ON A SMALL COLLECTION OF ASCIDIANS FROM THE PACIFIC COAST OF COSTA RICA¹⁾

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With 10 Text-figures

A small collection of ascidians was made in February to March 1970 in the shallow water at Playas del Coco on the Pacific coast of Costa Rica by Drs. Charles BIRKELAND and Tom M. Spight of the Smithsonian Tropical Research Institute at Coco Solo, Canal Zone, and submitted to me for identification. The place seems to be very significant to see how the boreal species abundant in the California Current are reaching southerly and how the Atlantic elements in the West-Indies are distributed across the isthmus. Moreover, the ascidian fauna in this area has never been studied wholly. The present material which was collected during frequent divings includes not only larger prominent forms but also smaller forms in it. Though the number of species is not so great, the material may be safely regarded to show the general aspect of the ascidian fauna at this place. Then, in view of the importance of the list showing the results of identification, it is decided to give the reasons for the present identification and to repeat the description as to the species which might be identified differently by other researchers or done with a considerable difficulty.

In all, 13 species and one unidentifiable form are included in the present material. They are:

- 1. Amaroucium constellatum Verrill, 1871
- 2. Polyclinum laxum VAN NAME, 1945
- 3. Didemnum (Didemnum) candidum SAVIGNY, 1816
- 4. Didemnum (Didemnum) moseleyi (HERDMAN), 1886
- 5. Lissoclinum fragile (VAN NAME), 1902
- 6. Lissoclinum caulleryi (RITTER & FORSYTH), 1917
- 7. Rhopalaea birkelandi Tokioka, 1971
- 8. Ascidia ceratodes (Huntsman), 1912
- 9. Ascidia sp. (unidentifiable)
- 10. Symplegma pizoni nom. nov. (for Protobotryllus viridis Pizon, 1908)
- 11. Polyandrocarpa (Eusynstyela) tincta (VAN NAME), 1902
- 12. Styela partita (STIMPSON), 1852

¹⁾ Contributions from the Seto Marine Biological Laboratory, No. 560.

- 13. Pyura sp. aff. vittata (STIMPSON), 1852
- 14. Pyura lignosa Michaelsen, 1908.

Before going into descriptions, I want to express here my hearty thanks to Drs. Birkeland and Spight for their kindness in giving me the chance of studying the present interesting material.

1. Amaroucium constellatum Verrill, 1871

(Fig. 1, 1-4)

A single colony in the material, roughly oval in outline but with a protrusion at one end (fig. 1), with somewhat even surface, 25 mm×22 mm in extent and 19 mm in height, and attached to the substratum by a part of the lower side of the colony (fig. 2). The coronal part or the thoracic and abdominal layers of the colony is roughly 7 mm thick and gelatious but with a considerable rigidity, translucent, and faintly pinkish zooids are seen through the milky white test. The basal portion is a little harder, encrusted with fine sand grains, and grayish yellow-white in colour. The surface is smooth and mostly free from sand grains, but partly encrusted with them sparsely; no definite systems of zooids are observed there, though some rough linear arrangement may be seen partially.

In an examined zooid (fig. 3), the thorax is 1.7 mm, the abdomen 1.4 mm, and the postabdomen 7.5 mm in length; in the living state the thorax must be much longer. The branchial aperture 6-lobed, the atrial languet is simple and long, and issued at the level of the first transverse vessel. There are 12–14 stigmatal rows, roughly up to 10 stigmata in a row on each side; the anus is opened at the 8th or 9th transverse vessel. Tentacles could not be counted exactly because of strong contraction, dorsal languets are displaced to the left by 2 stigmata.

The stomach is globular in outline, situated near the middle of the abdomen or a little anteriorly, and with 20 to 25 plications on the surface, some of them are irregular or incomplete. The hind-stomach and middle-intestine are defined very distinctly, the latter passes to the intestine at the posterior end of the abdomen, the proximal end of the intestine forms a pair of horns, protruded posteriorly.

The postabdomen (fig. 4) is very long. The ovary is situated apart from the posterior end of the intestinal loop by a half of the length of the loop. Testicular follicles are arranged in two rows.

Remarks: Putting aside the problem of the identity of A. constellatum with Alcyonidium? pellucidum Leidy, the distinction between A. constellatum and either of A. californicum Ritter and Forsyth, 1917 and A. exile Van Name, 1902 is rather exact. Zooids of the present colony have a little more stigmatal rows and longitudinal plications on the stomach than A. californicum. The present colony is attached to the substratum by much narrower basal portion and postabdomens of zooids in the present colony are

longer than in A. exile. These are the reasons for the present provisional identification, though the taxonomical significance of such differences should be checked strictly in future studies.

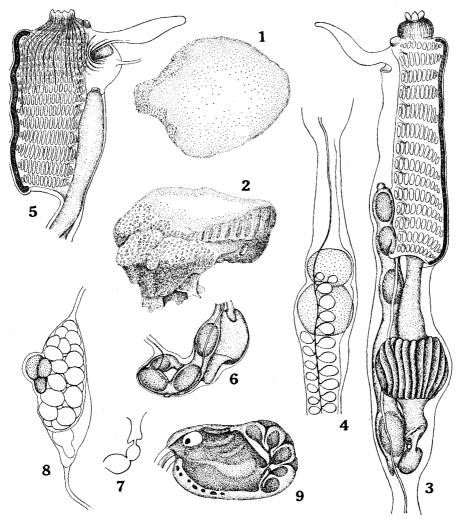


Fig. 1. Amaroucium constellatum Verrill (1-4). 1: upper surface of colony. 2: the same colony, lateral side. 3: thorax and abdomen, right side. 4: anterior portion of postabdomen.

Polyclinum laxum Van Name (5–9). 5: thorax, left side. 6: abdomen. 7: hind-stomach and middle-intestine. 8: postabdomen. 9: embryo, ×73.

2. Polyclinum laxum VAN NAME, 1945

(Fig. 1, 5–9)

Two colonies, 45 mm \times 28 mm - 45 mm \times 35 mm in extent and 15–20 mm in

height, and attached to the substratum by the whole underside. Colonies are grayish yellow to brown, but they must be deeply reddish orange in the living state, as formalin used as the preservative is coloured deep yellow. The colony surface is nearly smooth and more or less encrusted with sand grains. The test is gelatious, considerably hard on the surface, but in the inner part of the colony it is soft, translucent, and quite free from sand grains. Zooids are arranged in groups, but distinct systems can not be discerned, probably because of sand incrustation. Common cloacal apertures are round or oval and 1–2 mm in diameter. The zooidal layer occupies the surface 5–7 mm stratum.

