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Three New *Loxosomella* (Entoprocta: Loxosomatidae) from Coral Reef Shore in Okinawa, Ryukyu Archipelago, Japan

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ABSTRACT—Three new species of solitary entoprocts of the genus *Loxosomella*, *L. monocera* sp. nov., *L. lappa* sp. nov., and *L. aloxiata* sp. nov., are described from coral reefs in Okinawa Island and Sesoko Island, Ryukyu Archipelago, Japan. This is the first report on entoprocts from this archipelago. Whereas most of *Loxosomella* species are known to be epizoic, the three species were found on stones and shell remains in the shallow reef flat or on the slide glasses settled there. It is thus probable that the present species do not depend on host animals unlike most other congeneric species.

INTRODUCTION

Entoprocts (=Kamptozoans) are solitary or colonial, sessile animals which occur in intertidal zone to deep seas of more than 500 m depth. Only one species, *Urnatella gracilis*, is reported from fresh waters. They are known to be phylogenetically enigmatic animals. Recent molecular studies suggest that this phylum is related to Cycliophora, Annelida, Mollusca, and other groups having trochophore larvae (Mackey *et al.*, 1996; Zrzavy *et al.*, 1998). To date, a total of approximately 160 species are recognized for this phylum, of which ca. 50 species are colonial (the order Coloniales) and the remainder are solitary (the order Solitaria). The taxonomical study of this group is still at an early stage, and there seem to be a number of entoprocts yet to be described (e.g. Nielsen, 1982).

From Japan, 29 entoprocts have been reported, of which 17 (10 *Loxosomella* and 7 *Loxosoma*) are solitary and 12 (2 *Pedicellina*, 7 *Barentsia*, 1 *Pseudopedicellina*, 1 *Loxosomatoides*, and 1 *Urnatella*) colonial (Oka, 1895; Toriumi, 1949, 1951; Yamada, 1956; Konno, 1971, 1972, 1973, 1974, 1975, 1976, 1977a, b; Ikeda *et al.*, 1977; Oda, 1982). All these species are reported from around the main islands of Japan: no entoprocts have ever been reported from the Ryukyu Archipelago, a chain of over 100 islands ranging between Kyusyu of Japan and Taiwan, surrounded by coral reefs. This area enjoys tropical climate, which contrast to temperate climate in most of the rest of Japan.

In other part of the world, Entoprocta have been reported from tropical waters. For example, 19 species including both

solitary and colonial ones are known from the Indo-Malayan region (Harmer, 1915), and three solitary species of the genus *Loxosoma* from Thailand (Nielsen, 1996). It is thus likely that entoprocts actually occur in the Ryukyus, and that the absence of records of this group there merely reflects an absence of surveys in the Ryukyus by its taxonomist.

I have surveyed entoprocts in shallow waters of Okinawa Island, the largest island of the Ryukyu Archipelago, and of an adjacent small island. As a result, three forms of solitary entoprocts, each representing an undescribed species, have been collected from their reef flats as below.

MATERIALS AND METHODS

Sampling was carried out in Mizugama (Okinawa Island) and Sesoko (Sesoko Island) (Fig. 1). Slide glasses were immersed in reef flats, and were collected after two months. Stones and shell remains were also collected in these reef flats. Epifaunas on these objects were examined under a binocular stereomicroscope. Some of these stones and glasses, as well as shells, were examined immediately after collection, whereas others were kept in a bucket of seawater with glass plates for 1–2 months in order to obtain asexually proliferated individuals attached on the glass plates.

To observe budding modes and liberated buds, some animals, removed from surfaces of these objects, were temporally cultured in petri dishes. When the specimens had large buds, the buds grew up and dropped off from the mother within a few days. When the specimens did not have large buds, prolonged culture was carried out by adding sufficient amount of microalgae (Marine Chlorella 100, Marine-bio Co., Japan) to each dish one time a day. Within 30 min after each treatment, stomach of each animal was filled with the algae. Then, the seawater of the dish was exchanged. The buds grew up on mother animals and were liberated after one week or so. In some species, the newly liberated buds metamorphosed and settled on the dishes within a few days.

0.37M of MgCl₂, up to equal volume to seawater, was added to the sample for narcotization, and then, the specimens were fixed in

