

Didemnid-Algal Symbioses: Host Species in the Western Pacific with Notes on the Symbiosis

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Abstract—Collections from Palau, Philippine and Caroline Islands, Guam and the Great Barrier Reef contain 15 (including 2 that are new) of the 20 didemnid species known to contain algal symbionts. With the exception of the Fijian species (*Diplosoma multipapillata*) and two species known only from the Atlantic and eastern Pacific (*Trididemnum solidum* and *T. della vallei*), all the known species have a wide geographic range in the western Pacific and some extend into the western Indian Ocean. Populations of several of the species suggest a degree of isolation, but not speciation. The known geographic ranges of all but the most conspicuous species are affected by patterns of collecting. A key to identification of species is based on features of both the zooids and the colony and its contained spicules. Species descriptions complement those of Kott (1980).

Introduction

Algae-bearing didemnid ascidians of the Indo-West Pacific region have been reported on by Kott (1980, 1982). Records from Palau and the Philippine Islands referred to in those reports were based on published accounts (Van Name 1918; Tokioka 1942, 1950, 1955, 1967). This account is based principally on more recent collections from these two locations in the western Pacific and from the Great Barrier Reef at Lizard Island and to the north. Small collections from Guam and the Caroline Islands (Truk and Pulusuk) are also included.

Fifteen of the 20 species known to have prokaryotic algal symbionts are present in these collections. Two additional species (*Echinoclinum triangulum* and *Trididemnum strigosum*) have been recorded previously from a range of locations in the western Pacific including the Philippines (Kott 1980, 1982). Further collecting in the Indian Ocean will very likely demonstrate an Indo-West Pacific range for all these species with the exception of *Diplosoma multipapillata*, which is thought to be endemic, having been recorded only from a specialised habitat in Fiji, and *Trididemnum solidum* and *T. della vallei* known only from the Atlantic and the eastern Pacific, respectively.

Intraspecific morphological variation in these species is generally not conspicuous, despite the vast distances between their populations. Diversity in the appearance of the colonies most often results from variations in the crowding of spicules in the surface test that shade the contained algal symbionts (e.g., *Lissoclinum*

voeltzkowi in Kott 1980 and *Lissoclinum bistratum* in Kott 1982). Olson (1980) has shown a similar increase in the density of spicules in the surface test and a withdrawal of plant cells away from the surface as a response to increased light in the Atlantic species, *Trididemnum solidum*.

These responses, by which the host ascidian orders the arrangement of its spicules in order to satisfy the needs of the contained algae, clearly demonstrate the symbiotic nature of the ascidian-algal relationship. They are the result of environmental rather than genetic factors. Variability in the distribution and intensity of carotenoid pigments is also apparently the result of environmental factors (cf. *L. bistratum* at Lizard Island in Kott 1980).

There are also variations in colony size and shape that result from age, substrate, and lobulation (subdivision). Small rounded colonies of many of the species are usually maintained by lobulation, as in *Diplosoma virens* (Thin et al. 1981). In some populations of certain species, lobulation does not occur (e.g., *Lissoclinum bistratum* specimens from Mooloolaba in Kott, 1980, and *L. voeltzkowi* specimens from the Palau Islands, see below) and the colonies form sheets rather than small rounded cushions. This may be genetic and reflect population adaptations rather than environmental factors. The relatively large larvae with a multiplicity of adhesive organs of *Diplosoma virens* at Lizard Island are also a likely population adaptation reflecting only a degree of isolation, rather than complete reproductive isolation and speciation.

Thus, morphological variations resulting from genetic isolation are rare and the present collections tend to support the proposition that a remarkable pattern of larval recruitment links the islands and reefs of the tropical Indo-West Pacific to maintain flow between populations of these widely ranging species (Kott 1980).

A description of the morphology of each species is available in Kott (1980) which supplements this account of the more recently collected material set out here. The material is deposited in the Queensland Museum (QM) with duplicate specimens in the collection of the Marine Laboratory, University of Guam.

Identification of Ascidian Specimens

The spicules and cloacal systems comprise the most easily observed and reliable taxonomic characters. However, observation of branchial sac and gonads will also be necessary to obtain definitive identification of most genera and species. It should be stressed that the distinction between the genera *Didemnum* and *Trididemnum* is based exclusively on the number of rows of branchial stigmata. These are often difficult to determine in these small zooids and microscopic examination of the cleared, relaxed pharynx is essential to distinguish between *Didemnum viride* and *Trididemnum nubilum* which otherwise have very similar colonies, zooids and spicules.

Colour photographs of many of the species in situ have been reproduced with this report. These demonstrate clearly that many of the species have a close superficial resemblance. Further, many grow in similar and often the same habitat, sometimes in

close association. Species identification based on the colour, shape, or other macroscopic features of specimens is unreliable. Dissection of colonies and microscopic examination of spicules and zooids must be undertaken in order to establish species identity.

Dissection of a didemnid colony is most readily achieved by cutting a thin vertical wedge or slice from a common cloacal aperture to the outer border of the colony. The organisation of the common cloaca, the relationships of the zooids to it and to the surface of the colony, and the distribution of the spicules may then be readily observed and zooids may be removed without mutilation.

KEY TO ALGAE-BEARING DIDEMNIDS OF THE INDO-WEST PACIFIC

1. Calcareous spicules absent from the test (*Diplosoma*) 2.
- 1'. Calcareous spicules present in the test 4.
 2. Colonies sheeting, not lobulated; retractor muscle from posterior end of thorax *Diplosoma similis*
 - 2'. Colonies oval or rounded, lobulated; retractor muscle from part way along esophagus 3.
3. Stigmata 6/row; retractor muscle moderate length; cloacal canals at thoracic level complicated; green *Diplosoma virens*
- 3'. Stigmata 5/row; retractor muscle very short; cloacal canals at thoracic level not complicated; blue *Diplosoma multipapillata*
 4. Stigmata in 3 rows (*Trididemnum*) 5.
 - 4'. Stigmata in 4 rows 11.
5. Atrial siphon present 6.
- 5'. Atrial siphon absent 7.
 6. Spicules up to 0.04 mm diameter; colonies with single systems....
..... *Trididemnum clinides*
 - 6'. Spicules up to 0.08 mm diameter; colonies with more than one system *Trididemnum paraclinides* n. sp.
7. Endostylar pigment cap present; plant cells in common cloacal cavity .. 8.
- 7'. Endostylar pigment cap absent; plant cells embedded 9.
 8. Vas deferens with $9\frac{1}{2}$ coils; colonies sheeting
..... *Trididemnum paracyclops*
 - 8'. Vas deferens with $5\frac{1}{2}$ coils; colonies oval *Trididemnum cyclops*
9. Spicules spherical (with flat-ended rays) *Trididemnum miniatum*
- 9'. Spicules stellate (with pointed rays) 10.

10. Spicules up to 0.05 mm, sparse in middle layers of test *Trididemnum nubilum*
- 10'. Spicules up to 0.08, dense throughout *Trididemnum strigosum*
11. Vas deferens spirals around the male follicle (*Didemnum*) 12.
- 11'. Vas deferens runs straight 14.
12. Colonies flask- or dome-shaped; mucus secretion copious; plant cells confined to common cloaca *Didemnum molle*
- 12'. Colonies not flask- or dome-shaped; mucus secretion not apparent; plant cells embedded in test 13.
13. Colonies sheeting; spicules stellate with 7 to 11 conical rays in optical section *Didemnum viride*
- 13'. Colonies lobulated; spicules stellate with 5 to 7 almost cylindrical rays in optical section *Didemnum etiolum* n. sp.
14. Spicules flattened with rays of unequal length *Echinoclinum triangulum*
- 14'. Spicules stellate or spherical with rays of equal length (*Lissoclinum*) 15.
15. Spicules form capsule around zooid; colonies slimy, delicate *Lissoclinum punctatum*
- 15'. Spicules do not form capsule around zooid; colonies neither slimy nor delicate 16.
16. Spicules absent from basal test and from thickened surface ridges *Lissoclinum pattella*
- 16'. Spicules present in basal test; no thickened spicule-free surface ridges; variable density of spicules in surface test 17.
17. Spicules spherical *Lissoclinum bistratum*
- 17'. Spicules stellate *Lissoclinum voeltzkowi*

Systematic Account

Didemnum molle (Herdman)

Figs. 1, 2a-f, 19a

Diplosomoides molle Herdman, 1886: 310.

Didemnum molle: Kott, 1980: 2 and synonymy.

NEW RECORDS: Fiji (Mumbualau, on seagrass, 2 m). Philippines (Calatagan,

Batangas, 15 m, QM GH493; Apo Island, 20 m, QM GH493; lagoon, S.E. Apo Island, 15 m, QM GH453, 493; Mactan Island, Cebu, 2-8 m, QM GH522, 391; Puerto Galera, Verde Island, 2 m, QM GH390-392). Palau Island (*mult. loc.*). Lizard Island (Eagle Island, 1 m, QM GH141-2; Bird Island, 1 m, QM GH139, 140, 551; QM G12673-75; off. end of I., 11 m, in sand, QM GH320). Northern Great Barrier Reef (Martha Ridgeway, Deltaic Reef, QM GH267, 245; Raine Island 20 m, QM GH298). Guam (Pago Bay, underside of rocks, lowtide mark, QM GH695, juvenile colonies). Caroline Islands (Truk, reef flat, QM GH811).

RANGE: The species is common at all locations from Zanzibar to Fiji, north to Okinawa, and south to Cockburn Sound in Western Australia and to Heron I. in the Great Barrier Reef. It occurs from the low tide mark down to 69 m, on seagrass and on coral or rocky substrates where it is not exposed to a great degree of turbulence.