Zooids are rather small, the thorax (fig. 5) is about 1.7 mm, the abdomen (fig. 6) 1 mm, and the postabdomen (fig. 8) 1.3 mm long in the smaller colony. The branchial aperture is 6-lobed, and the atrial languet is prominent and simple or bifid at the tip. About 10 longitudinal muscles converging to the branchial aperture on each side in the anterior half of the thorax, thus the anterior half of the thorax is usually contracted to some extent, while the posterior half including posterior 5–6 stigmatal rows is left expanded. A fine vascular prominence is issued from the dorsal side just posterior to the atrial aperture. There are 12 to 15 stigmatal rows and up to 20 stigmata in a row on each side. The anus is opened at the level of the 8th transverse vessel. Dorsal languets are displaced to the left side by 3 stigmata. The hind-stomach and middle-intestine are defined very distinctly. The heart is situated at the distal end of the postabdomen, the ovary at the centre of the testis which consists of up to 30 follicles. About five embryos arranged in a series of developmental stages were found incubated in the atrial cavity. The larval trunk is about 480μ and the whole embryo (fig. 9) still enveloped in the egg capsule is about 500μ in length.

Remarks: According to the description given by VAN NAME (1945, p. 71), P. laxum seems to have a little fewer stigmatal rows than in P. planum (RITTER and FORSYTH), 1917, and the colony surface is more or less encrusted with sand grains in the former, but quite free from sand grains in the latter. However, the number of stigmatal rows may increase to some extent with the size of zooid, and the size of zooid will become larger in the waters of lower temperature. Thus, it is not impossible that P. laxum is nothing but only a regional form of planum, living in the warmer waters and on the sandy floor; P. planum seems to live in the cooler waters and on the non-sandy bottom.

3. Didemnum (Didemnum) candidum SAVIGNY, 1816 (Fig. 2, 10-12)

Three specimens in the material. The colonies of Specimens No. 1 and No. 2 are snowy white, 0.5 to 1 mm in thickness, somewhat brittle as the spicules are distributed very densely throughout the colony from the surface to the bottom, and the superficial spiculeless layer is indiscernible; the lacuna system is indistinct in No. 1

specimen, but thoracic lacunae are distinct in No. 2 specimen. Spicules are small, larger ones are $26-33\mu$ and 30μ on an average in diameter in No. 1 specimen (fig. 10) and $23-33\mu$ and 26μ on an average in No. 2 specimen (fig. 11), rays on the equatorial plane are 10-12 in No. 1 specimen and 9 (rarely 8)–10 in No. 2 specimen. Zooids are very small in these specimens, the thorax is $200-220\mu$ long in No. 1 and $200-280\mu$ and 245μ on an average in No. 2 specimen in a strongly contracted state; the retractile muscle is very short; the abdomen is $240-250\mu$ long in No. 1 and 410μ in No. 2 specimen; a mean distance between zooids is 465μ in No. 2 specimen. The thoracic organ is distinct, the testicular follicle 1 and the proximal part of the vas deferens coils 5-6 times in both specimens.

The No. 3 specimen is 15 mm \times 10 mm in extent and 1.5 mm in thickness, rather soft, and pale purplish gray in colour as the superficial spiculeless layer is discernible and spicules are sparse in thoracic and bottom layers, though they are dense in the surface layer. Spicules are a little larger (fig. 12) than in the other two specimens, larger ones are $32-36\mu$ and 34μ on an average in diameter, and rays on the equatorial plane are 8–11 and 9 on an average. In addition to thoracic lacunae, hypoabdominal lacunae are discernible. The thorax is 500μ and the retractile muscle is 570μ in a relatively expanded state, and the thoracic organ is prominent; the abdomen is 500μ long; a mean distance between zooids is 415μ . The testicular follicle 1 and the proximal part of the vas deferens coils up to 7 times.

Remarks: Specimens Nos. 1 and 2 may be safely identified with candidum, as their zooids and spicules are small. Spicules of No. 3 specimen are a little larger than those of Specimens Nos. 1 and 2, but still smaller than those of colonies referable to moseleyi. Zooids are also somewhat larger. The size of abdomen will differ primarily with the age after budding and secondarily with the development of testicular follicle, while the size of thorax will differ much according to the degree of contraction. It must be noted that thoraces are found rather expanded in No. 3 specimen. I have distinguished candidum and moseleyi from each other mainly by combination of the spicule size, the number of spicule rays, and the size of zooids after some previous researchers. Although the majority of colonies are identifiable more or less distinctively, there occur truly some colonies, though much fewer than identifiable colonies, which show some intermediate states. The present No. 3 specimen seems to be situated near such intermediate colonies.

4. Didemnum (Didemnum) moseleyi (HERDMAN), 1886 (Fig. 2, 13-15)

There are three groups of colonies in the material. Specimen No. 1: faintly grayish and irregularly lobated encrusting colonies, less than 1 mm in thickness. In some parts of the colony, groups of zooids are clearly definable through the test. The

test surface is somewhat raised up to form a small prominence just by the branchial aperture in some part of the colony. Thoracic lacunae are very spacious. Specimen No. 2: two pieces of snowy white colonies, respectively $15 \text{ mm} \times 23 \text{ mm}$ and $14 \text{ mm} \times 7 \text{ mm}$ in extent and 1-1.5 mm in thickness. The colony is partially divided into a number of small, 1-2 mm long oval areas including respectively several to a dozen zooids, though these small areas are not defined so clearly as in the Specimen No. 1. The superficial spiculeless layer is indiscernible, the test surface is locally protruded out to form a small projection by the branchial aperture. Thoracic lacunae are very

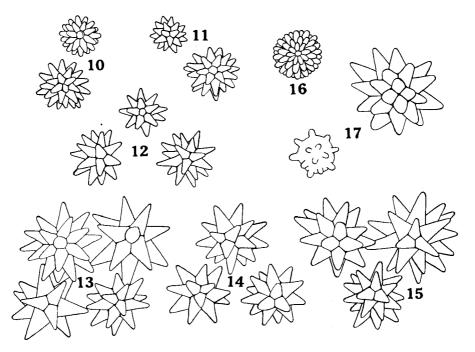


Fig. 2. Didemnum (Didemnum) candidum Savigny (10-12), spicules, ×440. 10: no. 1 specimen. 11: no. 2 specimen. 12: no. 3 specimen. Didemnum (Didemnum) moseleyi (Herdman) (13-15), spicules, ×440. 13: no. 1 specimen. 14: no. 2 specimen. 15: no. 3 specimen. Lissoclinum fragile (Van Name) (16), spicule, ×440. Lissoclinum caulleryi (Ritter & Forsyth) (17), spicules, ×440.

spacious and extending to the bottom of the colony. Specimen No. 3: a single colony, $27 \text{ mm} \times 12 \text{ mm}$ in extent and up to 1.5 mm in thickness. The structure of the test is just the same as in the Specimen No. 2, but spicules are much less dense and the colony looks pale purplish gray.

Spicules are rather large, larger ones are $35{\text -}44\mu$ and 40μ on an average in Specimen No. 2 (fig. 14), $36{\text -}56\mu$ and 47μ on an average in Specimen No. 3 (fig. 15), and $42{\text -}55\mu$ and 50μ on an average in Specimen No. 1 (fig. 13); rays are 7–10 on the equatorial plane and 8 or 8–9 on an average.

The thorax is 300 to 470μ , 400μ on an average, in length in a contracted state and the retractile muscle is nearly as long as the contracted thorax, about 6 stigmata in a row on each side. The abdomen is $360-400\mu$ long in examined zooids. The testicular follicle 1, very rarely 2, and the vas deferens coils 6–7 times. The embryos in earlier stages are about 350μ in length, pigment spots of the sensory organ are arranged antero-posteriorly.

