iseto, Tohru, Sugiyama, Nagisa, and Hirose, Euichi

Source: Zoological Science, 25(11) : 1171-1178

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.25.1171

ZOOLOGICAL SCIENCE **25**: 1171–1178 (2008)

A New Sponge-Inhabiting *Loxosomella* (Entoprocta: Loxosomatidae) from Okinawa Island, Japan, with Special Focus on Foot Structure

Tohru Iseto[1*†], Nagisa Sugiyama[2] and Euichi Hirose[2]

[1]*University Education Center, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*
[2]*Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

A new solitary entoproct, *Loxosomella plakorticola* sp. nov., was found on a sponge, *Plakortis* sp., on a coral reef slope on the western coast of Okinawa Island, Ryukyu Archipelago, Japan. This species has a medium-sized body (up to about 1.2 mm), slender proportion (the stalk is 0.83–1.76 times longer than the calyx), a slug-like foot with a foot gland and foot groove, and 14 to 18 tentacles. Small black pigment granules are visible only in the living stage in the calyx, stalk, and buds. This is the first report of a commensal loxosomatid from the Ryukyu Archipelago and the second species inhabiting sponges reported from Japan. Detailed morphological observations indicate that this species attaches to sponges by narrowing the foot groove; the sponge surface is pinched in the deepest part of the groove, which is free of the cuticle layer but covered by microvilli of epidermal cells. The accessory cells lining the foot groove have long been believed to be gland cells, but they are not gland cells in ultrastructure, at least in this species.

Key words: commensal species, coral reef, groove accessory cells, Kamptozoa, solitary entoproct, ultrastructure

INTRODUCTION

Entoprocts are small benthic animals that live mostly in marine waters. Scientists currently recognize about 180 species, of which 50 are colonial and the remainder solitary. All solitary species belong to the family Loxosomatidae, and most loxosomatids associate with larger animals such as polychaetes, sponges, bryozoans, and sipunculans (Nielsen, 1964; Soule and Soule, 1965). Commensal entoprocts attach to the body surface of animals or the inner surface of animals' tubes. Commensal association benefits entoprocts by providing a safe habitat (Iseto, 2005) and water currents generated by the host animals, which provide loxosomatids with food particles and fresh water for respiration (Nielsen, 1964). However, recent reports have indicated that non-commensal species are also common in this taxon (Iseto, 2005) and are found on stones, shells, algae, and man-made materials such as plastic panels and glass slides. On the shallow coral reef shores of Okinawa Island, the abundance of non-commensal loxosomatids exceeded 1800 individuals per glass slide in August (Iseto et al., 2007).

Loxosomatids have a "foot" attached to the substratum. Because this foot is the only organ that has direct contact with the substratum, it likely plays a pivotal role in substrate selection and attachment. For commensal species, the foot may establish and maintain the specific association with the host. Moreover, the foot is key in the generic classification of loxosomatids: Each genus is characterized by a specific foot structure and budding mode (see Iseto, 2002). Therefore, investigating the detailed structure of the foot is important for better understanding the ecology and taxonomy of entoprocts.

All 12 species reported to date from Okinawa Island and its vicinity are non-commensal and have been found on stones, dead coral fragments, shell remains, and glass slides immersed along the coast (Iseto, 2001, 2002, 2003, 2006). Here we provide the first report of a commensal loxosomatid from the Ryukyu Archipelago: a new *Loxosomella* species found densely on a sponge on a reef slope at a depth of about 10–15 m. Foot structure was studied histologically and ultrastructurally to determine how the loxosomatid attaches to the sponge surface.

MATERIALS AND METHODS

Loxosomatids were collected from a demosponge, *Plakortis* sp., at a depth of about 11 m on a reef slope at Manza, Onna Village, on the western coast of Okinawa Island (26°30'13.70"N, 127°50'32.39"E) by SCUBA diving. Underwater photographs of colonies of loxosomatids on the host sponge were taken in the natural

* Corresponding author. Phone: +81-739-42-3515;
Fax : +81-739-42-4518;
E-mail: tohru_iseto@hotmail.com

† Present address: Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, 459 Shirahama, Nishimuro, Wakayama, 649-2211 Japan
doi:10.2108/zsj.25.1171

habitat. Loxosomatids with the host sponge were anesthetized by adding 0.37 M $MgCl_2$ to the seawater and then fixed with 2% formalin in seawater (Iseto, 2001). All drawings were made from fixed specimens under a light microscope equipped with a camera lucida. Type specimens were stored in 2% formalin in seawater and deposited in the National Science Museum, Tokyo (NSMT). Dr. Yuji Ise (Misaki Marine Biological Station, University of Tokyo) kindly identified the host sponge to genus.

Specimens were fixed with 2.5% glutaraldehyde with 0.45 M sucrose in 0.1 M cacodylate buffer (pH 7.4) for 1 h at room temperature, briefly rinsed with 0.45 M sucrose–0.1 M cacodylate, and postfixed with 1% OsO_4 in the same buffer on ice. The specimens were dehydrated through a graded series of ethanol treatments. For scanning electron microscopy, the specimens were immersed in *t*-butanol and freeze dried. The dried specimens were sputter-coated with gold and examined under a scanning electron microscope (JEOL JEM-6060LV). For histology and transmission electron microscopy, specimens were embedded in low-viscosity epoxy resin, sectioned to 0.5–1 μm thick, and stained with 1% toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under a transmission electron microscope (JEOL 1011).