DESCRIPTION: Mature colonies are from about 1 cm to 10 cm in diameter. At the southern and eastern limits of the range of this species (Capricorn Group, Great Barrier Reef and Fiji respectively) only small colonies have been recorded. At all other locations very large colonies are commonly found and it is the most

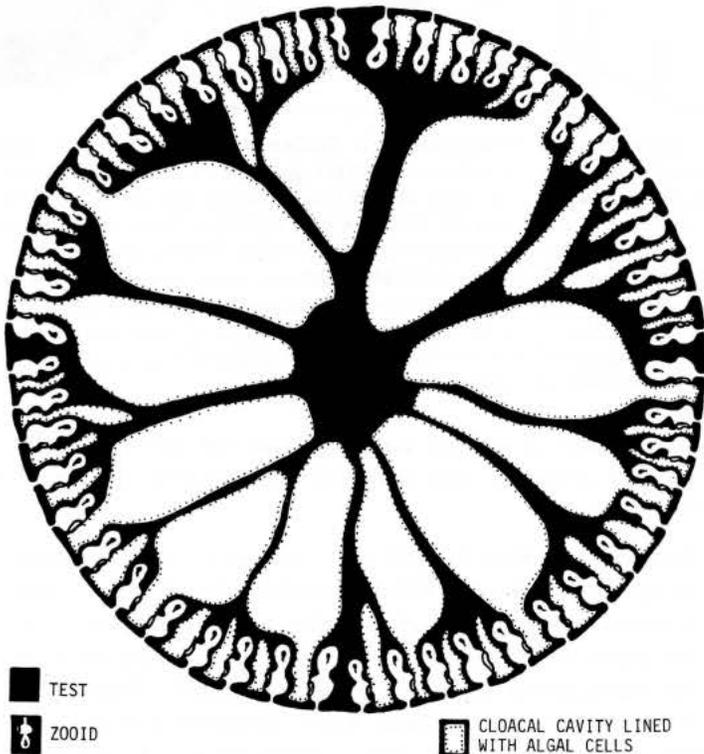


Fig. 1. Diagrammatic section through a *Didemnum molle* colony.

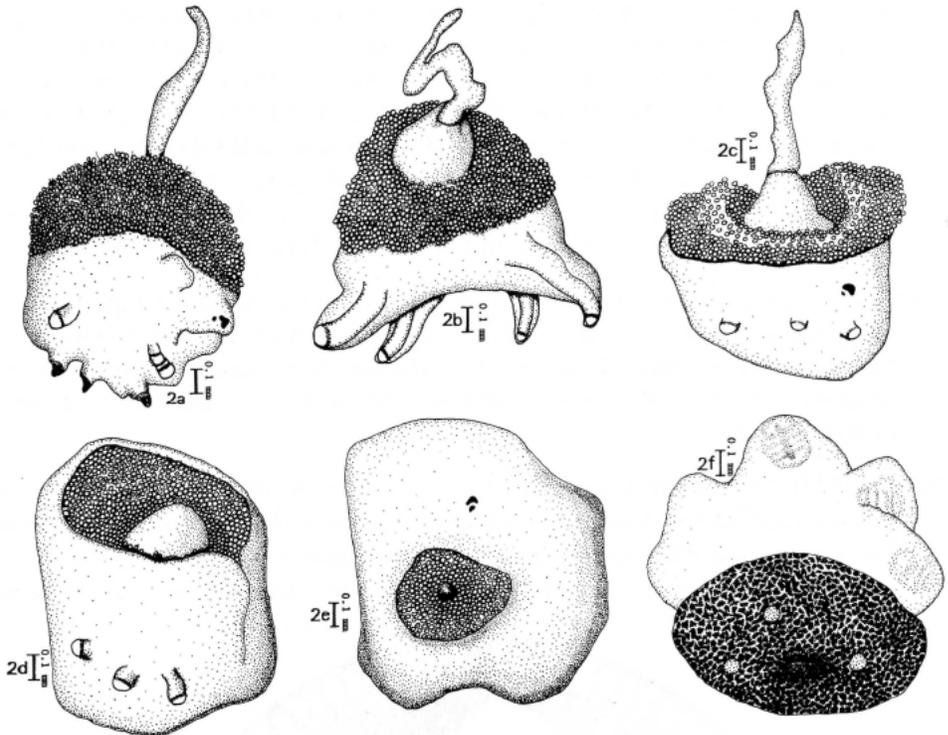


Fig. 2. Stages in larval metamorphosis of *Didemnum molle*. (a) adhesive organs protrude, tail begins to withdraw; (b) trunk becomes foreshortened, ectodermal ampullae protrude ("white" type colonies), and posterior part of trunk with plant cells adhering is withdrawn into the trunk; (c) "pigmented" type colony in which ectodermal ampullae do not extend as in "white" type; (d) tail and posterior end of larval trunk with plant cells completely withdrawn, larval test grows upwards to close over incipient cloacal cavity; (e) view from above into incipient cloacal cavity with sense organs of zooid showing through the test; (f) ventrolateral view of juvenile "pigmented" type colony showing basal pigmented saucer interrupted in circular areas over the ectodermal ampullae.

conspicuous ascidian in shallow water coralline habitats. It is not cryptic and apart from the dramatic size range of the colonies, colour is the most conspicuous variation.

Living specimens may be light grey, or almost white, or dark grey with patches of light brown. Some colonies are entirely light brown or dark chocolate brown or occasionally purple or even almost black. The colour is more intense around the upper surface of the colony and fades toward the base where the white of the spicules predominates. The brown pigment is contained in spherical, oval or irregular-shaped cells above, below or mixed with the spicules in the surface test. The colour is more intense where the spicules are less dense. The darkest colonies, or parts of colonies, occur where the spicules are absent or

when pigment is dense above the spicules. Pigment cells can fill the interstices between the bladder cells of the superficial layer of test and are forced into irregular fusiform or stellate shapes that form a three-dimensional trabecular pattern. Thus there is a wide range in density and distribution of pigment and spicules in the surface test that causes the colour variations in this species. The single cloacal aperture is usually in the center of the upper surface, although the larger colonies are usually assymmetrical. Colonies have been observed dividing across the cloacal aperture (M. Cowan, pers. comm.)

The colonies are prolific and well developed larvae are found free in the common cloaca and are readily released from the colony before fixation. In the metamorphosing larva the posterior half of the larval trunk, with the plant cells that surround it, rolls inwards around the base of the tail and is depressed into the larval trunk. The posterior end of the posterior haemocoelic cavity becomes conical and protrudes from the center of this cavity. At this stage the larva is very much reduced in length anteroposteriorly. Anteriorly the trunk is rounded, with adhesive organs and ectodermal ampullae protruding from it. Posteriorly the tail protrudes from the center of a concavity that is filled with plant cells. The larval test over the posterior end of the larva appears to be differentiated into hair-like strands that entangle the plant cells in the same way as the hairs of the diplosomid rastrum (Kott 1980). These hairs hold the plant cells in place in the posterior concavity. At the anterior end the adhesive organs protrude to make the first contact with the substrate. It is the ectodermal ampullae which subsequently reinforce the fixation. Withdrawal of the tail into the posterior haemocoelic cavity is sometimes before, but usually immediately after, the adhesive organs make their first contact with the substrate. After the tail is withdrawn, the larval test extends upwards over the posterior concavity and its contained plant cells, leaving an opening to the exterior that becomes the common cloacal aperture in the center of the upper surface of the colony.

Dark brown colonies with pigment in the outer layer of test and few spicules produce juveniles in which the basal surface (formerly the anterior end of the larva) becomes enveloped in brown pigment cells interrupted only at 4 points where the ectodermal ampullae formerly protruded. This localized saucer of pigment cells has not been observed in juveniles from grey to white adult colonies.

Didemnum viride (Herdman)

Figs. 3-5

Leptoclinum viride Herdman, 1906: 340

Didemnum viride: Kott, 1980: 4 and synonymy.

NEW RECORDS: Philippines (Apo Reef, 10 m, QM GH488; Calatagan, 16 m, QM GH483). Palau (QM GH498, 501).

RANGE: The new records extend the known range of this species from the Indian Ocean (Malagasy and Sri Lanka) into the western Pacific.

DESCRIPTION: The colonies form investing sheets, 2 to 12 cm in maximum dimension and from 0.3 to 1.0 cm thick. The zooids are restricted to the upper part of the colony and variations in thickness are a result of variations in

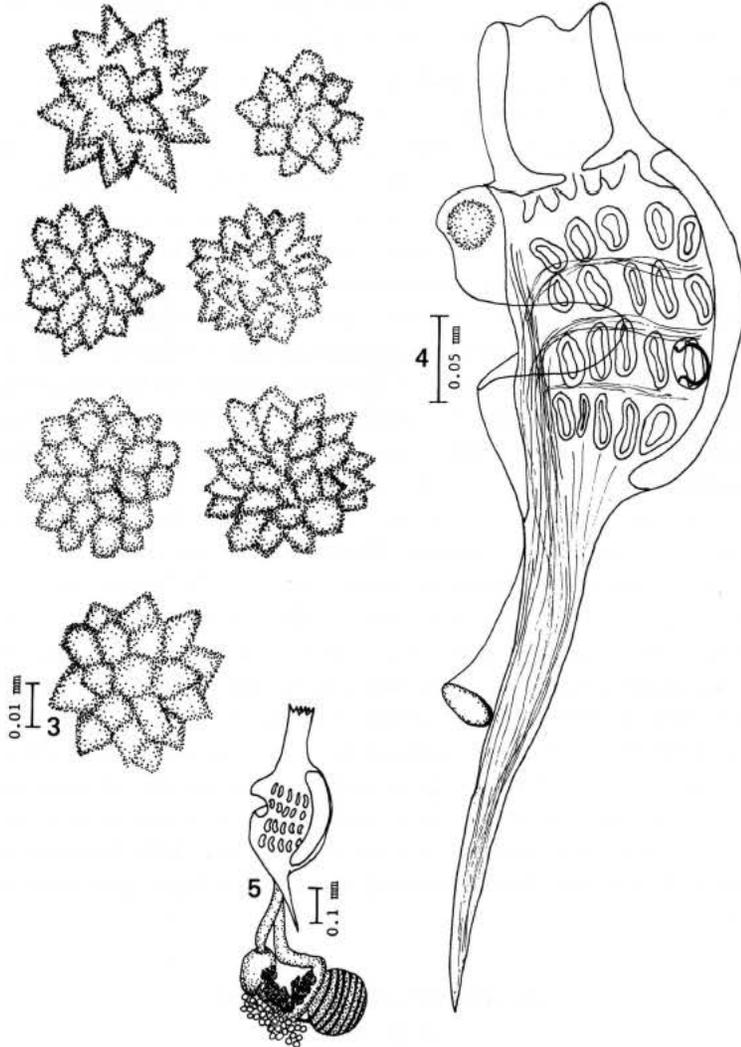


Fig. 3. Spicules of *Didemnum viride* (QM GH483, 488).

Fig. 4. Thorax of *D. viride* (same specimen).