Relatively larger spicules and smaller number of spicule rays may be enough reasons to identify these colonies with *Didemnum moseleyi*, and the existence of a prominence by the branchial aperture, though locally, seems to indicate that the colonies show a weak trend to forma *granulatum* TOKIOKA.

Two colony pieces in the material, respectively 15 mm \times 7 mm and 20 mm \times 8 mm in extent and less than 1 mm in thickness. The colony is very soft, as the lacunae are very extensive and not only thoraces but also whole abdomens are exposed in the space, each being supported only by a single trabecula which is issued from the left dorso-posterior corner of the abdomen and attached to the floor by the other end. The colony surface is irregularly grooved and partially shows a complicatedly lobated appearance. Spicules are very dense throughout the colony; they are very small (fig. 16), larger ones are $18-32\mu$ and 24μ on an average in diameter, and bear 20 or more rays on the equatorial plane. A mean distance between zooids is 520μ .

The atrial aperture is huge, because the thoracic mantle is rather narrow and with muscles weakly developed, about 7 stigmata in each of 4 rows on each side, the anus opens at the dorso-posterior corner of the thorax; the abdomen is 490μ long in an examined zooid. The testis is situated on the left side of the abdomen near the base of the trabecula, but it is so rudimentary that it is impossible to see whether it is a single lobe or consists of a pair of lobes.

In typical colonies of *fragile*, abdomens are usually laid horizontally on the floor with the right side beneath. Zooids in the present colony differ in this feature from the typical ones. At present, it is not known whether or not such a variation in the situation of abdomen can be allowed in a single species of *Lissoclinum*. The extensive lacunae, the appearance of spicules, and the structure of thorax, characteristic to species of *Lissoclinum* and shared by the present specimen, are the reasons for the present identification.

6. Lissoclinum caulleryi (RITTER & FORSYTH), 1917 (Fig. 2, 17)

A grayish 11 mm × 9 mm colony, about 1 mm in thickness. Comparatively large

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spicules are distributed densely only on the surface of the abdominal stratum, but scarcely are seen in the surface layer and completely absent inside the abdominal stratum to the bottom. Larger spicules are 52 to 67μ and 60μ on an average in diameter, rays are stout but rather short and are 8–11 and 10 on an average on the equatorial plane. Thus, the central spherical core of spicules is relatively large; this is seen evidently in some smaller spicules (fig. 17). In the test partially, and on the thoracic or abdominal mantle from zooid to zooid or from part to part, bluish black pigments are distributed in different densities and this gives the colony a grayish tint.

The thorax is $370-420\mu$ long and the retractile muscle is very short in a contracted state; the thoracic mantle is coloured grayish with bluish black pigments except the mid-ventral line over the endostyle. The atrial aperture is huge. The abdomen is 620μ long in an examined zooid and embedded horizontally in the abdominal stratum with the right side toward the substratum. As the bottom layer of the abdominal stratum is quite devoid of spicules and bluish black pigments, the stomach and intestine are seen clearly through the bottom of the colony. The mantle of the upper side, namely the left side, of the abdomen is coloured darkly; this is due to the black pigments covering a pair of testicular lobes, especially deeply the posterior one of the pair. The present specimen can be identified with caulleryi very easily and definitely.

7. Rhopalaea birkelandi TOKIOKA, 1971

According to the communications from Dr. BIRKELAND and his transparency of living individuals in their natural environments, this ascidian is brilliantly blue when alive and one of the most important members of the benthic communities at the place because of its abundance and longevity.

8. Ascidia ceratodes (Huntsman), 1912 (Fig. 3, 18-24)

Three specimens, all attached to the substratum by the whole left side; they are respectively 30 mm×18 mm in extent and 10 mm thick, 26 mm×15 mm in extent and 6 mm thick, and 17 mm×14 mm in extent and 5.5 mm in thickness. The body is clongate oval in shape (fig. 18), and both apertures are quite sessile; the branchial aperture terminal and the atrial near the middle of the dorsal side. The test is gelatinous, rather hard, faintly milky white but transparent, and about 1.5 mm thick in the largest specimen; the surface is smooth and quite free from any foreign matters. Siphons are of a moderate length on the mantle body, the atrial siphon is slightly directed backwards; the branchial aperture is 8-lobed and the atrial is 6-lobed. The mantle body is whitish throughout, but faintly reddish orange in siphonal areas. It is wholly reticulated on the right side with muscle fibres which are mainly transverse (fig. 19). In the preserved and then somewhat contracted state, the ventral part of

the visceral mass is protruded out of the ventral edge of the muscular right side of the mantle body. The anterior end of the intestinal loop reaches anteriorly beyond the middle of the anterior half of the mantle body between both siphons (figs. 20–21). The second loop is very deep, its axis passing through the proximal portion of the intestine. As the oesophagus opens to the branchial sac at the level very near the base of the atrial siphon, the posterior part of the branchial sac is extended much

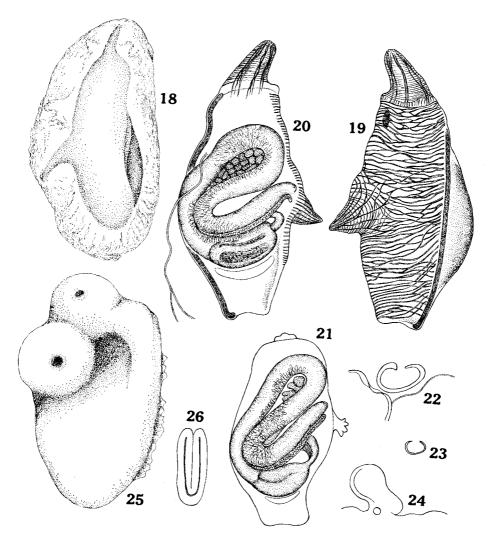


Fig. 3. Ascidia ceratodes (Huntsman) (18-24). 18: 26 mm long specimen. 19: mantle body of the same specimen, right side. 20: the same, left side. 21: 17 mm long specimen, mantle body, left side. 22: 26 mm long specimen, ciliated groove. 23: 17 mm long specimen, ciliated groove. 24: 26 mm long specimen, one of papillae on the inner surface of branchial sac.

Ascidia sp. (25-26). 25: 47 mm long specimen. 26: ciliated groove.

posteriorly beyond the stomach edge. The heart is seen clearly along the posterior edge of the stomach which is oval and with a few folds on the left side. A vessel to the test is issued from the antero-ventral side of the visceral mass. The ovary is situated mainly in the first intestinal loop and testicular follicles are spread over the intestinal wall around the first loop and also around the bottom of the second loop. Even the smallest specimen is matured already.