DESCRIPTION OF THE NEW SPECIES

Genus *Loxosomella* Mortensen, 1911
Loxosomella plakorticola sp. nov. Iseto & Sugiyama
(Figs. 1–6)

Material examined

Holotype: NSMT-Ka 87. Found on *Plakortis* sp. 10 March 2002, on a reef slope at Manza (Onna Village), Okinawa Island, Japan, at a depth of 11 m.

Paratypes: NSMT-Ka 88; 23 adults collected with the holotype. NSMT-Ka 89; one adult collected with the holotype.

Type host: NSMT-Po 1342. An individual of *Plakortis* sp. on which the holotype and paratypes were attached.

Etymology

The specific epithet indicates that the species inhabits *Plakortis* sp.

Description

Lives at high densities on sponges on reef slopes (Fig. 1A–C). In the living animals, black pigment granules (ca. 2 μm in diameter) scattered in tentacles, calyx, and buds, but absent in stalk and foot (Fig. 1D–E). Pigment disappears after fixation. Total length (from basal attachment of foot to uppermost part of tentacle membrane) 461–1226 μm. Calyx length (from lower end of stomach to uppermost part of tentacle membrane) 245–461 μm. Stalk 0.83–1.76 times as long as the calyx. Tentacles number 14 or 15 at the bud

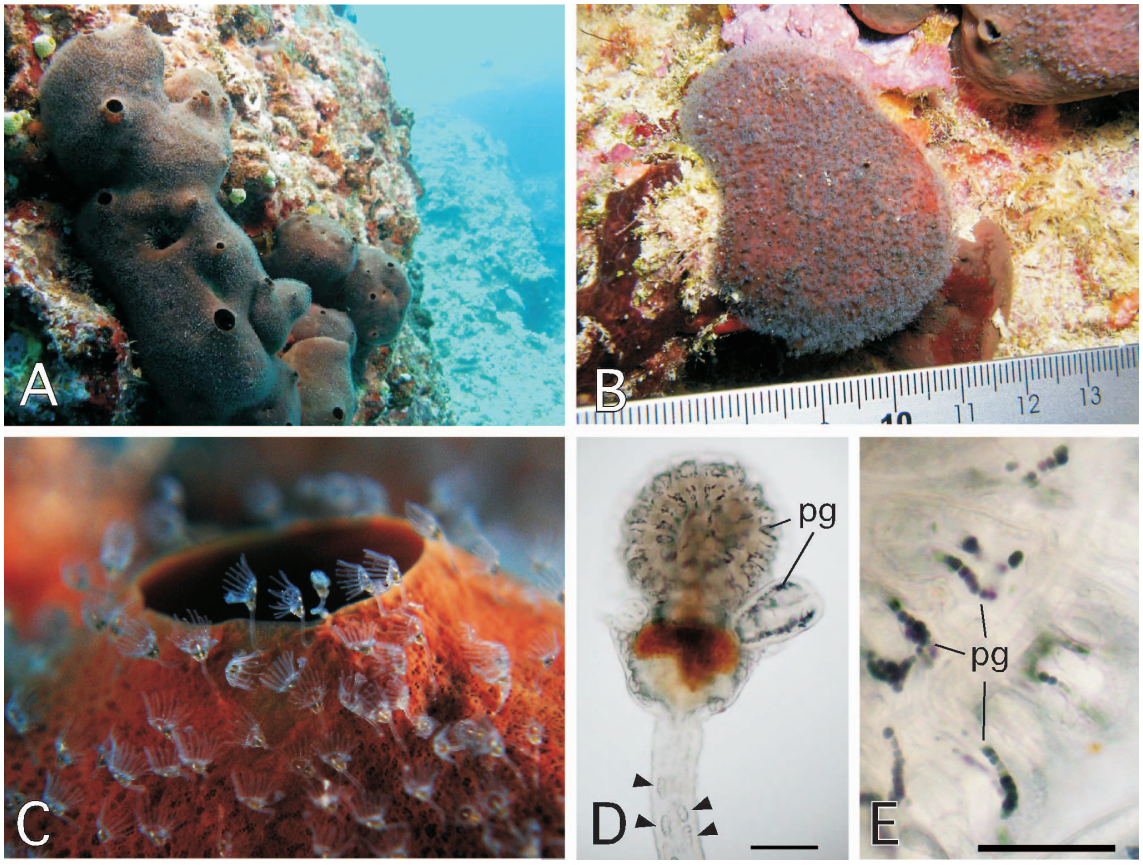

Fig. 1. *Loxosomalla plakorticola* sp. nov. found on *Plakortis* sp. **(A)** Large *Plakortis* sp. found on a reef slope at a depth of 11 m. The sponge was completely covered by *L. plakorticola*. **(B)** Smaller *Plakortis* sp. covered by *L. plakorticola*. **(C)** Higher magnification of *L. plakorticola* found on *Plakortis* sp. showing individuals extending their tentacles. **(D)** Living individual of *L. plakorticola* with small- (left) and medium-sized (right) buds. Tentacles are contracted. The upper half of the stomach is brownish. **(E)** Higher magnification of the margin of the tentacle crown in (D). pg, black pigment granules; arrowheads, granular cells. A–C, photographs taken in the field. Scale bars: 100 μm (D), 30 μm (E).