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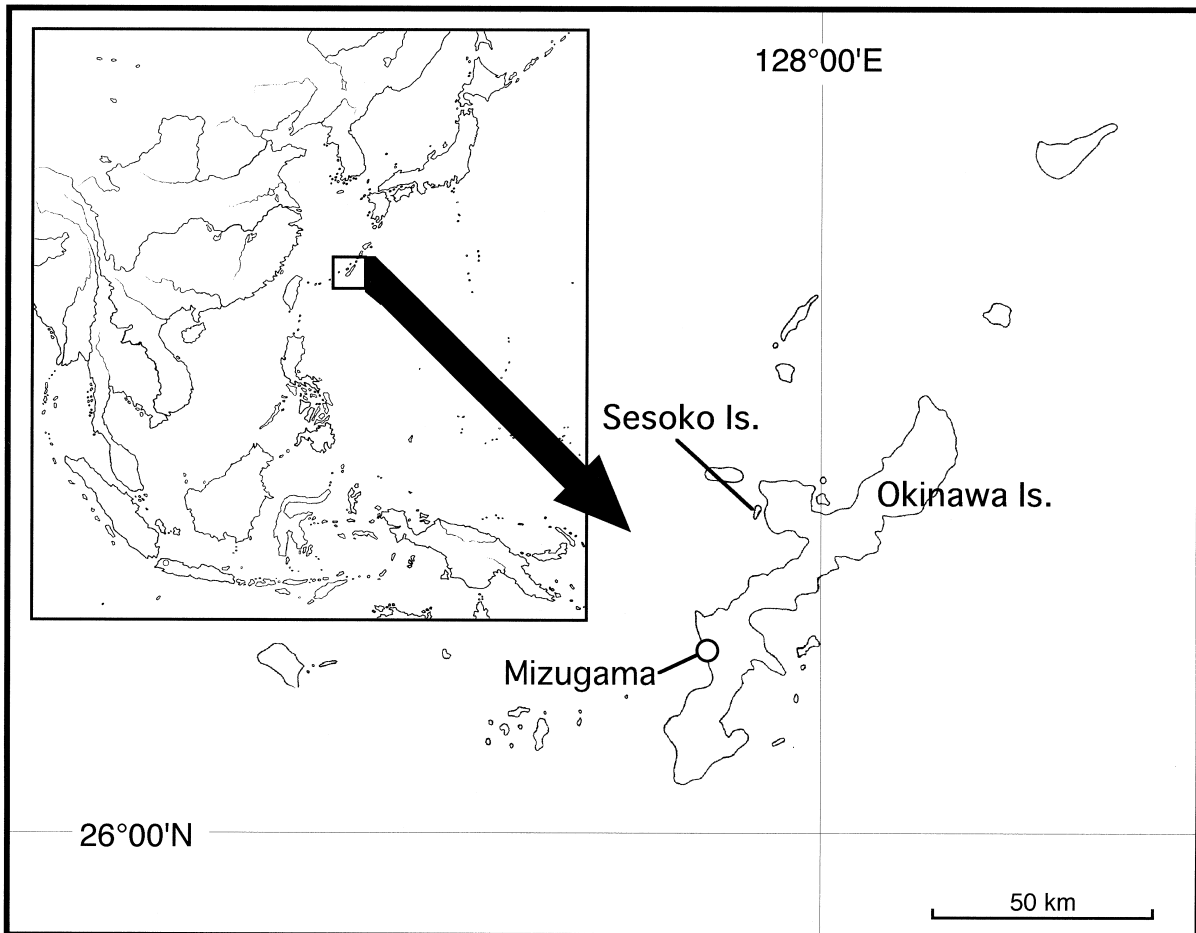


Fig. 1. Map of Okinawa Island and its vicinity, showing geographic locations of Mizugama and Sesoko Island, the type localities of the species described here.

2% formalin in seawater. All the drawings were prepared on the basis of fixed specimens under a light microscope equipped with a camera lucida. Type specimens are deposited in the National Science Museum, Tokyo (NSMT).

DESCRIPTION

Family *Loxosomatidae* Hincks, 1880
Genus *Loxosomella* Mortensen, 1911

Loxosomella monocera sp. nov.
(Figs. 2 and 3)

Material examined.

Holotype: An adult (NSMT-Ka 20), derived from budding from an individual found on a shell remain from the reef flat at a depth of ca. 1 m in front of the Sesoko Station of the Tropical Biosphere Research Center (University of the Ryukyus), Sesoko Island, Okinawa, Japan (26°38'N, 127°52'E) on 12 July 1999.

Paratypes: Five adults: NSMT-Ka 21, the mother individual of the holotype; NSMT-Ka 22, sibling individuals of the holotype derived from NSMT-Ka 21 by budding. Two liberated buds: NSMT-Ka 23, derived from NSMT-Ka 21; NSMT-Ka 24, derived from an individual collected from reef flat in

Mizugama (estuary of Hija River), Okinawa Island, Okinawa, Japan (26°21'N, 127°45'E), on 12 September 1999.

Etymology: The specific name "*monocera*" is composed of two Greek words, "*monos*", meaning "single", and "*keras*" meaning "horn", in reference to the characteristic single appendage on the abfrontal side.

Diagnosis.

Adult: Total length (from basal tip of stalk to uppermost part of tentacle membrane) up to 420 μ m. Tentacle number invariably 10. Stalk short: about half as long as calyx. No foot in adult. Lateral sense organs absent. Lateral lobe of stomach absent. One conspicuous appendage, ca. 50 μ m in length, on abfrontal midline of calyx. **Liberated bud:** Slug-like appearance with no boundary between calyx and stalk or between stalk and foot. Total length 350 μ m in NSMT-Ka 23, 420 μ m in NSMT-Ka 24. Tentacles folded. Foot groove present. Lateral sense organs absent. Appendage present at posterior, outer edge of lophophore, probably homologous to adult abfrontal appendage.