Tentacles are very numerous, evidently more than one hundred and probably attaining to 120. The ciliated groove is simply C-shaped opening anteriorly (figs. 22–23), the dorsal ganglion is situated just near the ciliated groove, and the dorsal lamina is ribbed except for the anterior beginning portion, but ribs are never projected out distally beyond the margin of the lamina. There are 37 internal longitudinal vessels on each side in the 26 mm long specimen and about 30 ones in the 17 mm long specimen, and about 110 transverse vessels in the former and about 70 ones in the latter. Plications are roughly as many as internal longitudinal vessels. About 12 stigmata on each plication. Papillae (fig. 24) at crosses between the transverse and longitudinal vessels are of the usual shape, but rather small; intermediate papillae are practically absent, though found exceptionally at some places.

9. Ascidia sp. (Fig. 3, 25–26)

A single specimen, 47 mm long, 24 mm wide, and 12 mm in thickness; attached to the substratum by the left side. Siphonal areas are each swollen to a dome-shaped prominence (fig. 25), the branchial aperture terminal and the atrial roughly at the level of the anterior one third, the latter opens toward the right side. The test is hard gelatinous, yellowish white slightly with a grayish tint, translucent, smooth on the surface, and quite free from any foreign matters. The mantle body is heavily mutilated, probably because the specimen was preserved long after it died. The right side of the mantle body is wholly (?) reticulated with a dense musculature. The atrial siphon is short and situated with its posterior base at the middle of the mantle body.

The oesophagus opens to the branchial sac near the posterior end of the body. Branchial tentacles are found strongly coiled, the ciliated groove is U-shaped (fig. 26), but narrow and deep. The dorsal ganglion is apart from the groove by two times the ganglion length, the dorsal lamina is ribbed. No plications are found on the wall of the branchial sac. Stigmata are rather long, about one stigma in each mesh formed by internal longitudinal and transverse vessels; parastigmatic vessels are present and thus there are intermediate papillae.

Remarks: The partial structure of the branchial sac, the existence of only a single stigma in a mesh and of parastigmatic vessels and then intermediate papillae, seems to be unique. If these are true in perfect specimens, then these will be enough to

establish a new species. But, at present, these features are seemingly not decisive, as the mantle body is quite mutilated. The distribution of Ascidia ceratodes (Huntsman) covers a wide range of the west coasts of North, Central, and South Americas, and of course the Pacific coast of Costa Rica is included within. Remarkably swollen siphonal areas of the test body of the present specimen seem rather peculiar, but the body appearance of A. ceratodes is known to be very variable. The short atrial siphon is issued from near the middle of the mantle body in the present specimen, so that there is no essential difference in the relative situations of the branchial and atrial siphons between the present specimen and A. ceratodes. If the internal structure of any perfect specimens of the present test appearance is found in future to conform to that of A. ceratodes, somewhat strange appearance of the test body of the present specimen must be regarded as taxonomically insignificant.

10. Symplegma pizoni nom. nov. (Fig. 4, 27–29)

Protobotryllus viridis, Pizon 1908. Botryllocarpa viridis, Hartmeyer 1909.

A single colony in the material. It is $35 \text{ mm} \times 25 \text{ mm}$ in extent and about 1.5 mm in thickness; pale grayish orange in colour shortly after preservation, but later faded to pale yellow. Zooids are arranged partially more or less linearly as in species of *Botrylloides*, but never forming any definite systems. They are embedded wholly in the common test, obliquely or nearly perpendicularly, and both apertures open directly to the exterior.

Zooids are 3 mm long in a contracted state, very probably 4 mm or more when alive; the branchial aperture is circular and quite sessile, while the atrial aperture is round, too, but opens on the small short siphon issued from the dorsal side slightly anterior to the middle; no atrial tentacles are definable, probably because of strong contraction. The frontal side of the zooid facing the colony surface is elliptical in outline. The posterior end of the branchial sac reaches the rear end of the body. Tentacles are 6. There are eleven stigmatal rows on each side, the second row does not reach the dorsomedian line. Stigmata are arranged near the middle of the branchial sac between three internal longitudinal vessels as follows:

The anterior end of the intestinal loop attains to the 5th transverse vessel (fig. 27), the anus is plainly margined and slightly projected out from the point of attachment to the 6th transverse vessel. The second intestinal loop is very prominent, very deep, and as wide as the first loop, its axis passes through the cardiac half of the stomach. The oesophagus opens to the stomach near the posterior end of the body. The stomach occupies about two thirds of the ventral branch of the first intestinal loop and is

furnished with 11–13 longitudinal plications and a prominent pyloric coecum, the tip of which is connected with the intestine by a vessel which is forked shortly after it leaves the coecum (fig. 28). A single gonad is situated on each side just along the endostyle, between the first transverse vessel and the middle of the 6th stigmatal row on the left and between the second and 5th transverse vessel on the right side. Each gonad (fig. 29) consists of an ovary and a testis, the latter is divided antero-posteriorly into a pair of testicular lobes which are each subdivided into several lobules and the former is situated between the pair of testicular lobes; the vas deferens is long and thin. So far as the examined zooids are concerned, no more than three large egg cells are found in the ovary.

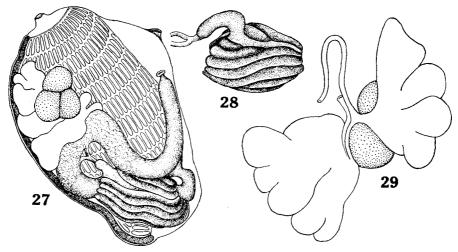


Fig. 4. Symplegma pizoni nom. nov. 27: zooid, left side. 28: stomach. 29: gonad, exposed surface.

Remarks: The general structure of zooids of the present specimen resembles closely that of botryllids, as zooids are rather standing and provided with three internal longitudinal vessels on each side of the branchial sac. The direct opening of the atrial aperture to the exterior is known in Botryllus primigenus Oka, 1928, though this might be only a passing phenomenon in the course of asexual reproduction. However, a closer examination revealed that the gonad is constructed just the same as in Symplegma. Morphological differences between the present specimen and general species of Symplegma are only the existence of merely three internal longitudinal vessels on each side of the branchial sac and somewhat standing feature of zooids in the present specimen; the latter is, however, met with frequently in some Symplegma colonies growing in some limited spaces and cannot be a taxonomically significant feature. The point must be whether or not the existence of only three internal longitudinal vessels in place of four vessels can be worthy to define a separate genus which was

named originally Protobotryllus by Pizon in 1908 but amended by Hartmeyer in 1909 to Botryllocarpa. As seen for instances in the genera, Polyzoa and Metandrocarpa, the number of internal longitudinal vessels is never strictly fixed in respective genera. As the morphology of the gonad seems taxonomically much more significant than the decrease of internal longitudinal vessels by only one, it may be very reasonable that the diagnosis of the genus Symplegma is extended to include Botryllocarpa (=Protobotryllus Pizon) by admitting the existence of three internal longitudinal vessels. Further, as the number and morphology of the gonad are fundamentally very constant throughout the known species of Symplegma, the present specimen with the gonad of the Symplegma-type is better admitted as a species of this genus than be attributed to any other genus. As to the specific identification, the present specimen resembles so closely Protobotryllus viridis Pizon from Amboina that the former is identified at present tentatively with the latter. Zooids of Pizon's species are 4 mm long and bear 10 stigmatal rows, the second row never reaching the dorso-median line, three internal longitudinal vessels on each side, 12-14 plications on the stomach, and testicular lobes subdivided into a few to several lobules. They are lying but not standing in the colony, and provided with distinct atrial tentacles. The only prominent difference between the present specimen and Pizon's species is the existence of distinct atrial tentacles in the latter, but such fine tentacles will easily become undefinable in a strongly contracted state; thus this difference seems to be of a minor significance. Then the present specimen would be treated as Symplegma viride (PIZON), if the specific name were not preoccupied by Herdman in 1886 for the form from Bermuda. It is now requested to give a new name for Pizon's species to include it in the genus Symplegma, and here pizoni nom. nov. is proposed for viridis.