Fig. 5. The whole zooid of *D. viride* (same specimen), showing the position of the V-shaped mass of pigment cells in the inner curve of the gut loop and a clump of plant cells outside the gut loop.

thickness of the basal test. Zooids are arranged in double rows around circular areas of test and appear as dark points in the living colony and as light points in the narcotized, preserved material. Living colonies are white to grey, depending on the density of the plant cells embedded in the surface layer of test. Colonies often have a "marbled" appearance resulting from variations in density of spicule distribution. The test is tough and is usually packed with spicules. Spicules are absent only from the thin superficial layer of test.

The spicules are 0.03 to 0.04 mm and occasionally to 0.06 mm in diameter with 9 to 11 conical, pointed or rounded rays that break up fairly readily. The plant cells are usually found embedded in the superficial test. Beneath this they are found in reduced density mixed with spicules in the upper layer of test, and lining the cloacal canals. They are invariably absent from the basal test. In some colonies they are also absent from the superficial layer of test. Plant cells are 0.008–0.010 mm in diameter.

The common cloacal canals are thoracic and rather restricted. In the largest colonies there are also some narrow posterior abdominal canals and these are also lined with plant cells. There are relatively large oval masses of dark green pigment cells in the basal test.

The zooids are very small, less than 1 mm in length. The branchial siphon is long and muscular and sometimes as long as the pharynx. The branchial lobes around the aperture are relatively shallow. These small branchial lobes are not outlined by spicules in the surface test. There are 4 rows of 6 rather rounded stigmata. The short retractor muscle is free from halfway down the long esophageal neck. There is a lateral organ opposite the third row of stigmata on each side of the endostyle. The atrial aperture is an incised opening across the middorsal surface of the thorax. The gut loop is flexed and there is a V-shaped mass of dark greenish pigment cells on the ventral side of the inner curve of the gut loop. There appears also to be a clump of plant cells close against this part of the abdomen. There are $7\frac{1}{2}$ coils of the vas deferens around the single male follicle.

A single embryo was found in the test of the specimen from Apo Reef (QM GH488). The trunk is 0.52 mm long and is almost spherical. The tail is wound only half way around the trunk. The embryo is surrounded with a coating of plant cells that are absent only from a circular area over the sense organs and an elongate area down the center of the anterior end of the trunk where the 3 adhesive organs are exposed.

REMARKS: The colony is strongly reminiscent of *Trididemnum nubilum* and the species are distinguished only by the zooids. The coating of plant cells around the embryo is also similar to that found in *Trididemnum miniatum*, *T. cyclops* and *T. clinides*.

Didemnum etiolum n. sp.

Figs. 6-9

RECORDS: Northern Great Barrier Reef, epizooic on *Polycarpa cryptocarpa* (Martha Ridgeway Reef, syntypes QM GH247; Deltaic Reef, paratypes, QM GH272). Philippines (Apo Reef, 20 m, paratypes QM GH459).

RANGE: The species is small and inconspicuous. The few records available suggest a wide range in the western Pacific.

DESCRIPTION: The colonies are small, and circular or oval, up to 1 cm in length, but more often 0.5 cm or less. They are soft, with a central cloacal aperture, slightly projecting. The zooids are arranged in a circle around the periphery of the colony, forming slight rounded protruberances on the surface. The surface test is thin, there is a thoracic cloacal cavity and a relatively thick basal test in

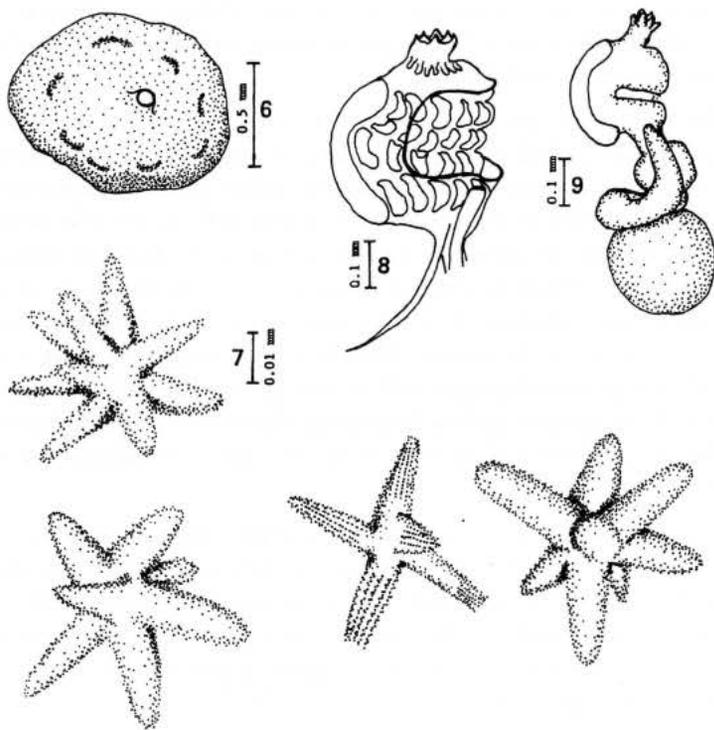


Fig. 6. *Didemnum etiolum* colony (QM GH459).

Fig. 7. Spicules of *D. etiolum* (same specimen).

Fig. 8. Thorax of *D. etiolum* (same specimen).

Fig. 9. A whole zooid of *D. etiolum* (same specimen).

which the abdomina are embedded. Spicules are found throughout, although they are most crowded in the basal test. Plant cells (0.008–0.01 mm in diameter) are found embedded in the test. These become less dense basally. In some of the colonies there are dark pigment cells (0.001–0.008 mm) in the body wall, especially over the abdomen and in the surface layer of test. The distinctive spicules are stellate, generally about 0.045 mm in diameter with 5 to 7 long almost cylindrical rays in optical section.

The zooids are only about 0.5 mm long. The branchial siphon is of moderate length and the branchial lobes are distinct but not deeply separated. The atrial aperture is a deep transverse incision across the dorsal border of the thorax. There are 4 rows of 5 rectangular stigmata. A very fine retractor muscle separates from the posterior end of the thorax. There is a large oval lateral organ opposite the last row of stigmata on each side of the endostyle. The gut loop is flexed upwards. No mature testis follicles were observed.

REMARKS: These small soft colonies superficially resemble those of *Trididemnum clinides*. The species is distinguished from *Didemnum viride* by its smaller plant symbionts, by its small and soft colonies, by its spicules, and by its very fine retractor muscle from the posterior end of the thorax. Although mature male follicles are not present, the species appears to be closely related to *Didemnum viride* and is accordingly assigned to the genus *Didemnum*.

***Trididemnum nubilum* Kott**

Figs. 10–12

Trididemnum nubilum Kott, 1980: 9 and synonymy; 1981: 188.

NEW RECORDS: Philippines (Apo Reef, 20 m, QM GH539). Great Barrier Reef (Lizard Island, on *Acropora* rubble on sandy reef flat, QM GH150).

RANGE: The species is known from the Philippines, Fiji and the Great Barrier Reef (Lizard Island).

DESCRIPTION: The colonies are firm, flat (3 mm thick) and irregular in outline. Although previous records are of small colonies, up to 2 cm in maximum dimension, the colony from Apo Reef forms a larger investing sheet. The superficial layer of test has embedded plant cells and some scattered spicules. The superficial test varies in thickness to some extent and the plant cells are not found always in the surface, causing a marbled appearance. The thoracic cloacal cavity is very shallow and restricted. Spicules are present throughout the test in moderate density. They are 0.03–0.04 mm in diameter with 9–11 short conical rays. The plant cells (0.006–0.012 mm in diameter) are present in the upper layer of test, and lining the cloacal canals.

The zooids are about 0.7 mm long. The branchial siphon is of moderate

length with only very shallow lobes. The atrial opening is a limited middorsal incision, sometimes produced into a very short siphon. The branchial sac is short and relatively broad, with 6 rounded stigmata in each of the three rows. There is a long retractor muscle that is free from the anterior part of the esophageal region.

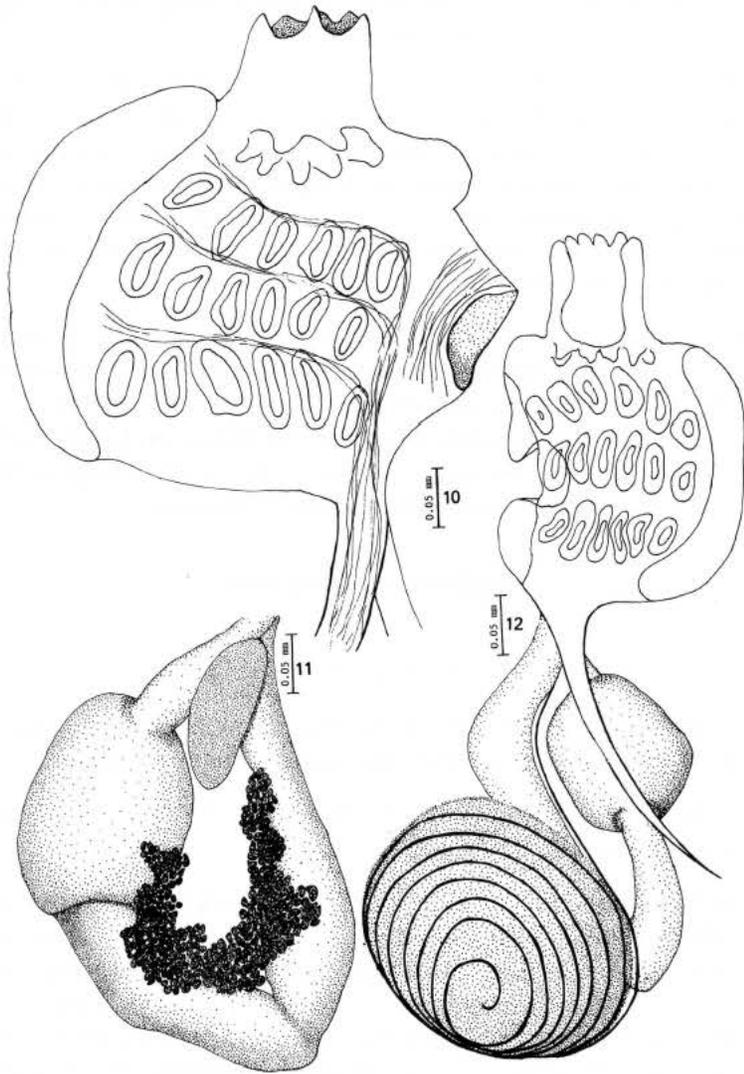


Fig. 10. Thorax of *Trididemnum nubilum* (QM GH539).