Reproduction: Buds emerge from the frontal area of calyx at level of the lower side of the stomach, attaching themselves to their mother's body by tips of their feet; as growing

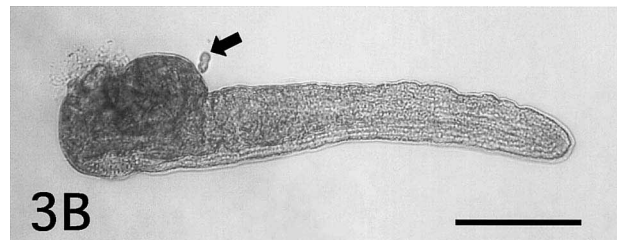
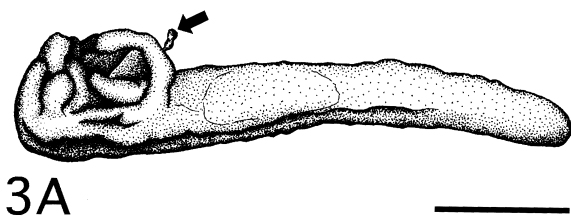
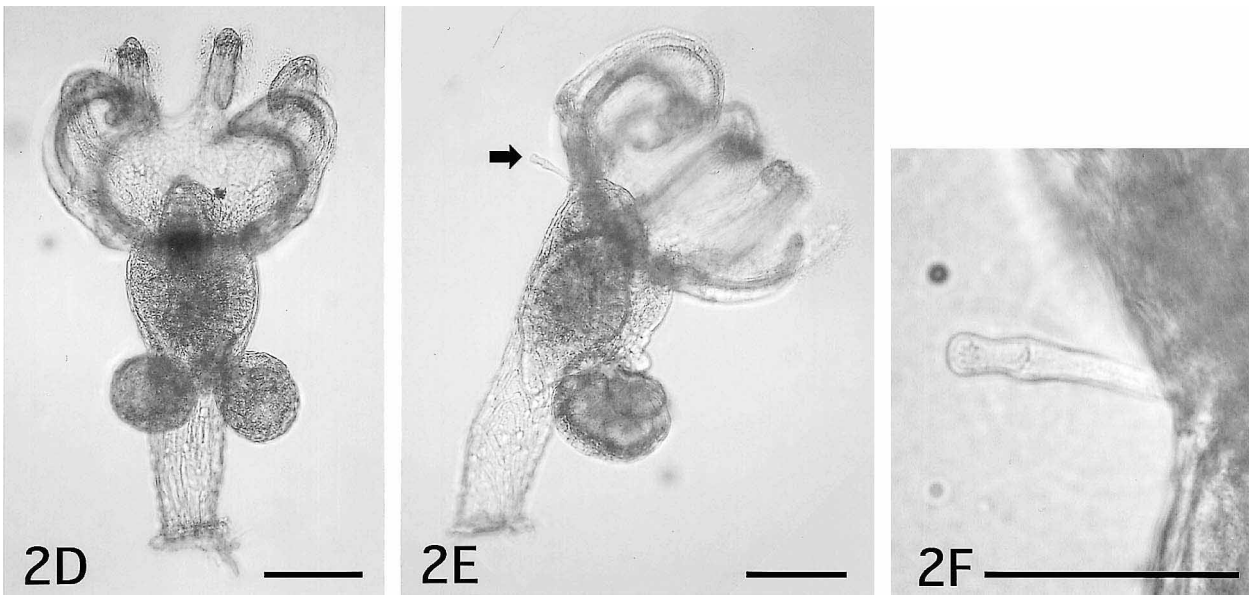
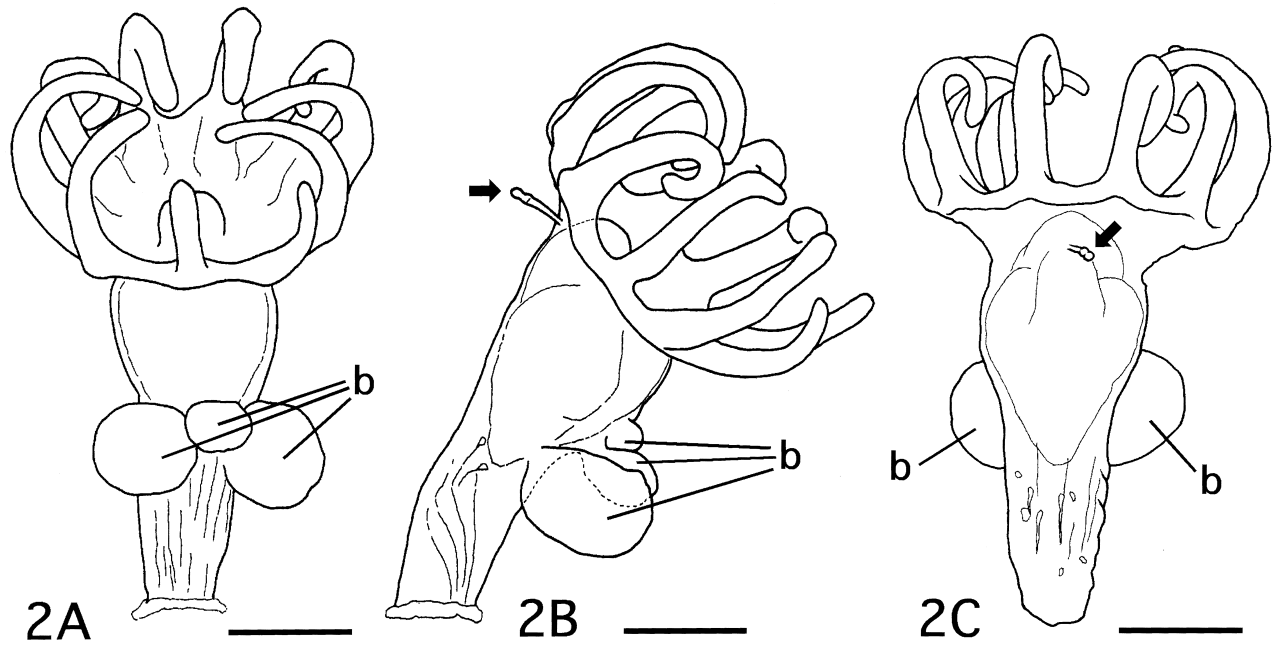