By those who are of the opinion to separate the group of Botryllus and Botrylloides as a family or subfamily distinct from other styelids, the group has been characterized by the formation of the common cloacal canal system, the structure of the gonad, and the mode of budding; in addition the existence of no more than three internal longitudinal vessels on each side of the branchial sac is generally given in the diagnosis of the group. The last feature is shared with some species of Polyzoa, the budding is essentially the same as in Symplegma, and the structure of the gonad is so variable in so-called social styelids that only the structure of gonad in the group of botryllids can not be regarded as of a taxonomical importance. Then the only feature characterizing the group of botryllids must be the formation of the common cloacal canal system; namely the indirect opening of the atrial aperture to the exterior. In Synoicidae, Sigillinaria and Euherdmania bear the atrial aperture opening separately; and in Polycitoridae, Hypodistoma has the atrial siphon issued from the posterior part of the thorax and opening to the exterior indirectly in contrast with *Eudistoma*, related very closely to Hypodistoma. Although there may be some doubt to quote the instances in remote Aplousobranchia to apply them to explain some relationship in Stolidobranchia, it seems to me now very difficult to draw a boundary of family between the

group of botryllids and *Symplegma*. Botryllids should be included in Styelidae. This does not mean that no further subdivision of this family is needed. At the present level of my poor knowledge about the compound and social styelids, I can but follow Ärnbäck, Michaelsen and others only traditionally to retain the separation of botryllids as a subfamily and feel vaguely a necessity of drawing some other boundary or boundaries to separate the forms, or some of them, without any branchial plications but only small numbers of internal longitudinal vessels from the forms with distinct branchial plications, from a quite different point of view.

Two colonies are found in the material, one is a 50 mm × 30 mm colony encrusting a cylindrical pebble and the other is a small 17 mm × 10 mm piece. The colonies shortly after preservation (in April, 1970) were coloured reddish orange, with a deeply coloured patch on each side of respective apertures as seen in fig. 30, but were faded into pale purplish gray in the end of July, 1971, when they were dissected. This colouration is due to the mantle, as the test itself is rather translucent and without any special colouration; it is somewhat cartilaginous, and 0.2 to 0.35 mm in thickness on the dorsal side of zooids, but thinner on the ventral side. Zooids are connected with one another by thick test substance so that they look embedded perfectly in the common test, the outline of respective zooids being never defined by the test morphology. Respective zooids are oval, up to 7 mm in length, 5 mm in width, and to 2 mm in height. Apertures are sessile, elongated laterally to form each a transverse slit in a contracted state, and situated to divide the body length into three, the branchial being situated in the narrower part of the body.

The mantle is very thin in the ventral half and bears several endocarps on the inner surface of the dorsal half. Tentacles are very irregular as seen in fig. 32, this might be partly due to strong and irregular contraction of this portion. The ciliated groove is a simple longitudinal slit. Four plications are found on each side of the branchial sac, internal longitudinal vessels are arranged in a 7 mm long zooid as:

Transverse vessels are alternating with parastigmatic vessels in the posterior part; two to five stigmata in a mesh, but up to 6–10 in larger meshes along the endostyle or the dorsal lamina. The loop of the alimentary canal is rather small (fig. 31), its anterior end never reaching the middle of the body. The stomach is oval, slightly longer than a half of the length of loop, and provided with 17 plications, exclusive of a typhlosole, and a comparatively small pyloric coecum which is connected with

the proximal part of the glandular area of the intestine by a vessel issued from the subterminal of the coecum. The glandular area occupies the proximal portion of the distal branch of the loop and coloured orange. The anus is plainly margined and opens at the level of about the middle of the intestinal loop and approximately the level of posterior one sixth of the branchial sac. Up to seven gonads on each side. The gonad consists of two testicular lobes on the side of attachment and the ovary on the exposed side, eggs are about 200μ in diameter.

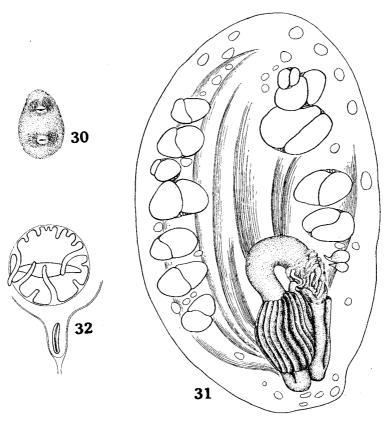


Fig. 5. Polyandrocarpa (Eusynstyela) tincta (VAN NAME). 30: zooid, dorsal. 31: zooid, ventral. 32: tentacular ring and ciliated groove.

Remarks: The present specimen conforms to P. tincta from the West-Indies in the feature of zooids embedded wholly in the common test and the outline of respective zooids being undefinable by the test morphology, and in the shape of the stomach which is not so long as in P. gravei Van Name, 1931. In P. floridana Van Name, 1921, respective zooids are defined very easily, as the test substance connecting zooids with one another is rather poor, and much more internal longitudinal vessels are found on the inner surface of the branchial sac.

12. Styela partita (STIMPSON), 1852 (Fig. 6, 33-36)

Two small individuals in the material; the larger one is 10 mm long and attached to the substratum by the whole left side, the smaller one is only 5 mm in length. In the larger individual (fig. 33), the branchial aperture is subterminal and the atrial aperture is situated on the dorsal side, with its posterior margin at the middle; both siphons are very short. The test is cartilaginous, whitish, and rather translucent, the outline of right gonads being defined through the test in the smaller and seen vaguely in the larger individual. It is up to 0.5 mm in thickness, the surface is nearly smooth and encrusted sparsely with fine sand grains, while the inner layer is transparent and

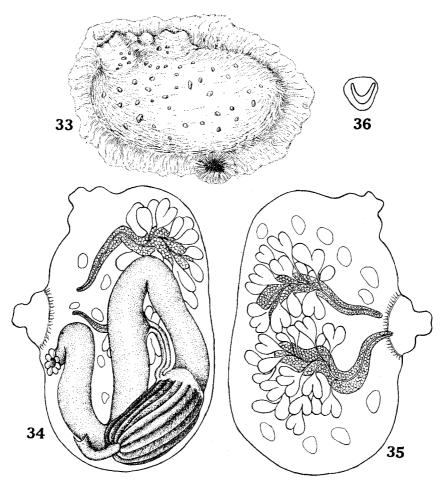


Fig. 6. Styela partita (STIMPSON). 33: 10 mm long specimen. 34: left half of mantle body, internal side. 35: right half of mantle body, internal side. 36: ciliated groove.

adhered so firmly to the mantle that it is very difficult to take the mantle body out of the test. The mantle is whitish, extremely thin in the ventral half, and furnished with endocarps on the inner surface; fine atrial tentacles are defined.