Fig. 11. The abdomen of *T. nubilum* (same specimen) showing the epicardial sac and the V-shaped mass of pigmented cells on the upper (ventral) side of the inner curve of the gut loop.

Fig. 12. A whole zooid of *T. nubilum* (QM GH150).

The gut loop is only slightly flexed upwards. There are $8\frac{1}{2}$ coils of the vas deferens around a rather flattened male follicle. There is a V-shaped mass of dark greenish black cells in the loop of the gut (as in *D. viride*) and oval masses of these cells in the basal test.

REMARKS: Colonies of this species bear a close resemblance to colonies of *Didemnum viride*. The spicules of the latter species have a greater size range, up to 0.06 mm and slightly longer rays, but they are of similar form to those of the present species and do not represent a reliable means of distinguishing the species. The double rows of zooids are more conspicuous from the surface of the colonies in *D. viride*, but the plant cells are the same size and there are similar abdominal masses of dark pigment cells in the basal test. Despite the presence of 4 rows of stigmata, the branchial sac in *D. viride* is smaller and relatively narrower than is the case in *T. nubilum*. The species are also distinguished by the long branchial siphon of *D. viride*, and by the retractor muscle, which in the present species is free from the proximal end of the esophageal neck, rather than half way down it as in *D. viride*.

T. clinides and related species are distinguished from *T. nubilum* by their atrial siphons and more delicate retractor muscles free from the posterior end of the thorax.

Tridemnum paraclinides n. sp.

Figs. 13–15

Tridemnum clinides: Kott, 1981: 186 (part, specimens from Mumbualau and Dravuni).

NEW RECORDS: Palau (Nemelis, 1 m, holotype, QM GH575).

RANGE: The species has been recorded from Fiji (Mumbualau, paratype QM GH 144; Dravuni, paratype QM GH 91) in high energy parts of the reef. The Palau specimen was taken in shallow lagoonal waters protected from wave action.

DESCRIPTION: Colonies are firm, flat surfaced, irregular sheets up to 2 cm in greatest extent. Living colonies are green to blackish slate. In preservative they are clear green. The colour appears to persist for reasonably long periods in formalin and is contained in the plant cells that are embedded in the smooth superficial layer of test. There are no spicules in this layer which covers the surface and borders of the colony. Spicules are present in a single layer beneath the plant cells (immediately above the thoracic cloacal cavity) and throughout the basal layer of test. Spicules are large (0.03–0.08 mm) and stellate, with 9 to 11 short conical rays. Plant cells are 0.008–0.015 mm in diameter. They are present in greatest density in the superficial test and lining the quite extensive thoracic

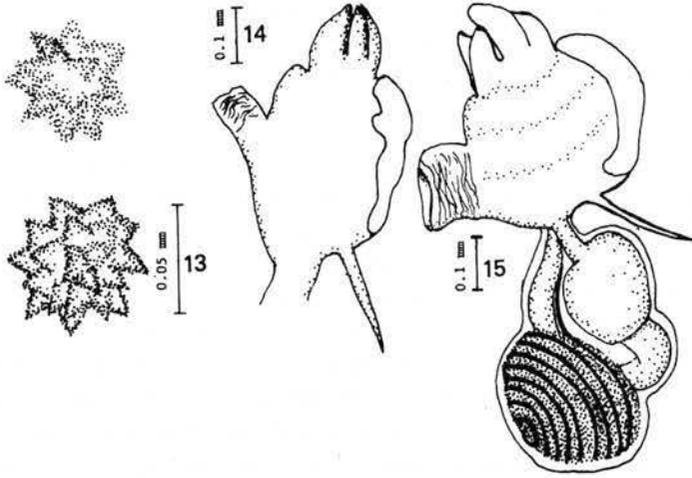


Fig. 13. Spicules of *Trididemnum paraclinides* (QM GH575).

Fig. 14. Thorax of *T. paraclinides* (same specimen).

Fig. 15. A whole zooid of *T. paraclinides* (same specimen).

common cloacal cavity. They are not present in the basal test beneath the common cloacal cavity. Minute dark pigment cells are scattered through the test and are present in oval accumulations in the basal test.

Zooids are only about 1 mm in total length. The thorax is slightly larger than the abdomen. The thorax is muscular and the branchial sac is never exposed. The atrial aperture is on a muscular siphon directed laterally or posteriorly. The branchial aperture has 6 large, deeply divided lobes. There is a very short, delicate retractor muscle from the posterior end of the thorax. There are 3 rows of 6 oval stigmata. The gut loop is relatively short and is only slightly flexed upwards. There are $8\frac{1}{2}$ coils of the vas deferens around the almost hemispherical male follicle.

The larval trunk is 0.7 mm long. There are 3 adhesive organs and the usual 4 pairs of ectodermal ampullae. The larval test has embedded plant cells, leaving only the sense organs and adhesive organs at the anterior end of the larva exposed.

REMARKS: The zooids of this species are almost indistinguishable from those of *T. clinides*. Their only distinguishing characters are the larger number of vas deferens coils ($8\frac{1}{2}$) in the present species and the embedded plant cells and the spicules of *T. paraclinides* which are both larger than those of *T. clinides*. The colonies provide principal distinction between the two species. *Trididemnum paraclinides* has more extensive, firm, flat topped colonies, whereas those of *T.*

clinides are small, soft, rounded cushions with single systems of zooids. Further the conspicuous superficial layer of test, without spicules, conferring a clear greenish jelly-like appearance to the outside of the colony, is quite distinctive. The species is distinguished from *T. nubilum* by its more extensive cloacal cavities, larger spicules, absence of spicules from the superficial layer of test, and atrial siphon.

It is this species that most closely resembles the eastern Pacific and Atlantic Ocean species *Trididemnum solidum*. The zooids are similar; there is a similarly directed atrial siphon; the plant cells are embedded in the superficial layer of test and the spicules are of the same size and form in both species. *T. solidum* appears to be distinguished only by its larger numbers of vas deferens coils and stigmata.

Trididemnum clinides Kott

Fig. 16

Trididemnum clinides Kott, 1977: 617; 1980: 5 and synonymy; 1981: 186 (part, not specimens from Mumbualau and Dravuni).

NEW RECORDS: Philippines (Tambuli Beach, 2 m, QM GH396). Guam (Pago Bay, underside of rocks, intertidal, QM GH696).

RANGE: The species has a wide range in the West Pacific, having been recorded from Heron Island at the southern end of the Great Barrier Reef and from the Philippines, Eniwetok and Fiji. It has not been recorded from the Indian Ocean, although it is small and inconspicuous and may have been overlooked there.

DESCRIPTION: The small, rounded, soft colonies of this species, with embedded plant cells and patches of spicules over the zooids, are quite distinctive. In preservative they are invariably brownish green. The plant cells are 0.006–0.008 mm in diameter and are very bright green and are found throughout the test. Cyanophyceae are embedded in the test of specimens from Guam, as reported for Philippine specimens (*T. viride*: Tokioka, 1967, zooids with atrial siphons < *T. clinides*, see Kott 1980).

The atrial siphon, that is often funnel-shaped or frilled when extended (see Kott, 1980, fig. 5) is quite distinctive.

REMARKS: *Trididemnum paraclinides* has been confused with the present species on the basis of the very similar zooids, branchial lobes, retractor muscle, thoracic cloacal cavity and embedded plant cells. In *T. clinides*, the small colony and characteristic distribution of the spicules in the surface test appears to represent a reliable distinction that is confirmed by size differences in the spicules and embedded symbionts and by a difference in the number of vas deferens coils.

T. clinides has been taken in very shallow (1–2 m) water, inshore, on

seagrass in association with small colonies of *Didemnum cuculliferum* (Kott 1982) at both Tambuli Beach, Philippines and Green Island in the Great Barrier Reef.

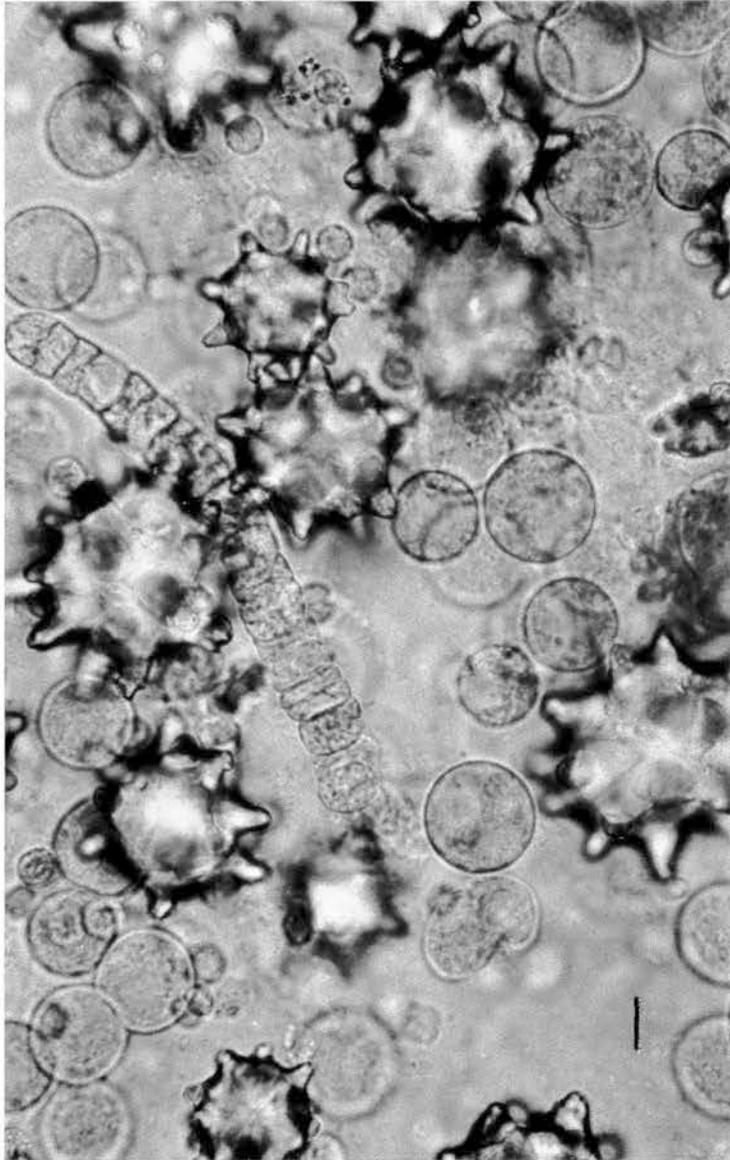


Fig. 16. Test inclusions of *Trididemnum clinides* (QM GH696) including spicules, *Prochloron*, and cyanophytes. Scale = 0.01 mm.