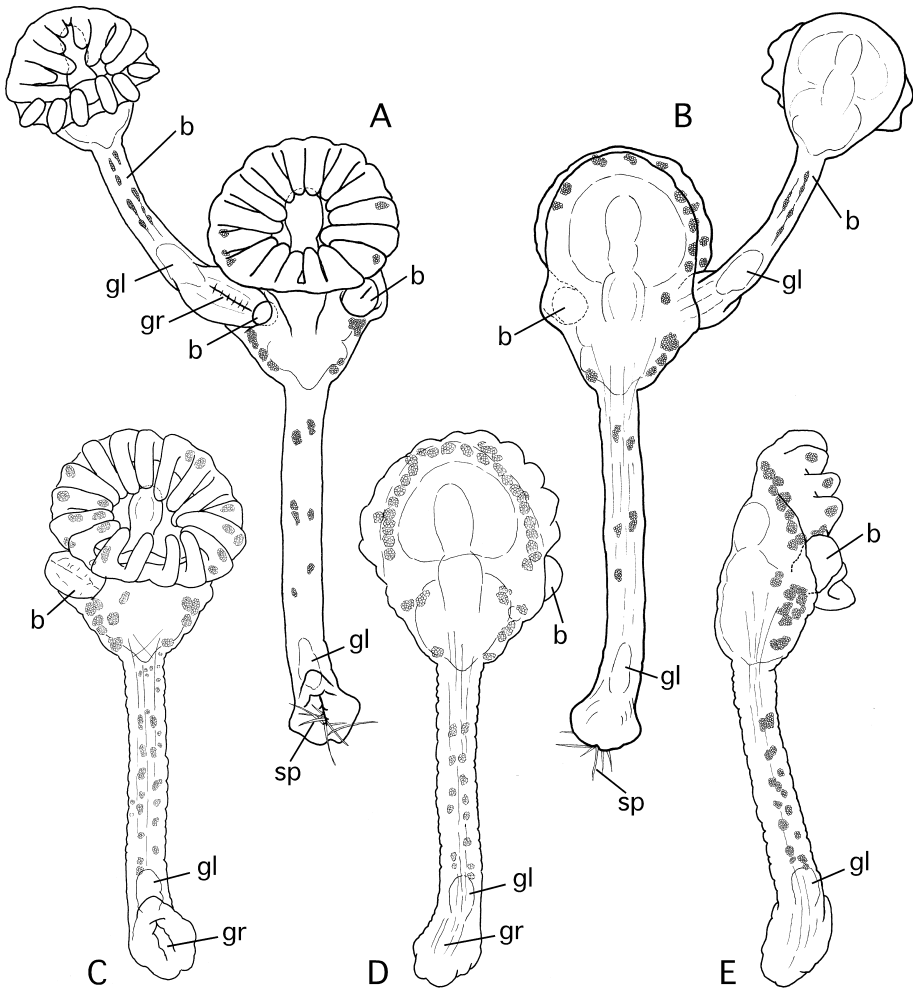

Fig. 2. Drawings of *Loxosomella plakorticola* sp. nov. **(A, B)** Holotype (NSMT-Ka 87) with a large bud and two small buds in **(A)** frontal and **(B)** abfrontal views of the whole body. **(C–E)** Paratype specimen (NSMT-Ka 89) with a small bud in **(C)** frontal, **(D)** abfrontal, and **(E)** right-side views. b, bud; gl, foot gland; gr, foot groove; sp, sponge tissue. Scale bar: 1 mm.

stage and increase after liberation from parent, but never exceed 18 (Fig. 4). Lateral sense organ on calyx absent. No conspicuous appendage anywhere on body. Transparent granular cells (about 10–15 μm in diameter) scattered on calyx, tentacles, and stalk (Figs. 1D, 3C–F), and the number of cells varies among individuals. Granular cells also present in buds (Fig. 3I) but distributed more densely than in adults. Foot with foot gland, as well as foot groove lined with accessory cells, present in both adult and buds (Figs. 2A–E, 3G–H). Foot size (from frontal end to posterior tip) about 160 μm in both adults and large buds. Bean-shaped foot gland about 90 μm long (Fig. 3G–H). Entire body free of covering detritus. Grown bud resembles parent in general shape, but is smaller in size (Figs. 2A–B, 3A).

Reproduction

Buds emerge from paired lateral pockets of the calyx at the level of the upper half of the stomach. Buds are attached to parent's calyx by the tip of the foot (Fig. 2A). Large buds have 14 to 15 tentacles (Fig. 4). Up to four buds occur simultaneously on a single parent; only one bud enlarges at a time. Sexual reproduction was not observed in our specimens.

Remarks

This species was found on *Plakortis* sp. on the slope of a coral reef at a depth of 10–15 m (Fig. 1A). *Plakortis* sp. is a common sponge at this site, and the loxosomatids were often found on it at high densities (Fig. 1A–C), although still many *Plakortis* sp. at the same locality were free of loxosomatids.