Branchial tentacles are 30 or more in larger individuals including large and small ones, but excluding minute ones. The ciliated groove is simply U-shaped (fig. 36). Internal longitudinal vessels of the branchial sac are arranged in the 10 mm long individual as:

Transverse and parastigmatic vessels alternate regularly, about 4 stigmata in a mesh, but up to 6 in larger meshes along the endostyle.

The alimentary canal (fig. 34) forms double loops, the second loop is as deep as the first and its axis passes through the cardiac end of the stomach; the anterior end of the first intestinal loop reaches the level of the anterior one fourth. The stomach is elliptical, shorter than a half of the ventral branch of the first loop and provided with about a dozen longitudinal plications on the exposed right side; no pyloric coecum is formed but a stout band including a vessel within it is connecting the inner pyloric corner of the stomach to the dorsal branch of the first loop at the level a little anterior to the middle. The anal margin is cut into several lobes.

Two gonads on each side, the ventral half of each elongate and sinuous ovary is surrounded by 6 (in smaller individual) to 10 (in larger individual) testicular follicles (fig., 35), some of which are bilobed or trilobate; gonads are already matured in the 5 mm long individual.

Remarks: A couple of small specimens were already collected by Van Name at To-bagilla Island, the Bay of Panama (Van Name 1945, p. 293). The present two specimens are also very small and yet they are fully matured. Probably, lesser body size may be a character general to the specimens occurring in the waters of higher temperature.

There are twelve specimens in all. It is somewhat difficult to distinguish the branchial siphon from the body proper very distinctly in every specimen, then the body length is given mostly inclusive of the branchial siphon extending anteriorly from the anterior end of the body. The smallest specimen is 13 mm in length, exclusive of the 7 mm long branchial siphon (fig. 43), while the largest one is 39 mm in length, 19 mm in width (exclusive of the short atrial siphon) and 15 mm in lateral thickness (fig. 37). The body is somewhat elongated and oval in shape. Siphons are rather prominent. The atrial siphon may be as long as the branchial siphon (fig. 38), but

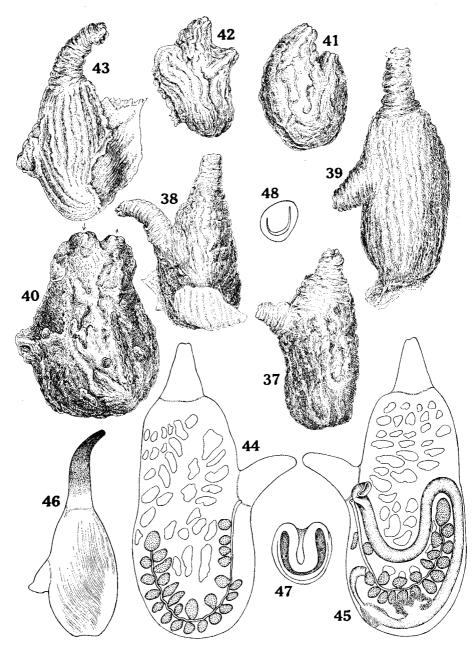


Fig. 7. Pyura sp. aff. vittata (STIMPSON). 37: 39 mm long specimen. 38: 37 mm long specimen. 39: 32 mm long specimen. 40: 31 mm long specimen. 41: 29 mm long specimen. 42: 24 mm long specimen. 43: 13 mm long (exclusive of branchial siphon) specimen. 44: 39 mm long specimen, right half of mantle body, internal side. 45: the same, left half of mantle body, internal side. 46: 13 mm long specimen, mantle body, right side. 47: 39 mm long specimen, ciliated groove. 48: 13 mm long specimen, ciliated groove.

usually is shorter than the branchial, and generally is issued from the dorsal side of the body, with its posterior base at the middle (figs. 37–39, 42, 43). In some specimens (figs. 40, 41), the atrial siphon may be directed anteriorly and the atrial aperture may come near the branchial aperture as seen in fig. 40. The animal attaches to the substratum generally by the left side, but by the right side or by the right posteroventral side in a few specimens. The test is hard, leathery and rather thin, less than 1 mm in thickness even in the largest specimen, though exceptionally attaining to 2 mm near the place of attachment in some specimens. The surface is fouled in some specimens by didemnids, sponges and other organisms; in somewhat contracted preserved specimens it is usually creased longitudinally and in some specimens transversely, too. The test is mostly yellowish with a brownish hue, especially on siphons, in a greater or less degree, but in some, rather reddish brown on the surface; the section is whitish; its inner surface is usually white and slightly glistening, but in a few specimens faintly with a greenish tint. In spite of repeated examinations, no spinules were found in the distal part of the inner surface of respective siphons. They might be extremely minute or limited very strictly to the end. The mantle is yellowish white and rather thin, as the whitish muscle fibres are comparatively thin and set very densely (fig. 46); siphons are prominent and coloured yellowish orange to reddish orange, deeply toward the tip; the atrial situated with its posterior base at the anterior one third to the middle. There are many endocarps of a medium size over the inner surface of the mantle.

Tentacles are 20 (in the smallest specimen) to 25–30 (in larger specimens) exclusive of minute ones, larger and smaller ones are arranged roughly alternately; branches in two orders, one or two small branches of the size of the second order may be issued from the tentacle stem in some internodes. The ciliated groove (figs. 47–48) is simply U-shaped throughout the specimens. Six branchial plications on each side. Internal longitudinal vessels are arranged as follows:

13 mm (body proper) long specimen

```
Left D 3 (17) 1 (6) 2 (11) 2 (9) 2 (7) 1 (4) 0 V
Right D 3 (16) 1 (8) 2 (12) 2 (12) 2 (8) 1 (4) 1 V
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31 mm (inclusive of the branchial siphon) long specimen

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Left D 3 (19) 3 (17) 4 (18) 5 (18) 4 (13) 4 (8) 3 V
Right D 4 (14) 4 (16) 4 (19) 5 (17) 5 (15) 5 (7) 3 V
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37 mm (inclusive of the branchial siphon) long specimen

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Left D 5 (20) 4 (11) 3 (15) 4 (11) 5 (7) 4 (5) 3 V
Right D 5 (18) 4 (11) 5 (12) 5 (12) 6 (7) 4 (6) 4 V
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39 mm (inclusive of the branchial siphon) long specimen

Left D 4 (23) 3 (14) 4 (18) 4 (15) 5 (10) 4 (7) 3 V Right D 6 (21) 3 (16) 4 (18) 4 (18) 6 (11) 5 (9) 4 V.

Naturally the arrangement of transverse vessels differs according to the specimen size. In the smallest specimen, thicker and thinner vessels alternate regularly or three thinner ones between each pair of thicker ones, parastigmatic vessels are absent, and 1–3 small stigmata in a mesh. In a 31 mm long specimen, parastigmatic vessels are found in some parts of the branchial sac and vessels are arranged as 1 p 3 p 2 p 3 p 1 where the numerals represent the order of the vessel thickness and p indicates a parastigmatic vessel; 4–5 stigmata in a mesh. In the largest specimen, however, parastigmatic vessels are again undefinable and their sites are taken by fine vessels of the 4th order of thickness; 3–4 small stigmata in a mesh.

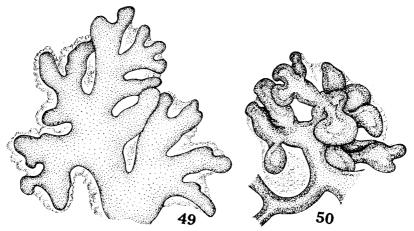


Fig. 8. *Pyura* sp. aff. *vittata* (STIMPSON), hepatic lobules of 37 mm long specimen, ×47. 49: lobules from cardiac lobe. 50: lobules from pyloric lobe.

The anterior end of the intestinal loop reaches the level of the posterior base of the atrial siphon (fig. 45), where the anus opens. The second loop is very distinct, its bottom at the level of the anterior one third of the posterior half of the body posterior to the atrial siphon, and its axis passes through the middle of the pyloric liver mass. The oesophagus opens to the stomach near the posterior end of the body, thus the first intestinal loop is very spacious. The gastric region is thick only at the cardiac end, but immediately becomes thinner and nearly as thin as the intestine near the pyloric end. The liver consists of the cardiac mass (fig. 49) fringing the anterior and posterior sides of the cardiac end of the gastric region and the pyloric mass (fig. 50) occupying the anterior side of the pyloric end of the gastric region. The cardiac mass consists of hepatic lobules which are rather thick, less dense, and greenish; the wall of lobules seems a little thicker, as the internal feature of lobules cannot be

seen through. The pyloric mass, on the other hand, consists of lobules which are a little smaller, somewhat rounded distally, very dense, and slightly yellowish or brownish; the wall of lobules seems a little thinner, as the wall thickness can be seen through. Hepatic lobules are enveloped by a soft frothy tissue in both masses.

A single gonad on each side, consisting of a chain of roundish genital capsules as in usual pyurids (fig. 44). There are 34 (right)—35 (left) capsules in a 31 mm long specimen, 18 (left)—21 (right) capsules in a 37 mm long specimen, and 20 (left)—26 (right) capsules in the largest specimen. The gonads in a 29 mm long specimen are fully matured.

Remarks: Several individuals are described above somewhat in detail, this is because to present some fundamental data for further taxonomic studies of P. vittata. The principal morphology of the present specimens conforms nearly completely to that of the forms generally treated under the specific name of vittata. However, I cannot understand properly the existence of so many endocarps over the inner surface of the mantle in the present specimens. Further, I have failed to find out any spinules on the inner surface of the distal part of siphons in the present specimens. As stated already by Van Name (1945, p. 325), I cannot wipe away from my mind the question whether more than one species is not being confounded in P. vittata. The number and arrangement of endocarps might vary with the size of specimens or according to the environmental conditions. Until this is confirmed by actual observations, I believe that the decisive identification of the present specimens with P. vittata should be left pending. Thus, they are treated here tentatively as a species affined to P. vittata. I don't like at present to establish a new species for them.

14. Pyura lignosa Michaelsen, 1908

(Fig. 9, 51–64 and Fig. 10, 65–70)

There are four specimens in the material; the smallest is a 11.5 mm long, nearly spherical specimen (fig. 51) attached to the substratum by the ventral side, while other three are all somewhat elongate and attached to the substratum by the ventral, right ventral, or by the whole left side; the largest specimen is 47 mm long, 22 mm wide (dorso-ventral), and 14 mm thick laterally (fig. 54). In the smallest specimen, the apertures are sessile and each definable, with some difficulty, by four small lobes; the branchial aperture is terminal and the atrial is situated at the middle of the dorsal side. The test is leathery, translucent and rather thick, attaining nearly to 1 mm in the posterior portion; the inner layer is whitish, soft, but very tough; the surface is divided into a number of small oval and pale brownish red areas (fig. 51) which are each encircled by white joint and become horny around the centre which may be slightly raised in some areas. In other three specimens, the siphons are very short (figs. 52–54), but the apertures are defined easily by many small protuberances sur-

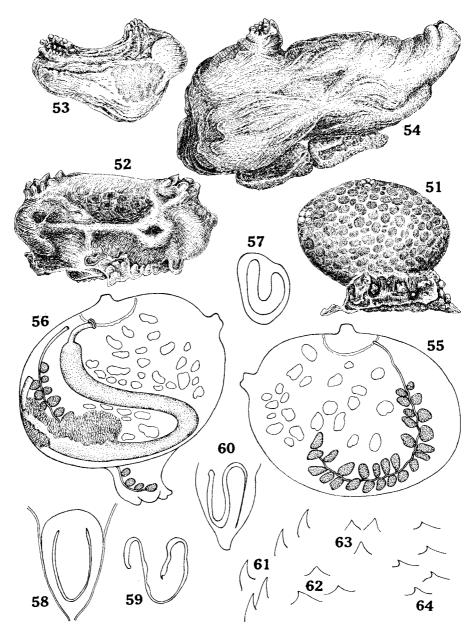


Fig. 9. Pyura lignosa Michaelsen. 51: 11.5 mm long specimen. 52: 24 mm long specimen. 53: 33 mm long specimen. 54: 47 mm long specimen. 55: 11.5 mm long specimen, right half of mantle body, internal side. 56: the same, left half of mantle body, internal side. 57: the same, ciliated groove. 58: 24 mm long specimen, ciliated groove. 59: 33 mm long specimen, ciliated groove. 60: 47 mm long specimen, ciliated groove. 61: spinules on the inner surface of the distal part of siphon, 11.5 mm long specimen. 62: same spinules, 24 mm long specimen. 63: same spinules, 33 mm long specimen. 64: same spinules, 47 mm long specimen. (61–64: ×440).

rounding them; the branchial aperture is nearly terminal, while the atrial is subterminal near the other end. The test is leathery, very hard, and rather thick, generally attaining to 1 mm, or to 2 mm or more in the posterior part of the largest specimen. The test surface is corrugated complicatedly, but longitudinal grooves or folds are more dominant. The scaly structure which is found typically on the smallest specimen is retained distinctly but partially on the 24 mm long specimen (fig. 52), but completely disappeared on the 33 mm and 47 mm long specimens (figs. 53–54). The test colour is reddish orange in the 24 mm long specimen, but yellowish brown or reddish brown in other larger specimens. The section of test is whitish, and the inner surface of the test is whitish and glistening. Spinules on the inner surface of the distal part of siphons are small, but distinct; about 20μ long on an average in the smallest specimen (fig. 61), about 9μ long in the 33 mm long specimen (fig. 63), and much smaller in 24 mm and 47 mm long specimens (figs. 62, 64).

On the mantle body (figs. 65–70), both siphons are distinct, though they are not remarkable in length, but in the 33 mm long specimen (figs. 67, 68). The mantle is thin in the smallest specimen, but rather thick and muscular in larger specimens; muscle fibres running obliquely and posteriorly from the antero-dorsal part of the body (thus, roughly converging to the branchial siphon) are the strongest and most prominent ones on the surface, while in the inner layer of the mantle, muscle fibres roughly converging to the atrial siphon are prominent. The mantle is whitish to yellowish on the body proper and faintly orange or deeply so at the tip of siphons. A number of small endocarps are scattered over the inner surface of the mantle in the smallest specimen (figs. 55, 56), but in three larger specimens they are found limitedly along the alimentary canal on the left side (figs. 66, 68, 69) and none or only a few to several ones mainly along the dorsal side of the gonad on the right side (figs. 65, 67, 70). The atrial velum is distinct.

Branchial tentacles are 22–24 in two smaller (11.5 mm and 24 mm long) and 32–35 in two larger (33 mm and 47 mm long) specimens, exclusive of minute ones, generally or at least partially larger and smaller ones are alternating. They are slender, though the stem membrane is rather prominent, and branches are in 2 (in two smaller specimens) or 3 (in two larger specimens) orders; branches of the last order are always very small and sparse, a few to several of them being issued from some internodes of the stem. The ciliated groove is simply U-shaped in two smaller specimens (figs. 57, 58), but one or both horns curl in or out in two larger specimens (figs. 59, 60). Dorsal languets are rather slender. Six branchial plications on each side, internal longitudinal vessels are arranged as follows:

11.5 mm long specimen