Trididemnum miniatum Kott

Trididemnum miniatum Kott, 1977: 617; 1980: 7 and synonymy.

NEW RECORDS: Northern Great Barrier Reef (Deltaic Reef, off *Polycarpa cryptocarpa*, QM GH269).

DESCRIPTION: The small, bright green colonies, with embedded plant cells and zooids less than 1 mm long are characteristic of the species. The algal cells are 0.007–0.01 mm in this species.

Trididemnum cyclops Michaelsen

Fig. 19b, c

Trididemnum cyclops Michaelsen, 1921: 19; Kott, 1980: 10 and synonymy; 1981: 188

Trididemnum symbioticum Pérès, 1962: 40.

NEW RECORDS: Palau (Urukthapel, Ascidian Lake, 1 m, QM GH580; Kamori Island, 10 m, QM GH581). Caroline Islands (Truk, lagoon, QM GH812).

RANGE: Indo-West Pacific. The species is found on weed or hard substrates in protected lagoonal and reef front habitats that are not too exposed. It is taken from the low tide mark down to at least 12 m.

DESCRIPTION: These small colonies from Palau are characteristic of the species. The spicules are less crowded in the basal test than in other parts of the colony. The plant cells are 0.008–0.012 mm in 5 colonies each from a different location in the range (Viti Levu, Great Astrolabe Reef, Heron Island, Lizard Island, and Kamori Island.) Thinh (1979) found plant cells in his specimens to be 0.012–0.019 mm.

Trididemnum paracyclops Kott

Fig. 19d

Trididemnum paracyclops Kott, 1980: 12 and synonymy.

NEW RECORDS: Philippines (Apo Reef, 1 m on coral rubble, QM GH503). Palau (QM GH507; Nemelis, 1 m on coral, QM GH582). Guam (Double Reef, 7 m, QM GH824).

RANGE: The species is also recorded from the whole extent of the Great Barrier Reef and from Fiji. It occurs on hard substrates and in shallow water, on reef flat and front where it extends down into the interstices of the reef frame and invests the flat surfaces of rubble.

DESCRIPTION: The branchial lobes of some of the zooids are especially well defined, with the most ventral lobes longer than the dorsal ones. There are dense clumps of spicules associated with these lobes that are seen as white patches on the surface, marking the branchial openings of each zooid. Spicules are absent from the basal test, although there may be a single layer against the substrate. Black pigment is present in the test and on the abdominal walls. The endostylar pigment cap is distinct. The plant cells are 0.010–0.015 mm.

The single colony from Guam has a characteristic black border. The zooids of this colony have an unusually long oesophageal neck. The free part of the retractor muscle is from the pyloric rather than the thoracic end of this neck.

The usual $9\frac{1}{2}$ coils of the vas deferens are present around the male follicle. Larvae are of the usual form and the trunk is 0.9 mm long (QM GH582).

REMARKS: The colonies in this collection are of moderate size, but do not form the large investing sheets that have been previously noted. The black pigment that has been observed around the border of the spreading sheets (Kott 1980) has not been recorded for these specimens.

Lissoclinum bistratum (Sluiter)

Fig. 19e, f; 20a

Didemnum bistratum Sluiter, 1905: 18.

Lissoclinum bistratum: Kott, 1980: 16 and synonymy; 1981: 189.

NEW RECORDS: Caroline Islands (Pulusuk, reef flat, QM GH814). Philippines (Apo Reef, 1, QM GH504, 505; Tambuli Beach, QM GH417). Smiths Reef, off Moreton Island, 5 m, QM GH370). Singapore.

RANGE: Moreton I. constitutes the most southerly record for this common species. It ranges widely in the Indo-West Pacific, from the Gulf of Aden to the Tokara Islands, Palau Islands, and Fiji.

Lissoclinum voeltzkowi (Michaelsen)

Fig. 20a–c

Didemnum voeltzkowi Michaelsen, 1920: 54.

Lissoclinum voeltzkowi: Kott, 1980: 13 and synonymy; 1981: 190.

NEW RECORDS: Caroline Islands (Pulusuk, reef flat, QM GH815). Philippines (Calatagan, Batangas encrusting *Enhalus*, 1 m, QM GH520; Apo Reef, 3–20 m, QM GH452, 505). Palau (Ngell Channel, 1 m, QM GH583, 502; Kamori Island, from *Enhalus*, intertidal QM GH586). Guam (Double Reef, 7 m, QM GH827).

RANGE: The species is common in shallow protected lagoonal habitats on sand, rubble, brown algae, *Enhalus* and *Halimeda* from the northern part of the Great Barrier Reef (north from Green Island) and from Fiji, Philippines, and Malagasy. It is one of the species with a range confined to lower latitudes in the Indo-West Pacific.

DESCRIPTION: The newly recorded collections contain characteristic colonies, with some variation in the density of spicules in the surface test.

Some of the colonies from Palau (QM GH502) form larger sheets than those previously known, with maximum dimension up to 9 cm. The surface test in these colonies has patches and flecks of dark pigment and spicules. The pointed, spicule-filled papillae occur at one side of the slit-like branchial apertures, especially around the borders of the colony, as previously described (Kott 1980). The spicules are only very dense beneath the superficial layer of test and in the test around the thoraces. They are sparse at abdominal level and absent altogether from the upper layer of basal test (below the posterior abdominal canal). These colonies differ from those previously described in that lobulation has not occurred to limit the size of the colony.

Some of the intertidal specimens from Kamori Island are irregular plates up to 3 cm and are within the size range previously recorded. Spicules are not dense in the surface test and the green plant cells show through very clearly. Spicules are also very sparse in the basal layer of test and, where that spreads out beyond the border of the colony, there is a translucent area with only sparse, scattered spicules.

Lissoclinum patella (Gottschaldt)

Fig. 19g

Didemnoides patella Gottschaldt, 1898: 653.

Lissoclinum pattelum: Kott, 1980: 18, and synonymy; 1981: 189.

NEW RECORDS: Philippines (Calatagan, 1 m, near base of *Enhalus* and on silt, QM GH514; Apo Reef, QM GH518; Tambuli Beach, QM GH420). Palau (QM G12679). Guam (Double Reef, 7 m, QM GH826). Northern Great Barrier Reef (Tijou Reef, QM GH289).

RANGE: The species is recorded from the east Indian Ocean and western Pacific. The most southerly records are at Cockburn Sound (Western Australia) and Heron Island. It is also recorded from Fiji. It occurs on hard substrates down to at least 10 m and is sometimes found over-growing living corals.

Lissoclinum punctatum Kott

Fig. 19h

Lissoclinum punctatum Kott, 1977: 620; 1980: 20 and synonymy; 1981: 190.

NEW RECORDS: Palau (Kamori Island, on *Lithothamnion*, intertidal, QM GH585). Singapore.

RANGE: This delicate species with soft test that readily disintegrates into mucus when removed from the substrate, is recorded only from the Great Barrier Reef (Heron Island to Lizard Island) and from Fiji and Palau. It is found on firm or hard substrates, and is usually cryptic, growing in the interstices of algal mats and coral rubble.

Diplosoma virens (Hartmeyer)

Figs. 17a-e; 20e-g

Leptoclinum virens Hartmeyer, 1909: 1455 (*nom. nov.* for *Diplosoma viride* Herdman, 1906: 341).

Diplosoma virens: Kott, 1980: 22 and synonymy; 1981: 193.

NEW RECORDS: Caroline Islands (Pulusuk, reef flat, QM GH816). Philippines (Apo Reef, lagoon on mangrove roots, QM GH447; Apo Reef, reef flat, encrusting coral rubble, QM GH469, 480). Palau (Koror-Babeldaup bridge, intertidal mud flat, QM GH579). Northern Great Barrier Reef (Lizard Island, reef between Palfrey and Lizard Islands, 1 m, QM GH589, 510; on *Acropora* rubble, Eagle Island, reef flat, 1 m, QM GH12, 591; Sue Island western reef flat, *Acropora* association, QM GH592).

RANGE: The species has a wide recorded range in the Indo-West Pacific, from Sri Lanka to Fiji, and the Marshall Is. It has been recorded from Heron I. at the southern end of the Great Barrier Reef, but is more often encountered at low latitudes. It is usually found in very shallow often intertidal habitats on weed or hard substrates or on mud. The specimens from Palau were taken from the lagoonal edge of mangrove flats, on aerial roots, submerged branches and driftwood.

DESCRIPTION: The colonies from all locations are irregularly shaped mats up to 5 cm, but usually only 1.0–1.5 cm in greatest dimension. They are always flat on the upper surface, with a distinct rounded border. The basal test varies in thickness and colonies are 2–8 mm thick. The test is very tough. There are the usual extensive posterior abdominal cloacal cavities, and a complex arrangement of canals at zooid level. Vascular ampullae can be seen in the test around the borders of the colonies and in the basal test. Zooids are fairly loosely spaced