Sponges are a major host for loxosomatids (Nielsen, 1964); to date, 16 species, all of which belong to the genus *Loxosomella*, have been found on various sponges. Many of these species have a conspicuous lateral lobe on the foot that is absent in the new species. Among the 16 *Loxosomella* inhabiting sponges, four (*L. bocki*, Franzén, 1966; *L. cochlear* (Schmidt, 1876); *L. museriensis* Bobin, 1968; and *L. vivipara*, Nielsen, 1966a) lack the lateral lobe on the foot. *Loxosomella plakorticola* is distinguished from these four as

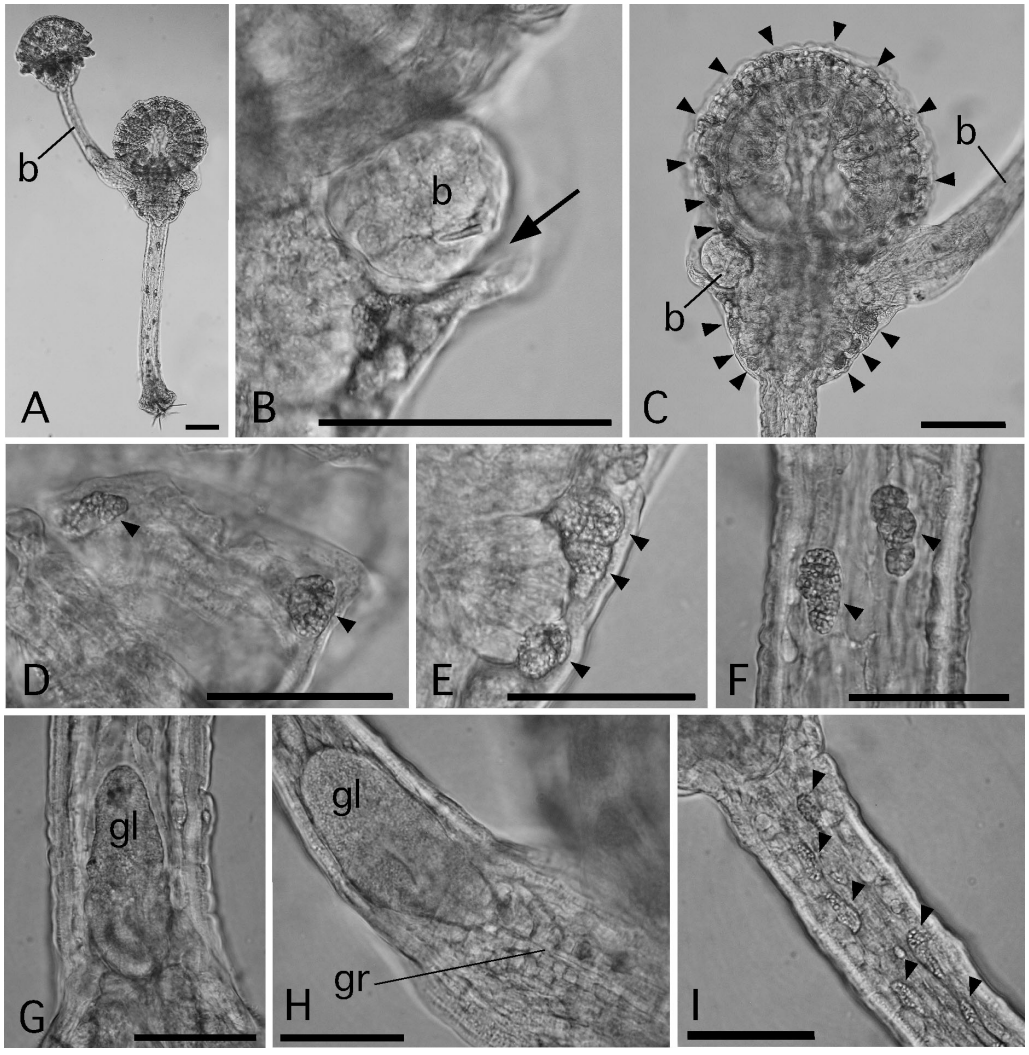

Fig. 3. Photomicrographs of *Loxosomella plakorticola* sp. nov. (Holotype, NSMT-Ka 87). **(A)** Frontal view of the whole body. **(B)** Lateral budding pocket and a small bud. **(C)** Abfrontal view of the calyx. **(D)** Granular cells in tentacles. **(E)** Granular cells in lateral side of calyx. **(F)** Granular cells in stalk. **(G)** Foot gland. **(H)** Foot of large bud. **(I)** Stalk of large bud showing granular cells more densely distributed than in the parent stalk. b, bud; gl, foot gland; gr, foot groove; arrow, lateral budding pocket; arrowheads, granular cells. Scale bars: 100 μm (A–C), 50 μm (D–I).

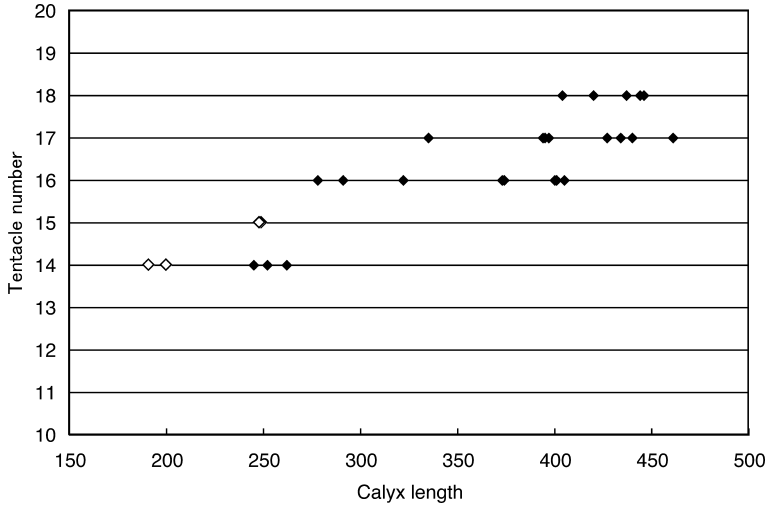

Fig. 4. Relationship between calyx length and number of tentacles in *Loxosomella plakorticola* sp. nov.