```
Left D 1 (15) 1 (10) 2 (12) 2 (12) 2 (8) 2 (5) 1 V
Right D 3 (14) 1 (11) 2 (14) 2 (14) 2 (12) 2 (6) 1 V
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24 mm long specimen

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Left D 1 (18) 1 (12) 2 (15) 3 (13) 1 (11) 1 (9) 1 V
Right D 1 (15) 2 (13) 1 (16) 2 (15) 2 (13) 2 (12) 2 V
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33 mm long specimen

47 mm long specimen

Transverse vessels are arranged generally as thick p thin p thick, p represents a parastigmatic vessel, and 3-4 stigmata in a mesh in the smallest specimen. In three larger specimens, vessels are arranged variably according to different parts of the branchial sac, as thick p thin p thick, thick p thin p medium p thin p thick, or seven thinner vessels and some parastigmatic vessels between each pair of thicker ones; there are 4-5 to 7-10 stigmata in a mesh.

The anterior end of the intestinal loop reaches anteriorly the middle of the anterior half of the body in the smallest specimen (fig. 56), but comes near the level of the posterior base of the branchial siphon and thus the loop becomes very elongated in larger specimens (figs. 66, 68-69). The second loop is shallow and its axis passes through the cardiac end of the gastric region to the middle of the oesophagus. The anus is plainly margined, but in the 33 mm long specimen the margin is cut into several lobules (fig. 68). The liver consists of the cardiac and pyloric masses, the former is composed of two lobes respectively bordering the dorsal and ventral margins of the cardiac portion, while the latter occupies the dorsal side near the pyloric end of the gastric region, at about the middle of the ventral branch of the first intestinal loop, and may be clefted into a few to several lobes in larger specimens. No differentiation is found between the cardiac and pyloric masses in the smallest specimen, but in three other larger specimens, hepatic lobules of the cardiac lobes are elongate and those of the pyloric lobes are shorter and smaller, thus the former bears superficially a kind of folded appearance, while the latter a granulated appearance. In the 33 mm long specimen, the cardiac lobes are divided into 4 smaller ones along the dorsal side and into 2 larger ones along the ventral side of the cardiac portion of the gastric region. (fig. 68).

The morphology of gonads is principally the same as in the preceding species; the only character noted may be the abundance of genital capsules, which are 24 (left) to 28 (right) even in the smallest specimen, up to 51 (right) in the 24 mm long specimen, to 55 (right) in the 33 mm long specimen, and to ca. 60 (left) to 64 (right)

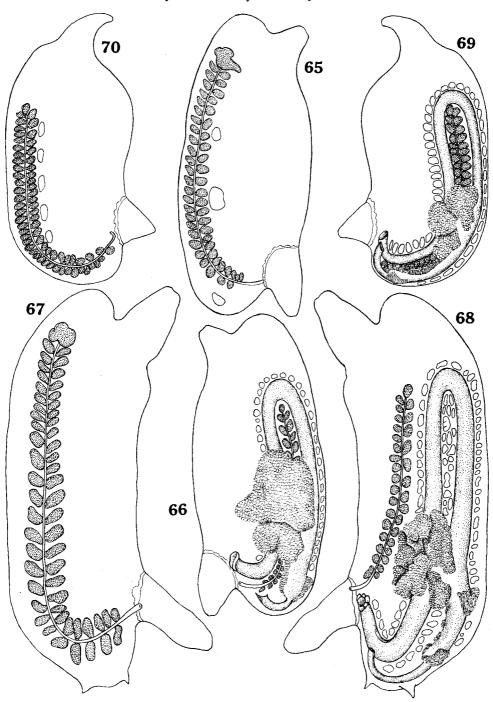


Fig. 10. Pyura lignosa Michaelsen. 65: 24 mm long specimen, right half of mantle body, internal side. 66: the same, left half of mantle body, internal side. 67: 33 mm long specimen, right half of mantle body, internal side. 68: the same, left half of mantle body, internal side. 69: 47 mm long specimen, left half of mantle body, internal side. 70: the same, right half of mantle body, internal side.

in the largest specimen. The distal capsule may be larger than others in some specimens (figs. 65, 67) and the distal end of some genital capsules may be extended into a single or two small elongate endocarps. The 33 mm long specimen shows a very peculiar anomaly in the situation of the left gonad which is stretching outside the intestinal loop along the dorsal branch of the loop (fig. 68). In the smallest specimen, the bottom part of the first intestinal loop is extended out a little and then the distal portion of the left gonad is included in this extension (fig. 56); this morphology is nothing but only an anomaly, too.

Remarks: It is evident that the present specimens conform to Van Name's specimens smaller than 32 mm in length (Van Name 1945, pp. 337–338). The 33 mm and 47 mm long specimens in the present material are devoid of any tesselated state, but the 24 mm long specimen bears partially such a state as shown by Van Name's specimens, and the smallest specimen is wholly squamous. The tesselated condition in younger stages seems to vanish with the growth and becomes invisible in specimens larger than about 30 mm in length. The number of internal longitudinal vessels in larger specimens of the present material seems to be an intermediate between those of Van Name's and Michaelsen's specimens.

A 4.1 mm long tesselated pyurid described from Philippine waters (Tokioka 1970, pp. 103–106) as a species affined to *lignosa* Michaelsen is very possibly an extremely young specimen of this species, as it resembles closely the smallest of the present specimens. If this is acceptable, then the distribution of endocarps may be changed with the growth, very scarce in extremely small specimens, then increased and scattered all over the inner surface of the mantle in small specimens, and lastly much decreased in larger specimens, but leaving those along the alimentary canal. Further, it may be said safely that *Pyura lignosa* occurs throughout the tropical Pacific.

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