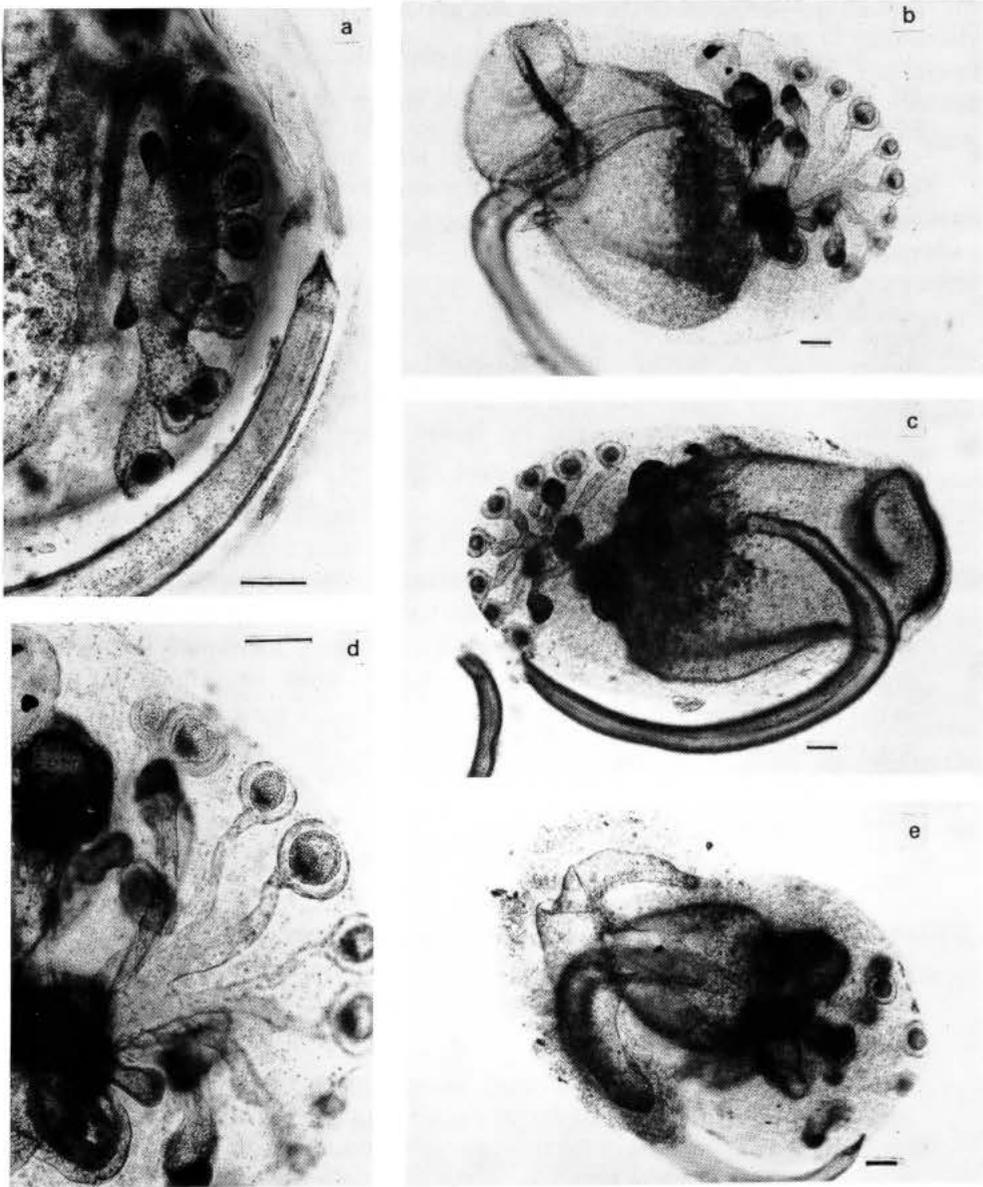


Fig. 17. Larvae of *Diplosoma virens* (QM GH590) from a Lizard Island population. (a) early larva showing adhesive apparatus constricted from front of larval trunk; (b-c) larva at stage of differentiation of rastrum; (d) anterior part of larval trunk showing adhesive organ replication; (e) fully developed larva. Scale=0.1 mm.

around the perimeter of the colony but are less crowded internally.

The zooids are slightly longer than 1 mm, excluding gonads posterior to the gut loop. The branchial siphon is rather long, with a distinct circular sphincter muscle. There are 6 oval stigmata in each row and the usual wide atrial opening exposes the branchial sac. A long slender retractor muscle separates from the proximal half of the relatively long oesophageal neck. The stomach is short and rounded, occupying only about half the length of the proximal limb of the horizontal gut loop.

Embryos are generally present in the thick basal test, although they are never numerous. They are especially robust at the stage when the rastrum has differentiated, but before it has developed its two horns. The trunk is up to 1.5 mm long (from the anterior end of the larva to the base of the tail). The tail is wound about half way around the larvae. More mature embryos with the rastrum very developed have an almost spherical larval trunk about 1.0 mm long. Before metamorphosis, the posterior end of the trunk narrows and is subsequently overgrown by the larval test.

Larvae from Philippine specimens usually have 3 median adhesive organs and 2–3 pairs of ectodermal ampullae (although there is a single specimen with 4). In immature embryos the anterior end of the larval trunk with the adhesive organs is constricted off from the main part of the trunk. In all the larvae found in the large numbers of colonies from Lizard Island, a greater number of adhesive organs than is usual for this genus is present. These result from subdivision of the 3 primary organs. In most cases there are 7–8 papillae, although a single larva with only 4 has been observed. These numerous adhesive organs are arranged in a single median line anteriorly. The rastrum is present in mature embryos (Kott 1980).

REMARKS: Most of these specimens are large and robust. They resemble colonies from Heron Island (Kott 1980) and from Mumbualau, Fiji (Kott 1981). In addition to the complex cloacal system and tough test, the solid and thick basal layer is characteristic.

Zooids are distinguished from those of *D. similis* by their long branchial siphons and by the origin of the retractor muscle from the esophageal neck rather than from the base of the thorax near the posterior end of the endostyle as in *D. similis*. Although in both species the retractor muscle varies in length, it is usually longer in *D. virens* than in *D. similis*. The stomach in *D. virens* is short and rounded, occupying only about half of the proximal limb of the gut loop, whereas in *D. similis* it is elongate and occupies the greater part of the proximal limb of the gut loop.

The larvae of *D. virens* are more robust than those of *D. similis*. The larvae of both these species become shorter as the rastrum separates from the trunk. At

this stage the larvae of *D. virens* are seldom less than 0.9 mm long from the anterior point of the trunk to the base of the tail and are more conspicuously spherical than those of *D. similis* which are only about 0.75 mm long. The tails of *D. virens* larvae are also robust and when wound around the trunk do not extend further than about half way while those of *D. similis* extend almost the whole way around.

The larvae of the specimens of *D. virens* from Lizard Island are unique in the multiplication of the adhesive organs. These are maintained in a median vertical line unlike those of *D. multipapillata* Kott, 1980, which form a rosette occupying the whole anterior end of the larva. The larvae of these specimens are also larger than those of other specimens of *D. virens*. The zooids of the Lizard Island colonies are also slightly larger than those in other populations of the species, but there are no other distinctions in either colonies or zooids. Subdivision of adhesive organs to multiply their number can be observed occasionally in other populations (Apo Reef, QM GH480) and it is likely that there is a propensity for this to occur in this species. Further, there are variations in the number and pattern of division in the Lizard Island populations, even within a single colony, so that although the propensity to subdivide may be genetic, the degree to which this occurs very likely varies with the individual and reflects the genetic diversity of this species.

Thus, although the condition of the Lizard Island larvae suggests a degree of isolation for the populations, it is probably not indicative of reproductive isolation and speciation. Such evidence of isolation is not often observed in the Great Barrier Reef, or even in the Indo-West Pacific. The adaptive advantages of the large number of adhesive organs may be associated with the shallow water habitats where tidal currents move between the line of islands that confine the lagoon of Lizard Island.

D. virens has been recorded from Lizard Island on only one previous occasion (QM G8593) (Kott 1977). These specimens, like colonies from Carter Reef (QM GH22), are juvenile colonies of *D. similis*.

Diplosoma similis (Sluiter)

Figs. 18; 20d

Leptolinum simile Sluiter, 1909: 77.

Diplosoma similis: Kott, 1980: 26 and synonymy; 1981: 191.

NEW RECORDS: Caroline Islands (Truk, lagoon, QM GH813). Philippines (Tambuli Beach, 2 m, QM GH414); Palau (Kamori Island, 3 m, QM GH576, 577; Arakabesong, 3 m, QM GH578). Guam (Double Reef, 7 m, QM GH825) Northern Great Barrier Reef (Sue Island, western reef flat, *Acropora* association, QM GH291; Lizard Island, reef between Palfrey and Lizard Islands, 1 m, QM GH589, 588; Carter Reef, QM GH22). Singapore.

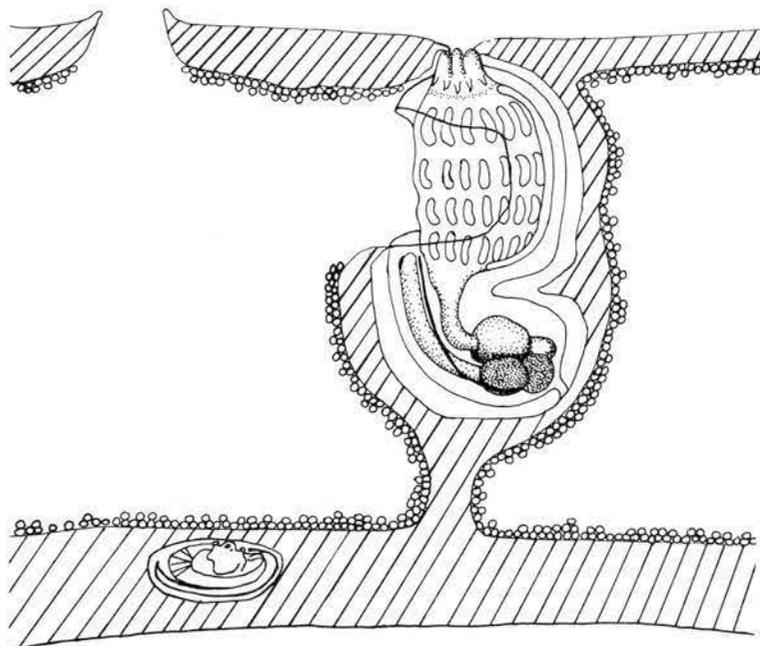


Fig. 18. Diagrammatic vertical section of part of a colony of *Diplosoma similis* showing algal cells lining the common cloacal canals.

RANGE: The species has a wide range in the West Pacific Ocean, from the Tokara Islands to Heron Island. Its most easterly record is from Hawaii. The species has not yet been recorded from the Indian Ocean. It is found on hard substrates and rubble, lining the interstices of the reefal framework, wrapped around the bases of coral skeletons and binding rubble together in pools in the outer high energy parts of the reef flat, at depths from low water to at least 7 m.

Biogeographic Notes

Recent collecting of this group of organisms has demonstrated a wide tropical range for most of the known species. The only known exception is *Diplosoma multipapillata* Kott, 1980, from Viti Levu, Fiji (Fig. 20h). The species occupies a very specialised habitat in a high energy location. Although similar habitats may occur in other tropical locations, the Fijian populations appear to be isolated and the species may be endemic. The known range of each species is set out in Table 1.