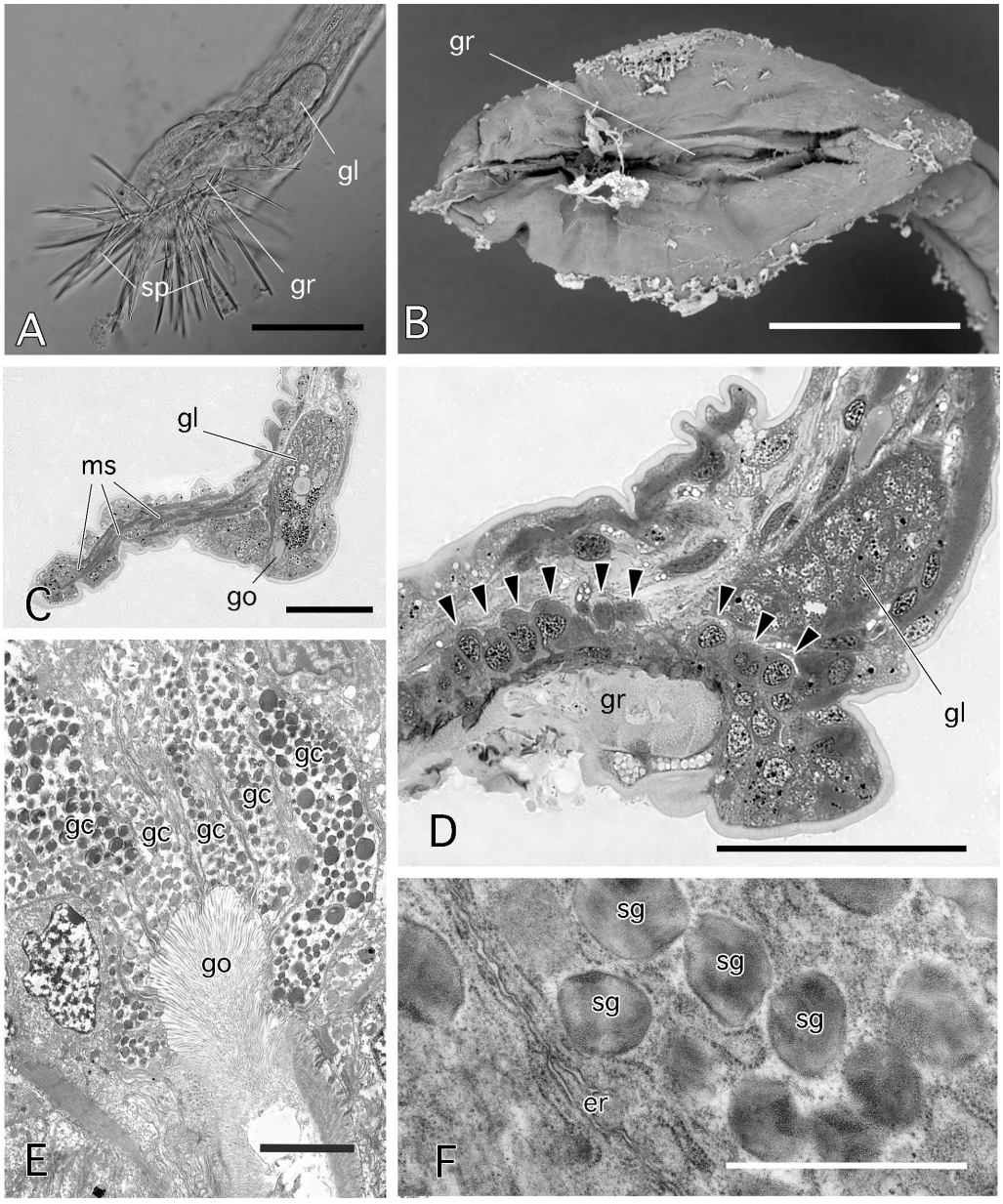

Fig. 5. Foot structure of *Loxosomella plakorticola* sp. nov. **(A)** Bottom-lateral side view of the foot with sponge tissue held in the foot groove (light microscopy). **(B)** Underside view of the foot showing longitudinal foot groove (scanning electron microscopy). **(C, D)** Semi-sagittal section of the foot (light microscopy). **(E)** Lower part of foot gland showing the opening position of the gland (transmission electron microscopy). **(F)** Higher magnification of a foot gland cell. er, rough endoplasmic reticulum; gc, foot gland cells; gl, foot gland; go, gland opening; gr, foot groove; ms, muscles; sp, sponge tissue. Scale bars: 100 μm (A), 50 μm (B, C, D), 5 μm (E), 1 μm (F).

follows. *Loxosomella bocki* produces "internal buds" with deep budding pockets that completely cover early-stage buds. The pockets of *L. plakorticola* are not as deep, and even small buds are visible externally (Figs. 2A, 3B). *Loxosomella cochlear* has only eight tentacles in the adult stage (Schmidt, 1876). Large specimens of *L. museriensis* have a very short stalk and the lobe on the lower, lateral part of calyx; these also differ from *L. plakorticola* in having conspicuous lateral sense organs in the calyx (Bobin, 1968). In general appearance, *L. plakorticola* most resembles *L. vivipara*, whose large cells in the calyx are possibly homologous to the granular cells found in the former species. However, the tentacles of *L. vivipara* number only 12 to 16. Moreover, some individuals of *L. vivipara* have a unique rounded structure on the back side of the calyx that is composed of more than 20 cells and a central hollow (Nielsen, 1966a). This structure was not observed in *L. plakorticola*.