Fig. 2. Holotype of *Loxosomella monocera* (NSMT-Ka 20) with three buds (b). All buds are still in early stage. Arrows indicate the abfrontal appendage characteristic for this species. A–C: Drawings of frontal, lateral and abfrontal views, respectively. D, E: Photographs of frontal and lateral views, respectively. F: Lateral view of abfrontal appendage in greater magnification. Bars=100 μ m.

Fig. 3. A liberated bud of *Loxosomella monocera* (NSMT-Ka 24, paratype). Arrows indicate an appendage which may be homologous to the abfrontal appendage in adults. Bars=100 μ m.

larger, they extend leftward or rightward. A maximum of three buds were observed simultaneously on a single mother. Nevertheless, only one bud grew large at a time. No larvae were found.

Remarks: From about 80 species hitherto described for the genus *Loxosomella*, *L. monocera* is distinct in having the characteristic abfrontal appendage. Also, morphology of the liberated bud of the present species is unique among the congeners. The liberated buds do not expanded their tentacles

before metamorphosis into adult form, and thus they never swam using the tentacular cilia as the liberated buds of other species do.

The function of the abfrontal appendage remains unknown. It does not seem to be comparable to the lateral sense organs found in many other species of the genus, because these organs, invariably located on both of the lateral sides of the calyx, are considerably shorter than the abfrontal appendage of *L. monocera*.