Table 1 shows the effects of more exhaustive collecting recently undertaken in shallow subtidal habitats in the Philippines, Palau, Fiji and Heron, Green and Lizard Islands on the Great Barrier Reef. The number of species recorded at each location appears to be directly related to collecting effort.

The most thorough survey for these species has been at Heron Island, one of the

Table 1. Recorded distribution of algae-bearing didemnid ascidians.
 × = Pacific Ocean location; ○ = Indian Ocean location

LOCATION	<i>D. multipapillata</i>	<i>D. viride</i>	<i>D. etiolum</i>	<i>T. strigosum</i>	<i>T. paracclinides</i>	<i>T. nubilum</i>	<i>L. voeltzkowi</i>	<i>E. triangulum</i>	<i>T. paracyclops</i>	<i>T. miniatum</i>	<i>L. punctatum</i>	<i>L. patella</i>	<i>D. molle</i>	<i>L. bistratum</i>	<i>T. cyclops</i>	<i>T. clinides</i>	<i>D. virens</i>	<i>D. similis</i>
Hawaii																	×	×
Line Is.																		×
Marshall Is.															×	×	×	×
Gilbert Is.															×	×	×	×
Fiji	×			×	×	×	×		×	×	×	×	×	×	×	×	×	×
Tonga														×				×
Tokara Is.														×				×
Okinawa													×					
Guam							×	×				×	×			×		×
Philippines		×	×	×		×	×	×	×	×		×	×	×	×	×	×	×
Palau Is.		×			×		×		×		×	×	×	×	×		×	×
Borneo												×						
Singapore										×			×					×
Indonesia								×				×	×	×			×	×
Darwin												×	×	×			×	
Martha Ridgeway Rf.			×						×			×	×		×			
Lizard Is.						×	×		×		×	×	×	×	×		×	×
Green Is.							×		×	×		×	×	×	×	×	×	×
Heron Is.								×	×	×	×	×	×	×	×	×	×	×
Mooloolaba													×					
Moreton Is.													×					
Cockburn Sound												○	○					
Sri Lanka		○															○	
Red Sea													○	○				
Zanzibar, Malagasy							○						○	○				

more southerly locations. Lack of records for any species from that site can therefore be considered valid. *Didemnum viride*, *D. etiolum*, *Trididemnum paracclinides*, *T. nubilum* and *Lissoclinum voeltzkowi* are accordingly the group of species that have the most restricted latitudinal range. *Didemnum etiolum* is the only species of this group that is likely to have been overlooked because it is so inconspicuous.

Diplosoma similis, *D. virens*, *Trididemnum cyclops*, and *T. clinides* have the widest latitudinal range.

The extended southern range (to Heron Island, 23°S) of so many species that only extend to 10°N may be indicative of the habitats provided by the southern part of the Great Barrier Reef. Table 1 also shows that the more conspicuous species are recorded from the greatest number of locations. *Didemnum etiolum*, *Echinoclinum triangulum*, *Trididemnum miniatum*, *T. clinides* and *Lissoclinum punctatum* are all extremely inconspicuous and this may be the reason for their scattered records. The

explanation for the patchily recorded distribution of *Didemnum viride*, *Trididemnum strigosum*, *T. paraclinides* and *T. nubilum*, all of which form conspicuous colonies, may be that their habitats, in high energy locations or in deeper waters, have not been adequately sampled.

There is no evidence of a barrier between Indian Ocean and Pacific Ocean populations and even species with the most limited latitudinal range have been recorded from the western Indian Ocean. The possibility that in due course the majority of species will be found to have a wide longitudinal Indo-West Pacific range should not be overlooked.

Identity of Eastern Pacific and Atlantic Species

Inferences that can be made from studies on the eastern Pacific and Atlantic species of symbionts are restricted because the ascidian host has not been identified. The Caribbean species *Trididemnum solidum* (Van Name) ($>T. cyanophorum$ Lafargue and Duclaux, 1979) has been confirmed as such a host. This species varies in colour from white or grey to green and dark purple, according to the distribution of plant cells and spicules in the colony (Olson 1980).

The specimen (QM G12702) from Baja California is probably of the same species as the colonies referred to by Lewin (Lewin 1975; Lewin, Cheng and Lafargue 1980). It appears to be a specimen of *Trididemnum della vallei* Ritter and Forsyth. It resembles *T. opacum* (from southern California) and is distinguished from *T. solidum* by its massive colonies in which the surface is raised into rounded ridges and lobes. The common cloacal apertures occur on these prominences. The zooids are arranged in groups surrounded by deep cloacal cavities that extend the whole length of, but rarely posterior to, the zooids and the variations in the thickness of the basal test cause the variations in thickness of the colonies. The zooids are about 1.2 mm long. They have brown-black pigment in the body wall, especially over the anterior end of the thorax. The atrial siphon is directed posteriorly and there is a short but rather thick retractor muscle from the posterior end of the thorax. The gut loop is flexed upwards and there are $10\frac{1}{2}$ coils of the vas deferens around the spherical male follicle as in *T. solidum*. The plant cells are embedded in the surface test, as in *T. solidum*. The spicules are large (up to 0.06 mm) and stellate with at least 11 conical pointed rays in optical transverse section, thus distinguishing it from *T. opacum* from southern California, which has smaller spicules with fewer rays.

Other specimens of which the identity is not known are ?*D. virens* and ?*D. candidum* from the West Indies (Lewin, Cheng and Lafargue, 1980).

Specimens of *D. virens* (Whatley 1977) thought to be from Mexico (Thinh 1978), are more likely to be from Hawaii where *D. virens* does occur.

Evolution of the Symbiosis

Many of the plant cell symbionts of these didemnids have been shown to be

prokaryotic and it is a reasonable assumption that symbionts in closely related hosts are also prokaryotic. The present status of knowledge regarding the nature of these symbionts is set out in Table 2.

A close morphological relationship between these prokaryotes and Cyanophyta has been demonstrated (Newcomb and Pugh 1975; Thinh and Griffiths 1977; Thorne et al., 1977). However, Lewin (1976, 1977) has proposed a new division of algae, the Prochlorophyta, distinguished from the Cyanophyta by the presence of chlorophyll *b* and the absence of bilin pigments. This division accommodates a single genus *Prochloron* Lewin.

Table 2. Nature of plant symbionts. Related didemnids are grouped.

Host Ascidian	Plant Symbionts	Reference	**Diameter Symbionts (μ)
<i>D. similis</i>	<i>Prochloron</i>	> <i>D. virens</i> : Newcomb & Pugh 1975; Thorne et al. 1977	10-15
<i>D. virens</i>	<i>Prochloron</i>	Thinh & Griffiths 1977; Thinh 1978; Thinh et al. 1981	7-15
<i>D. multipapillata</i>	?		8-10
<i>T. cyclops</i>	<i>Prochloron</i>	Thorne et al. 1977; Thinh 1979*	12-19*
<i>T. paracyclops</i>	<i>Prochloron</i>	D. Parry pers. comm.	8-12
			10-15
<i>L. bistratum</i>	<i>Prochloron</i>	> <i>L. molle</i> : Newcomb & Pugh 1975	10-15
<i>L. voeltzkowi</i>	<i>Prochloron</i>	Cox in press	10-15
<i>L. patella</i>	?		10-20
<i>L. punctatum</i>	?		15-20
<i>D. molle</i>	<i>Prochloron</i>	> <i>D. ternatanum</i> : Newcomb & Pugh 1975	15-26
<i>T. solidum</i>	Cyanophyta	> <i>T. cyanophorum</i> Lafargue and Duclaux 1979	8-24
? <i>T. della vallei</i>	<i>Prochloron</i>	Lewin 1975	
<i>T. paraclinides</i>	?		8-15
<i>T. clinides</i>	?		6-8
<i>T. nubilum</i>	Cyanophyta	R. Olson pers. comm.	6-12
<i>T. strigosum</i>	?		4-8
<i>T. miniatum</i>	?		7-10
<i>D. viride</i>	?		8-10
<i>D. etiolum</i>	?		8-10
<i>E. triangulum</i>	?		13-15

** Measurements were made on formalin preserved material in the Queensland Museum unless indicated (*).

It has been proposed by many authors (cf. Raven 1970) that chloroplasts evolved through intracellular chlorophyll-containing prokaryotic symbionts. The relationship of *Prochloron* with both Cyanophyta and Chlorophyta has led to the proposition that the genus has a phylogenetic relationship with such an ancestral symbiont (Thin 1979). It is therefore appropriate to examine the likely evolutionary history of the ascidian hosts for evidence of these affinities.

Microascidites are didemnid spicules in sediments. They are known from the Eocene to the Pliocene and from Mesozoic sediments (Monniot and Buge 1971; Hekel 1973). It appears, therefore, that the Didemnidae may have evolved sometime early in the Tertiary. The family is, in fact, one of the most highly evolved of the Ascidiacea. The plant cell symbionts are found in only relatively few of the tropical species of 5 cosmopolitan genera of the Didemnidae. Larval adaptations for the transference of the algae occur only in those species in which the symbiosis appears to be obligatory. These larval adaptations are different in each genus suggesting an independent origin and evolutionary history for the symbiosis at least once in each of the 5 genera (Kott 1980). Although (as evidenced by morphological adaptations of the larvae) the symbiosis in each of these genera has a long history, it cannot have occurred before the extant genera of the Didemnidae were isolated from one another, and it must have occurred after there had been some radiation within these lineages. The earliest date for its evolution therefore appears to be some time late in the Tertiary. The plant cells that were involved can be reasonably supposed to have been common in the shallow water marine environment at that time. If *Prochloron* are, indeed, relicts of the ancestors of green plant chloroplasts, it is hard to understand why they persisted in the marine environment until the Tertiary (or later) and then became extinct between the Tertiary and Quarternary Periods; while the Cyanophyta have persisted until today.

Further, algal symbionts of extant Didemnidae are not intracellular. Cheng and Lewin (1979) believe that the symbionts occur in the peribranchial cavity, against the wall of the branchial sac. They are never found in that position in living colonies. In both larval and adult ascidians they are exclusively associated with the test which is a largely acellular secretion of the animals it protects. They are found held against the wall of the cloacal cavity by fine extensions of test, or they are found embedded in the test. Occasionally they are found in both positions. Their distribution in the cloacal cavity is interrupted where the atrial aperture opens into that cavity. This opening exposes the whitish translucent branchial sac and its stigmata through which the excurrent ciliary stream passes to the cloacal cavity and to the exterior. Direct exchange of photosynthate from plant cells to zooid is therefore unlikely, although it could occur through the test vessels. The symbiotic relationship of *Prochloron* with the host is not homologous nor even analogous with that proposed for the ancestors of green plant chloroplasts.