Foot structure

Specimens of *L. plakorticola* sp. nov. remain attached to sponges even after fixation. When being removed from the sponge by needle or by jet current, specimens usually retain some sponge tissue in the foot groove (Fig. 5A), a longitudinal crevice on the underside of the slug-like foot (Fig. 5B).

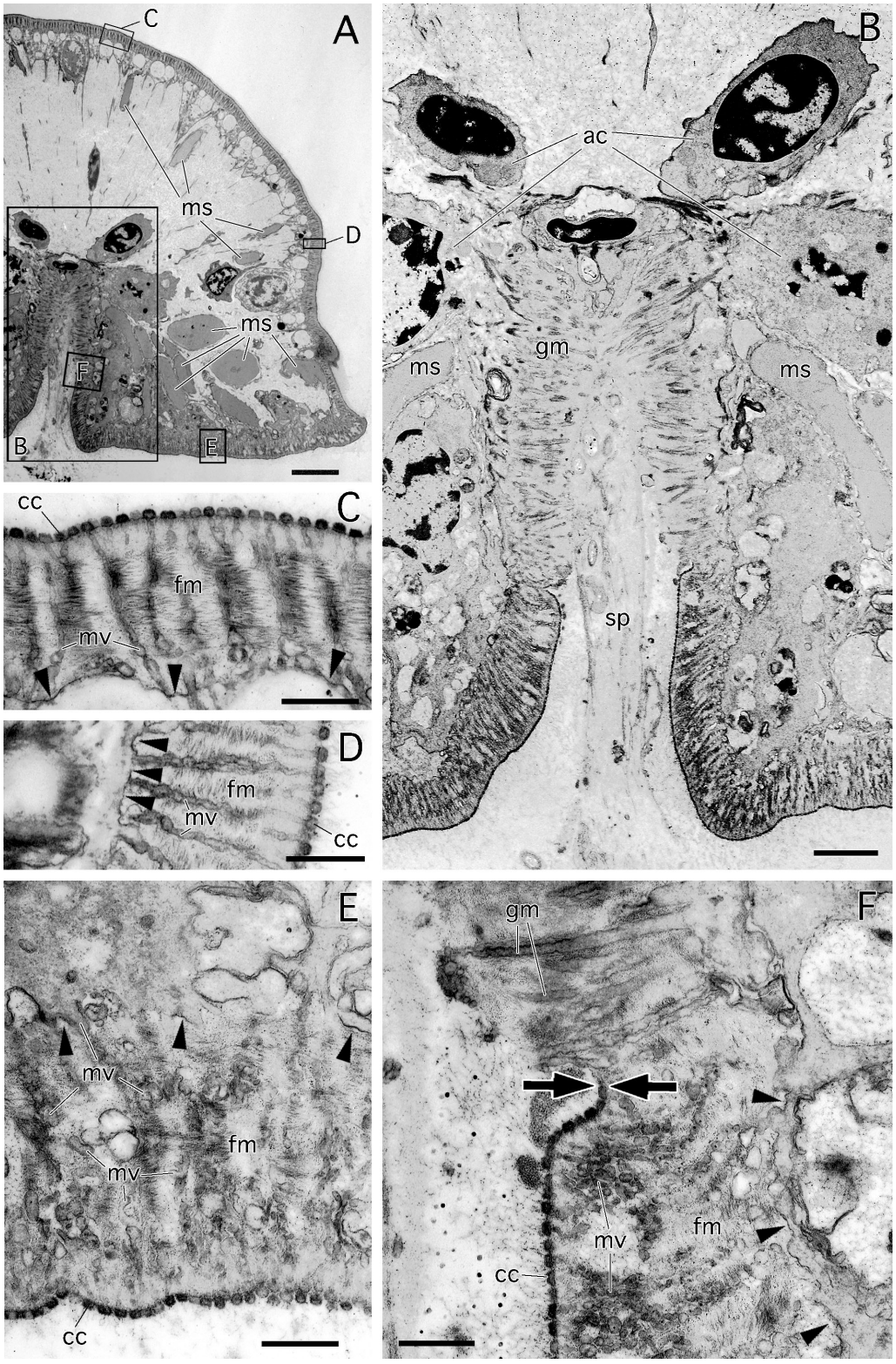

Fig. 6. Transmission electron photomicrographs of the foot of *Loxosomella plakorticola* sp. nov. All photos were taken from a single cross-section. **(A)** Low-magnification view of the section. Boxes indicate parts shown in other panels of this figure. **(B)** Higher magnification of the low–middle part of the foot showing the foot groove and associated cells. **(C–F)** Cuticle layers in different parts of the foot are shown at the same magnification. Arrowheads indicate the basal margin of epidermal cells. **(C)** Upper margin of the foot. **(D)** Lateral margin of the foot. **(E)** Bottom margin of the foot. **(F)** Cuticle layer extending into the foot groove. Arrows indicate the end of the cuticle layer at nearly half the depth of the foot groove. ac, groove accessory cells; cc, cuticle cap; fm, fibrous matrix in cuticle layers; gm, groove microvilli, ms, muscles; mv, microvilli in the cuticle layer; sp, sponge tissue. Scale bars: 5 μm (A), 2 μm (B), 500 nm (C–F).