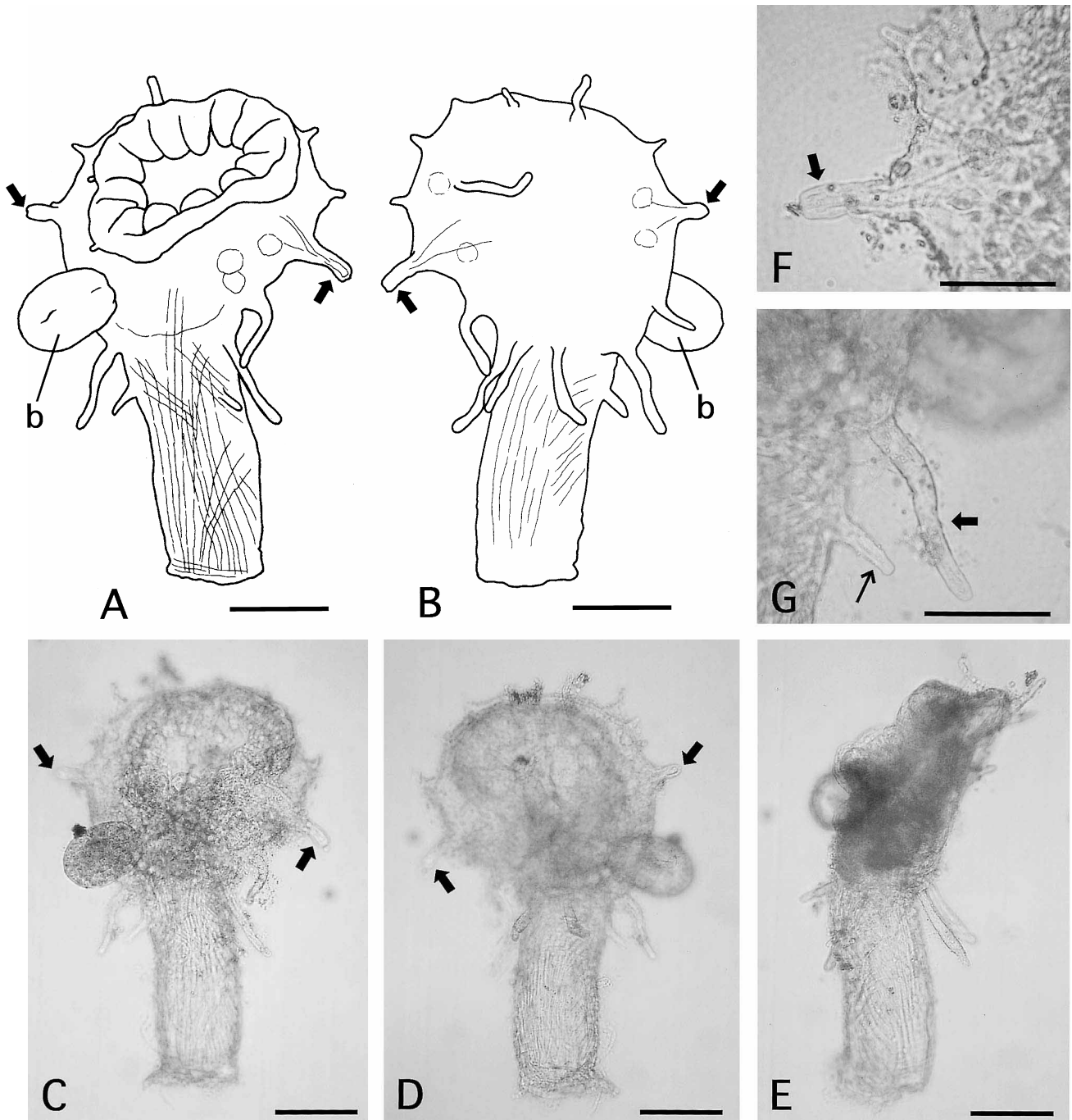


Fig. 4. Holotype of *Loxosomella lappa* (NSMT-Ka 25) with a small bud (b). A, B: Drawings of laterofrontal and abfrontal views, respectively. C–E: Photographs of laterofrontal, abfrontal and lateral views, respectively. F: Lateral sense organ. Arrows in A–F indicate lateral sense organs. G: Longer (thick arrow) and shorter (thin arrow) appendages at base of calyx. Bars=100 μ m (A–E), or 50 μ m (F and G).

This species was easily cultured in a petri dish. The liberated buds metamorphosed, settled on the substrata and generated the buds of the next generation.

Loxosomella lappa sp. nov.
(Figs. 4–6)

Material examined.

Holotype: An adult (NSMT-Ka 25) collected on 4 April 2000 from a glass plate that had been stored for two months in the laboratory with stones collected from the reef flat at a depth of ca. 1 m in Mizugama (estuary of Hija River), Okinawa Island, Okinawa, Japan (26°21'N, 127°45'E). **Paratypes:** 53 adults (NSMT-Ka 26), one young specimen (NSMT-Ka 27), and one liberated bud (NSMT-Ka 28) all collected from the glass plate described above.

Etymology: The specific name “*lappa*” is a Latin word meaning “bur”. This refers to the characteristic appendages covering the body of this species.

Diagnosis.

Adult: Total length (from basal tip of stalk to uppermost part of tentacle membrane) up to 480 μm . Tentacle number 14 in holotype, but obscure in paratypes due to severe contraction of lophophore during fixation. Stalk as long as calyx. No foot in adult. A pair of lateral sense organs at margin of calyx rim. Rim of the calyx expanded, forming a disc-shaped calyx. Several transparent granules, ca. 30 μm in diameter, in calyx. Appendages at margin of calyx rim, back of calyx, and

stalk. Some of appendages irregularly long, ca. 100 μm . Appendages smaller in number and shorter in young specimens than large individuals. Muscle fibers, running to many directions, conspicuous in stalk. **Liberated bud:** Total length (from posterior tip of foot to anterior-most point of tentacular membrane between two abfrontal tentacles) 330 μm . Tentacle number 10. Stalk very short. Foot with foot groove present. A pair of lateral sense organs present. Appendages comparable to those in adult specimens absent.

Reproduction: Buds emerge from laterofrontal areas of the calyx at level of the middle stomach, attaching themselves to their mother's body by tips of their feet. No larvae were found.

Remarks: The most characteristic feature of the present species lies in its possession of many appendages covering the body surface. Although some *Loxosomella* species are reported to have some appendages, such long appendages as in the present species are reported only in *L. cirrifera* (Harmer, 1915), *L. circularis* (Harmer, 1915), and *L. velata* (Harmer, 1915). The former two of these species are distinct from *L. lappa* in lacking expanded rim in calyx. The calyx rim of *L. velata* is much more expanded than that of *L. lappa*, reaching to the upper half of the stalk. In general shape, *L. lappa* resembles *L. ditadii* Marcus and Marcus, 1968, but the long appendages are not described for *L. ditadii* (see Marcus and Marcus, 1968).

This species was found on glass plates immersed in a bucket with stones collected from the reef flat. Thus, the origi-