An alternative explanation of parallel evolution of Cyanophyta within the ascidian hosts is supported by the morphology of the photosynthetic membranes of these symbiotic cells (Thin and Griffiths 1977; Thorne et al., 1977), and the

occurrence of Cyanophyta as symbiont in at least 2 of the species.

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- Fig. 19a. Small colonies of *Didemnum molle* in Palau.
- 19b. Colonies of *Trididemnum cyclops* with spicules in the surface test (3 m, from a coral reef, in the lagoon of Kamori Island, Palau).
- 19c. Colonies of *T. cyclops* with few spicules in the surface test (1.5 m), Ascidian Lake, Urukthapel Island, Palau).
- 19d. Typical colonies of *Trididemnum paracyclops* investing hard substratum (at Namanda, Fiji).
- 19e. Typical colonies of *Lissoclinum bistratum* with reduced spicule density in surface tests on the shaded side of a basalt boulder (Norfolk Island).
- 19f. A population of *Lissoclinum bistratum* on a sandy reef flat with dense surface spicules and with pink and yellow carotenoid pigments (2 m, Bird Island at Lizard Island, Great Barrier Reef).
- 19g. *Lissoclinum patella* near the low tide mark in a seagrass bed (Kamori Island, Palau).
- 19h. *Lissoclinum punctatum* with white spicules encapsulating the zooids seen toward the base of the colonies (Vuda Point, Fiji).
- Fig. 20a. Lobulated colonies of *Lissoclinum voeltzkowi* with crowded surface spicules (white) mixed with colonies of *Lissoclinum bistratum* at the top right of the view (intertidal, Fiji).
- 20b. Lobulated colonies of *L. voeltzkowi* with spicules relatively sparse in the surface test (on driftwood, Lizard Island, Great Barrier Reef).
- 20c. Sheet-like colonies of *L. voeltzkowi* on *Enhalus* (Kamori Island, Palau). Spicules are relatively sparse in the surface test.
- 20d. A typical colony of *Diplosoma similis* investing rubble (1 m, lagoon, Kamori Island, Palau).
- 20e. Typical lobulated colonies of *Diplosoma virens* on an intertidal mangrove branch (Koror, Palau).
- 20f. Close up view of Fig. 20e.
- 20g. Atypically large and robust colonies of *D. virens* with 7-8 larval adhesive organs (2 m, lagoon, Lizard Island, Great Barrier Reef).
- 20h. Colonies of *Diplosoma multipapillata* under cascades (Malevu, Fiji).

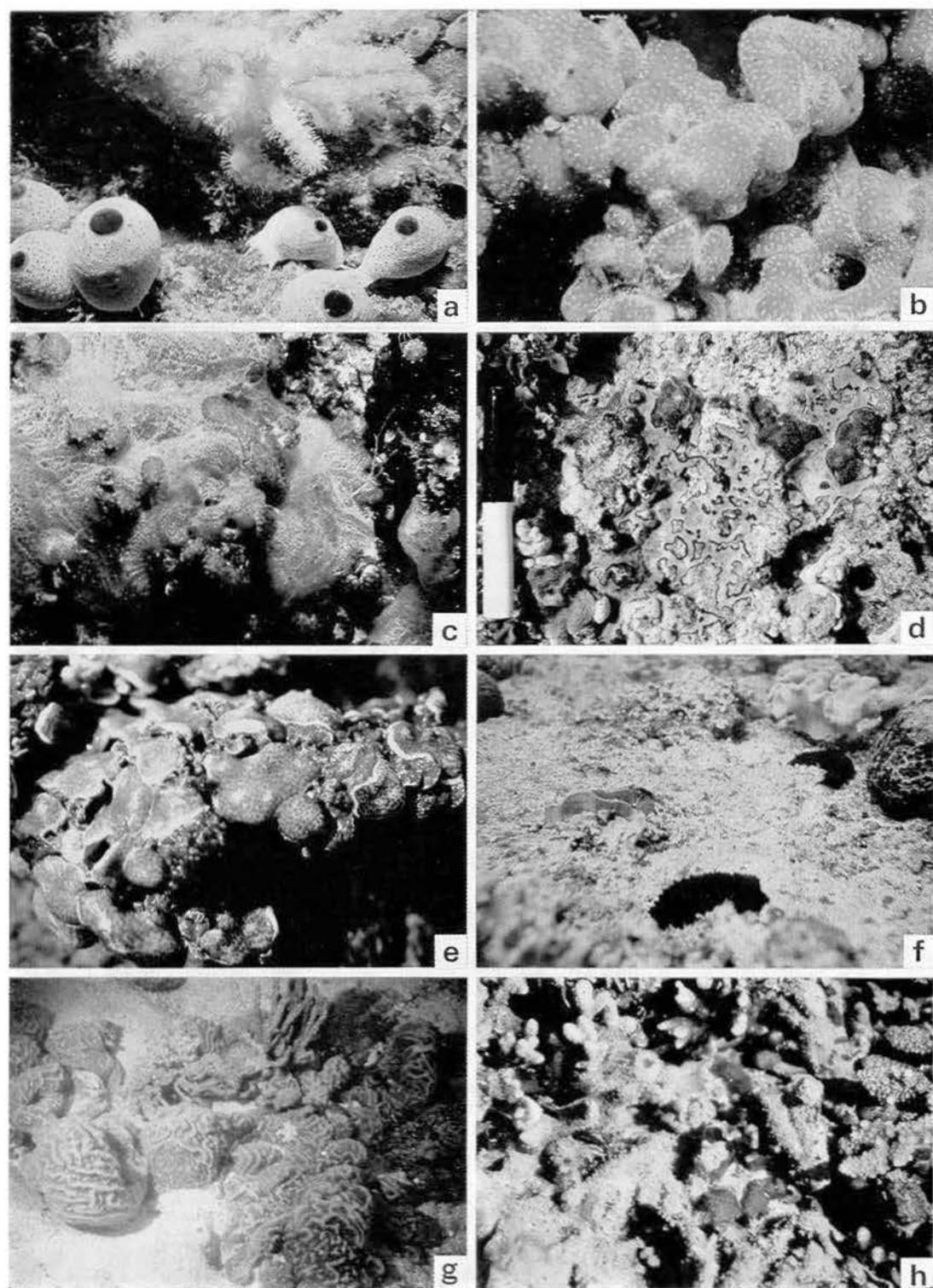


Fig. 19.

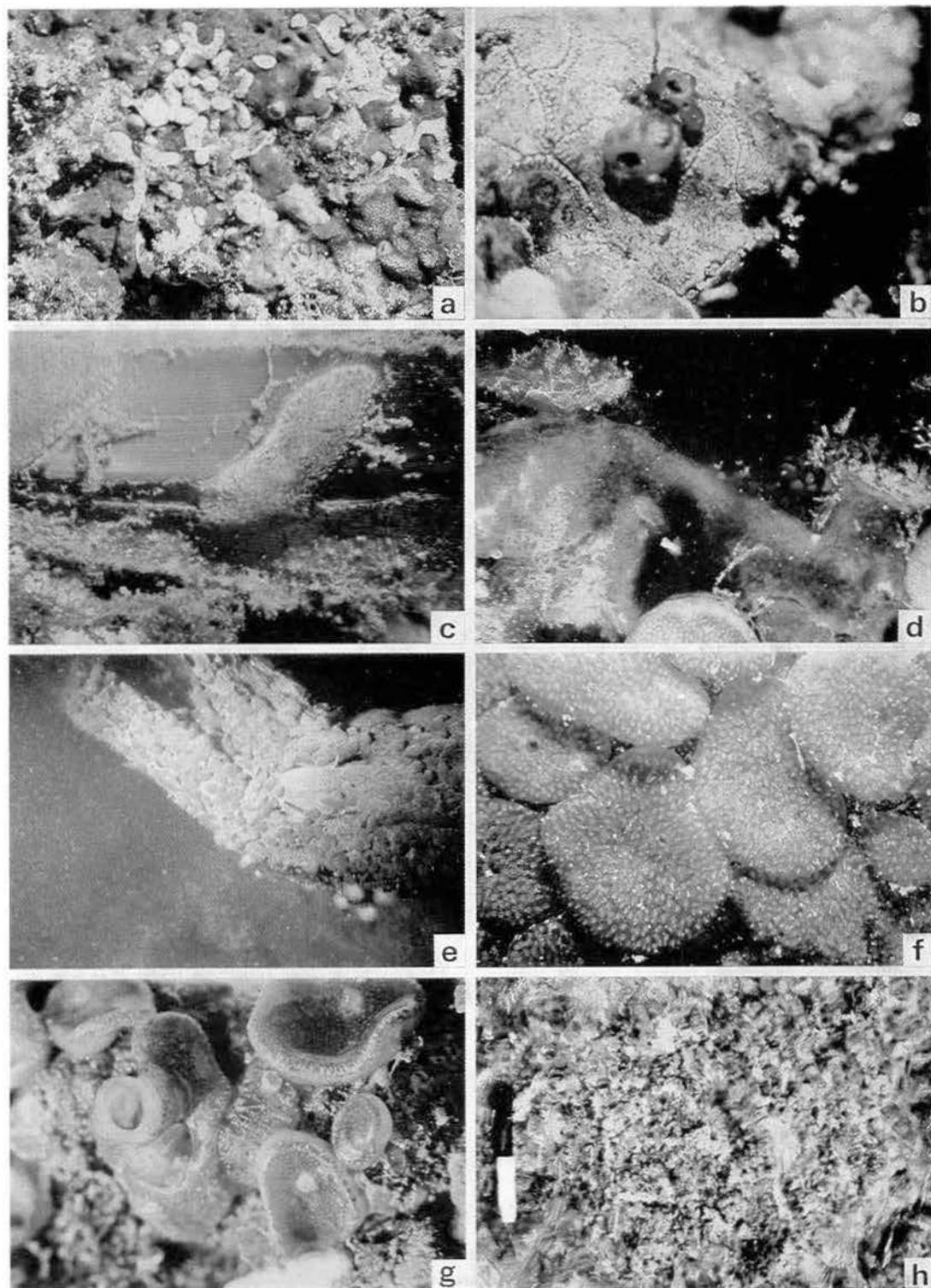


Fig. 20.

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