In a semi-sagittal section, the foot gland in the basal part of the stalk is elongated downward, nearly to the frontal end of the foot groove. Thick muscle fibers run from the backside of the foot gland toward the posterior tip of the foot (Fig. 5C). In another semi-sagittal section, a row of groove accessory cells (GACs) runs just above the foot groove (Fig. 5D). The foot gland is composed of several gland cells; at the opening of the gland, long gland cells are compactly aggregated, and each cell faces the opening of the multi-ciliated cell surface (Fig. 5E). Each gland cell contains many secretory granules (about 0.5 μm in diameter) and much rough ER (Fig. 5F).

A cross-section shows that the foot groove is nearly half the depth of the foot (Fig. 6A–B). Two pairs of GACs are positioned in the upper lateral area of the groove (Fig. 6B). Each GAC has a large nucleus, but the cytoplasm contains no secretory granules. There are muscle fibers beside the groove as well as in the upper and lateral sides of the foot (Fig. 6A–B). The groove wall is covered by microvilli of epidermal cells in the upper half and a cuticle layer in the lower half. The microvilli seem to hold the sponge tissue (Fig. 6B).

The cuticle layer covers all outer surfaces of the foot (Fig. 6C–F). This layer consists of the microvilli of the epidermal cells, a fibrous matrix that runs parallel to the epidermal surface, and an outer cuticle cap covering the tip of each microvillus. The microvilli are sometimes branched, but each tip is covered by a cuticle cap, and the caps uniformly cover the cell surface. The cuticle layer has a similar structure everywhere on the foot surface, but is thinner (about 0.79–1.20 μm) on the upper (Fig. 6C) and lateral (Fig. 6D) surfaces than on the underside of the foot (Fig. 6E) and in the groove wall (Fig. 6F; about 1.48–1.83 μm). Moreover, the microvilli are rather straight and the matrix fibers are denser on the upper and lateral surfaces compared to the underside and groove wall. The cuticle layer ends in the middle of the foot groove, and the upper part of the groove wall is covered with the long "naked" microvilli of the epidermal cells (Fig. 6F).

DISCUSSION

This is the first report of a commensal loxosomatid from the Ryukyu Archipelago; all species reported to date from this area have been non-commensal (Iseto, 2001, 2002, 2003, 2005, 2006). Previous surveys used attachment panels (mainly glass slides), which typically collect only non-commensal species. More surveys of host candidate animals such as sponges, polychaetes, and bryozoans are needed to further outline the loxosomatid fauna in this region.

The host specificity of loxosomatids inhabiting sponges is not very strict, as some loxosomatids species have been found on several different sponges. For example, *L. vivipara* has been reported on *Ircinia fasciculata*, *Tedania ignis*, *Aaptos aaptos*, *Chondrosia collectrix*, and *Chondrilla nucula* (see Nielsen, 1966a); and *Loxosomella tethyae* (Salensky, 1877) has been reported on *Tethya* sp., *Stylotella* spp., and *Microciona prolifera* (see Nielsen, 1964, 1966b). *Loxosomella plakorticola* may be found on other sponges, or other loxosomatids may be associated with *Plakortis* sp. No loxosomatid species associated with sponges have ever been found on any other animal group or non-living substrata; these species associate exclusively with sponges. This indicates that loxosomatid species have an obligatory relationship to the host sponge and may be specially adapted to living on sponges. For example, because sponges are often toxic, loxosomatids may have acquired tolerance to these chemicals. Also, because the foot is the only structure in direct contact with the sponge, it may be adapted to attach to or grab onto the sponge surface. The foot was examined histologically and ultrastructurally in this study. *Loxosomella plakorticola* has a slug-like foot that is characteristic of *Loxosomella* and *Loxocorone* species (Iseto, 2002). Observations of this type of foot (e.g., Schmidt, 1876; Franzén, 1966; Nielsen and Jespersen, 1997) are basically consistent with the foot structure of *L. plakorticola*, in that they describe a foot gland, foot groove, and so-called accessory gland cells along this groove. However, the accessory gland cells of *L. plakorticola* lack secretory granules, which clearly indicates that these cells are not gland cells. The accessory gland cell in *Loxosomella elegans* also lacks secretion granules (Fig. 18 in Nielsen and Jespersen, 1997). Whereas, these cells have long been called gland cells (Schmidt, 1876), we propose to call them "groove accessory cells" (GACs).