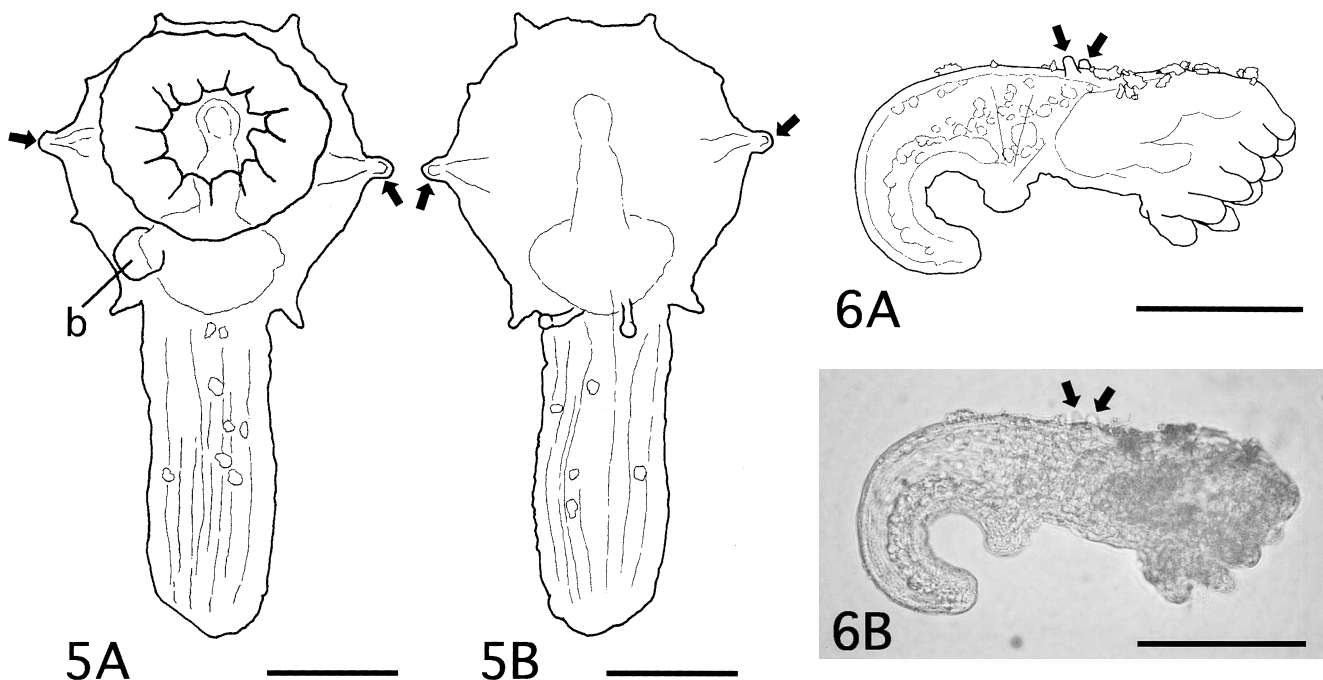


Fig. 5. Drawings of a young specimen of *Loxosomella lappa* (NSMT-Ka 27, paratype) with a very small bud (b). Note that the appendages are smaller in number and shorter than older holotype specimen (Fig. 4). A: Frontal view. B: Abfrontal view. Arrows indicate lateral sense organs. Bars=100 μm .

Fig. 6. Liberated bud of *Loxosomella lappa* (NSMT-Ka 28, paratype). Drawing (A) and photograph (B) of lateral view. Arrows indicate lateral sense organs. Bars=100 μm .

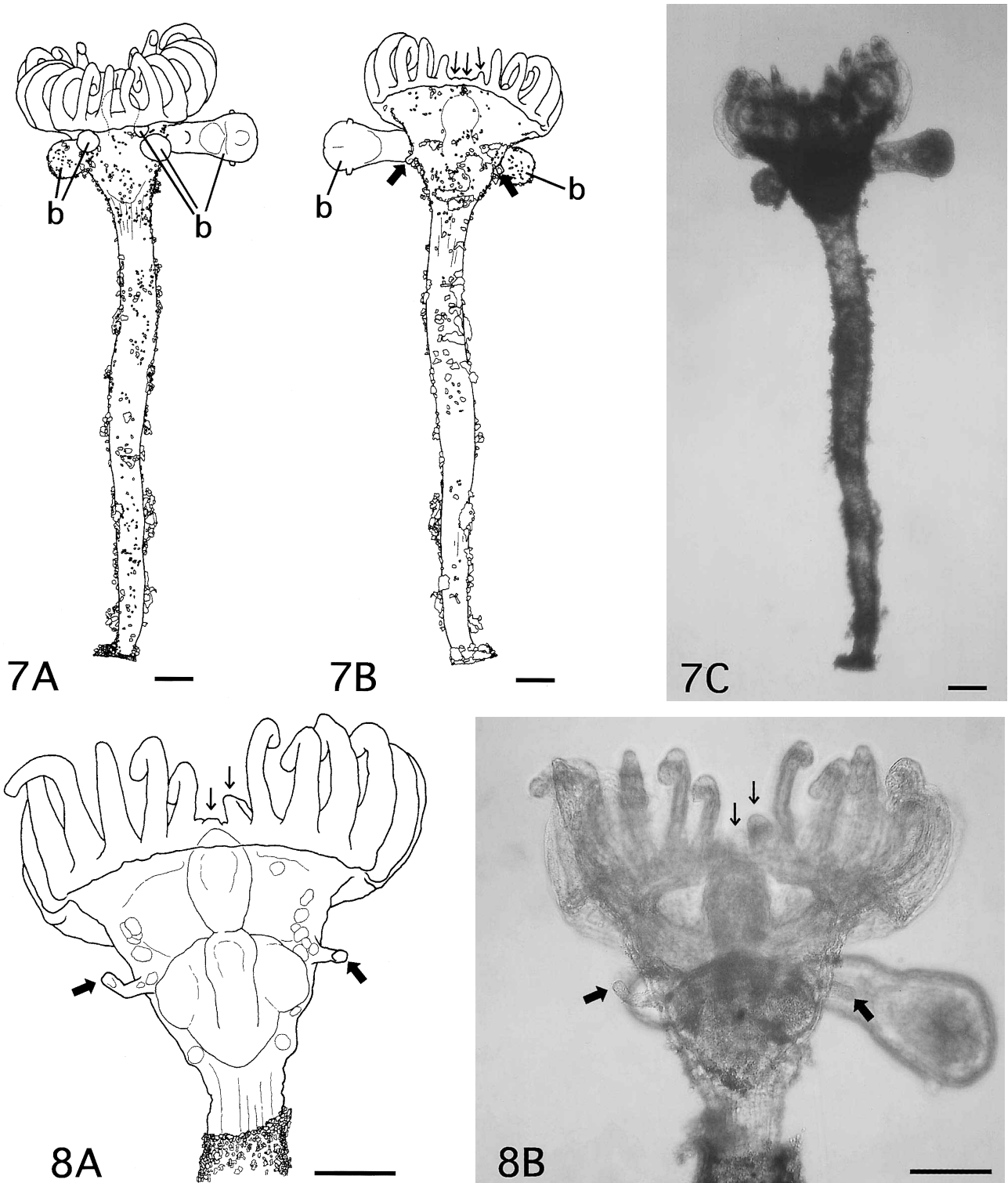


Fig. 7. Holotype of *Loxosomella aloxiata* (NSMT-Ka 29) with five buds (b) in various stages. A, B: Drawings of frontal and abfrontal views, respectively. Thin arrows indicate tentacles bending inward after fixation. Thick arrows indicate lateral sense organs. C: Photograph of frontal view. Bars=100 μ m.

Fig. 8. Drawing (A) and photograph (B) of calyx of *Loxosomella aloxiata* (NSMT-Ka 31, paratype) in abfrontal view. Detritus originally covering calyx was removed. Buds are omitted in A. Thin arrows indicate tentacles bending inward after fixation. Thick arrows indicate lateral sense organs. Bars=100 μ m.

nal individuals are likely to have occurred on stones as components of their epifauna.

Loxosomella aloxiata sp. nov.
(Figs. 7–9)

Material examined.

Holotype: An adult (NSMT-Ka 29) on a slide glass, settled on the reef flat for two months at a depth of ca. 1 m in Mizugama (estuary of Hija River), Okinawa Island, Okinawa, Japan (26°21'N, 127°45'E), collected on 5 July 2000. **Paratypes:** 14 adults (NSMT-Ka 30) collected with holotype specimen. An adult (NSMT-Ka 31) on a slide glass, settled for two months at the type locality, collected on 30 December 2000. Two liberated buds: NSMT-Ka 32, liberated from a specimen collected with holotype; NSMT-Ka 33, liberated from a specimen collected at the type locality on 23 June 1999.

Etymology: The specific name “*aloxiata*” is a Greek word meaning “not oblique”. This refers to the position of lophophore that is not oblique frontally and opens at upper most part of the calyx, whereas the lophophores of most other solitary species lean frontally.

Diagnosis.

Adult: A large species, total length (from basal tip of stalk to uppermost level of tentacle membrane) up to 1600 μm . Whole body often covered with detritus except for lophophore. Tentacle number 12 to 24. Stalk long; two to three times longer than calyx. No foot in adult. A pair of lateral sense organs, long enough to protrude from detritus covering, present. A pair of lateral lobes of stomach present. A few abfrontal ten-

tacles bending inward in fixed specimens; they expanding as other tentacles in live specimens. **Liberated buds:** Total length (from posterior tip of foot to anterior-most point of the tentacular membrane between two abfrontal tentacles) 470 μm in NSMT-Ka 32, 400 μm in NSMT-Ka 33. Tentacle number 12 in both specimens. Stalk very short. Foot with foot groove present. A pair of lateral sense organs present. Abfrontal part of lophophore bending inward in fixed specimens.

Reproduction: Buds emerge from laterofrontal areas of the calyx at level of the upper side of the stomach, attaching themselves to their mother's body by tips of their feet. A maximum of five buds were observed simultaneously on a single mother. No larvae were found.

Remarks: This species resembles large individuals of *L. antarctica* Franzén, 1973. The latter species, however, has a total of 12 tentacles. Moreover, lateral sense organs are rudimentary and buds emerge from middle of the stomach in *L. antarctica* (Franzén, 1973; Emschermann, 1993). *Loxosomella olei* du Bois-Rymond-Marcus, 1957 also resembles *L. aloxiata*, but is distinct from the latter in larger body size (up to 2600 μm long), much longer tentacles in adults, and presence of 14 tentacles in large buds (du Bois-Rymond-Marcus, 1957).

Loxosomella aloxiata is a large species, and the total length often exceeded 1500 μm , reaching 1600 μm . Although all of the type specimens were found on the slide glasses, this species was also found on the stones and dead coral fragments several times. This indicates that the natural habitats of this species include surfaces of stones and other non-living objects. The budding of this species was easily induced by feeding with microalgae. Nevertheless, most of the liberated buds failed to attach themselves to the surface of culture dish, leading long time culture in the laboratory to failure.