Our observations reveal that the foot is covered by a common cuticle layer except on the upper half of the foot groove, which is covered by naked microvilli (Fig. 6B, F). The area lacking cuticle is probably the most flexible part of the foot, and sponge tissue appears to be held in this area (Fig. 6B). Several longitudinal muscles run along the foot groove (Figs. 5C, 6A, B), indicating how *L. plakorticola* sp. nov. grasps the sponge surface. When the longitudinal muscles contract, the cross-section of the foot increases. Because the cuticle layer is thick and rigid except along the foot groove, the lateral walls of the foot groove are pushed due to the expansion of the cross-section (Fig. 7). The muscle contraction decreases the width of the foot groove, and the foot pinches the sponge surface (Fig. 7). At present, it is not clear that this way of attaching to the host is common among loxosomatids on sponges or those associ-

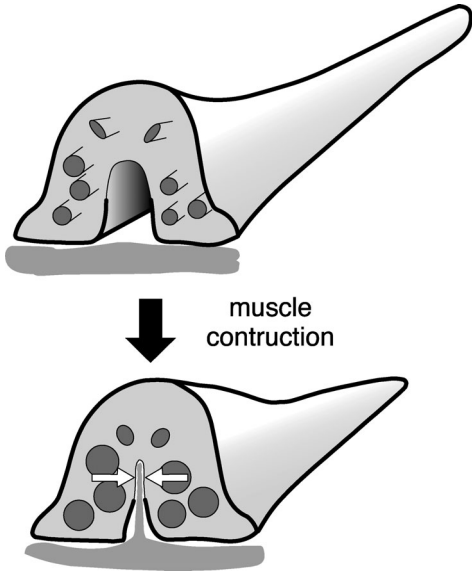

Fig. 7. Schematic drawings of the foot of *Loxosomella plakorticola* sp. nov., indicating how it grasps the sponge surface.

ated with soft-bodied hosts.

The foot structure is a very important feature discriminating loxosomatid genera; examples are the disc-shaped foot in *Loxosoma,* slug-like foot in *Loxosomella* and *Loxocorone* (cf. Iseto, 2002), the foot with a pair of "terminal wings" in *Loxomitra* (Nielsen, 1966b; Iseto, 2002), and the small swelling with several gland cells in *Loxomespilon* (cf. Bobin and Prenant, 1953). However, few studies have investigated the histology and ultrastructure of these feet. Future comparative surveys on foot structure will enable researchers to describe not only the function but also the taxonomy and phylogeny of loxosomatid entoprocts.

ACKNOWLEDGMENTS

We thank Dr. Y. Ise (University of Tokyo) for generic identification of the host sponge and Prof. M. Yokota (University of the Ryukyus) for valuable advice on nomenclature. This study was partly supported by the Fujiwara Foundation and the 21st Century COE Program of the University of the Ryukyus.

REFERENCES

Bobin G (1968) *Loxosomella museriensis* n. sp. Entoprocte Loxosomatidae de Mer Rouge. Israel J Zool 17: 175–189

Bobin G, Prenant M (1953) Deux Loxosomes nouveaux de Roscoff. Arch Zool Exp Gén 91: 25–35

Franzén A (1966) A new loxosomatid from the Pacific (Gilbert Islands) with a note on internal budding in Entoprocta. Ark Zool Ser 2 19: 381–390

Iseto T (2001) Three new *Loxosomella* (Entoprocta: Loxosomatidae) from coral reef shore in Okinawa, Ryukyu Archipelago, Japan. Zool Sci 18: 879–887

Iseto T (2002) *Loxocorone*, a new genus of the family Loxosomatidae (Entoprocta: Solitaria), with descriptions of two new *Loxomitra* (sensu stricto) and a new *Loxocorone* from Okinawa, the Ryukyu Archipelago, Japan. Zool Sci 19: 359–367

Iseto T (2003) Four new solitary entoprocts (Entoprocta: Loxosomatidae) from Okinawa Island, the Ryukyu Archipelago, Japan. P Biol Soc Wash 116: 1007–1020

Iseto T (2005) A review of non-commensal loxosomatids: collection, culture, and taxonomy, with new implications to the benefit of commensalism (Entoprocta: Loxosomatidae). In "Bryozoan Studies 2004" Ed by GI Moyano, JM Cancino, PN Wyse Jackson, Taylor & Francis, London, pp 133–140

Iseto T (2006) Two new non-commensal loxosomatids from Okinawa Island and Sesoko Island, the Ryukyu Archipelago, Japan (Entoprocta: Loxosomatidae). Species Divers 11: 33–43

Iseto T, Yokuta Y, Hirose E (2007) Seasonal change of species composition, abundance, and reproduction of solitary entoprocts in Okinawa Island, the Ryukyu Archipelago, Japan. Mar Biol 151: 2099–2107

Nielsen C (1964) Studies on Danish Entoprocta. Ophelia 1: 1–76

Nielsen C (1966a) On the life-cycle of some Loxosomatidae (Entoprocta). Ophelia 3: 221–247

Nielsen C (1966b) Some Loxosomatidae (Entoprocta) from the Atlantic coast of the United States. Ophelia 3: 249–275

Nielsen C (1989) Entoprocts. Synopses Br Fauna NS 41: 1–131

Nielsen C, Jespersen Å (1997) Entoprocta. In "Microscopic Anatomy of Invertebrates, Vol 13, Lophophorates, Entoprocta, and Cycliophora" Ed by FW Harrison, Wiley-Liss, New York, pp 13–43

Salensky M (1877) Études sur les Bryozoaires Entoproctes. Ann Sci Nat Ser 6, Zool Paleo 5: 1–60, Pls. 12–15

Schmidt O (1876) Die Gattung *Loxosoma*. Archiv Mikroskop Anat 12: 1–14

Soule DF, Soule JD (1965) Two new species of *Loxosomella*, Entoprocta, epizoic on Crustacea. Occas Pap Allan Hancock Found 29: 1–19

(Received February 4, 2008 / Accepted August 14, 2008)