DISCUSSION

This is the first report on Entoprocta from the Ryukyu Archipelago. Even in other parts of the world, the entoprocts in coral reefs have not been well studied taxonomically, and it is thus not surprising that all specimens examined here represented undescribed species.

Nielsen (1964) divided the genus *Loxosomella* (*sensu lato*) into two subgenera, *Loxosomella* and *Loxomitra*, on the basis of differential budding modes: buds of the nominotypical subgenus are attached to their mother's body by the tip of their feet, whereas those of the subgenus *Loxomitra* by the back of stalks or calyces. Soule *et al.* (1987) elevated the subgenus *Loxomitra* to the rank of full genus. I agree with this change, but since there are no other characters to distinguish these two genera, observation of budding modes is essential for a correct generic assignment of a given species. Therefore, if the specimen has no buds, culturing is required to induce budding. In the three species described here, buds were invariably attached to their mother's body by foot tips, and it is thus obvious that all these species belong to the genus *Loxosomella* (*sensu stricto*).

It is uncommon to collect entoprocts using settlement

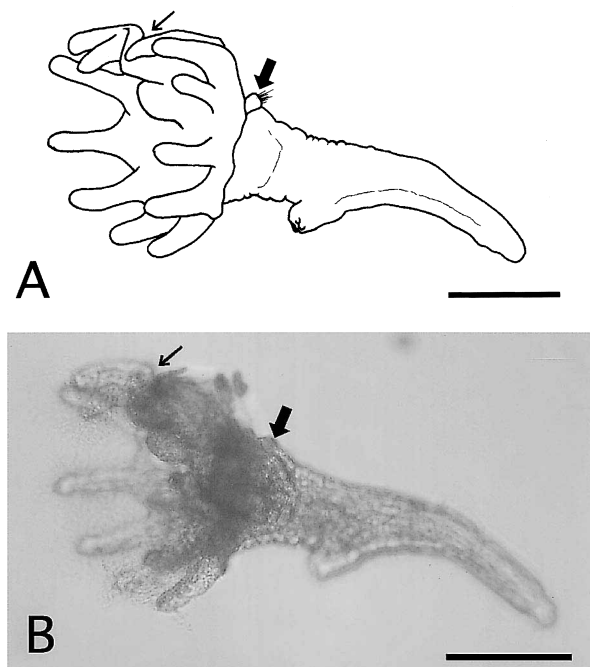


Fig. 9. Drawing (A) and photograph (B) of left lateral view of liberated bud of *Loxosomella aloxiata* (NSMT-Ka 33, paratype). Thick and thin arrows indicate lateral sense organ and a part of lophophore bending inward after the fixation, respectively. Bars=100 μm .

panels. Ryland and Austin (1960) immersed panels in a dock in Swansea, U. K., and found *Loxomitra kefersteinii* (Claparède, 1867) and *Loxosomella antedonis* Mortensen, 1911 attached on the panels some time later, and, to my knowledge, this is the only case of application of this method for the sampling of entoprocts in the field. In the present study, *Loxosomella aloxiata* was found abundantly on settled slide glasses. It is probable that this method enables us to discover additional undescribed species in future studies.

Solitary entoprocts are usually known to inhabit on other animals as epizoics or commensals (Hyman, 1951; Ruppert and Barnes, 1994), and a variety of animal groups (e. g., polychaetes, sipunculans, bryozoans, and sponges) are known to be their hosts (summarized in Nielsen, 1964; Soule and Soule, 1965). Only a few solitary entoprocts have been found from non-living objects. In *Loxosomella*, for example, only *L. olei* du Bois-Reymond-Marcus, 1957 lives on stones (du Bois-Reymond-Marcus, 1957), and *L. hispida* Marcus and Marcus, 1968 and *L. shizugawaense* (Toriumi, 1949) on algae (Toriumi, 1949; Marcus and Marcus, 1968; Konno, 1971). Furthermore, only one species, *L. isolata* Salvini-Plawen, 1968, is known to be non-epizoic among 25 species of the genus *Loxosoma* (Salvini-Plawen, 1968, 1986; Nielsen, 1996), and in *Loxomitra*, only *L. kefersteinii* (Claparède, 1867) is found on non-living substrata, as well as on other animals (Claparède, 1867; Nitsche, 1875; Ryland and Austin, 1960; Nielsen, 1966). In this regard, the present species seem to be rather exceptional, because all of them were found only on non-living substrata. I examined some of the host candidates, such as polychaetes, sipunculans, and sponges collected from the type localities of the present species, but any solitary entoprocts were not found on them.

Nielsen (1964) suggested that the solitary entoprocts not associated with other animals "may hardly be regarded as quite natural", because they were found under more or less artificial conditions, such as in aquaria or on settlement plates. However, because *Loxosomella monocera* and *L. aloxiata* were found on stones and shell remains directly collected from the reef flat, they probably have no host animals even in nature. Although it is possible that these species inhabit not only on non-living substrata but also on other animals, the non-living substrata seem to be common habitats for solitary entoprocts at least in the Ryukyus. It is probable that solitary entoprocts without host animals will be also found in shallow waters in other tropical areas.

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