TWO NEW SPECIES AND ONE POSSIBLY NEW RACE OF SOCIAL STYELIDS FROM SAGAMI BAY, WITH REMARKS ON THEIR LIFE HISTORY, ESPECIALLY THE MODE OF BUDDING[1]

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With 10 Text-figures

The first author has reared three prominent social styelids in these years on glass slides at the Shimoda Marine Biological Station of Tokyo Kyoiku University to make a series of comparative observations of their sexual and asexual reproduction. These ascidians seemed to him to be quite new faces in the Japanese waters and then they were submitted to the second author for taxonomical identification, together with their behavioural data. As the general morphology of social styelids is usually very similar among species of respective genera, minor or very exact characters are widely adopted as specific criteria. And the real usability of some of such minor characters might become very clear, if they are checked together with some behavioural characters. In hoping this, the two authors agreed with each other to make both the morphological and behavioural descriptions to define the above-mentioned three ascidians taxonomically. Checking these carefully, they have come to the conclusion that all the three social styelids described in this paper should be new to science.

Before going to the descriptions, the authors want to present their sincere thanks to Dr. Masashi YAMAGUCHI of the Faculty of Agriculture, Tokyo University and Mr. Hifumi UEDA of the Shimoda Marine Biological Station, Tokyo Kyoiku University for their kindness in submitting the interesting specimens to the authors.

Polyzoa vesiculiphora TOKIOKA, 1951

race *2l-5r* nov.

(Figs. 1-3)

Ref.: *Polyzoa vesiculiphora*—TOKIOKA (1951), Publ. Akkeshi Mar. Biol. Stat., 1, pp. 11–12, fig. 9; Pl.
       II, 1–2.
       Polyzoa pacifica—TOKIOKA (1951), Publ. Seto Mar. Biol. Lab., 1 (4), pp. 173–175, fig. 4.
       (1962), Publ. Seto Mar. Biol. Lab., 10 (1), pp. 15–16: Pl. III, 31.

A kind of styelid social ascidian which was collected in 1970 by Dr. Masashi
YAMAGUCHI in the vicinity of the Misaki Marine Biological Station, Tokyo University,
has since been reared continuously on glass slides by the first author at the Shimoda
Marine Biological Station of Tokyo Kyoiku University. A slide was picked out from
each of the summer and winter stocks for morphological examination.

Morphology: So far as the examined specimens are concerned, zooids are roughly
ovoid in shape (Fig. 1 a, b), up to 5 mm long, 3.6 mm wide and nearly as high as
wide, and are attached to the glass slide surface by the left ventral side; namely they
are lying rather than standing. Many stolons are issued from the periphery of re-
spective zooids and some of them are found forming new zooids, but they are always
cut off before any complicated network of stolon is formed. Zooids are nearly colour-
less and translucent or faintly yellow-brownish in both living and preserved specimens.
The test is thin, rather gelatinous, and adheres firmly to the mantle; its surface is
usually covered wholly with minute hydrozoan polyps and some other organisms
and thus shows somewhat a hairy appearance. The branchial siphon is subterminal,
and the atrial is situated posteriorly on the dorsal side, with the posterior base just
at the middle; both siphons are short and both apertures are 4-lobed (Fig. 1 b).

The mantle body can be taken out of the test with some difficulty. The mantle
itself is thin. A vessel to the test is issued from the postero-ventral side of the first
intestinal loop, but ramified into a few branches just after it leaves the mantle surface.
The branchial siphon is encircled with 18–20 sphincters. A large elongate vesicular
endocarp, about $500\,\mu$ long and $150\,\mu$ wide in an extended state, is found on the inner
surface of the mantle on each side.

Branchial tentacles are seemingly arranged in typical cases as 6 large and 6
small ones alternating, with a minute one in interspaces; in preserved specimens some
of small to minute tentacles may be contracted to an indiscernible size. The ciliated
groove is a small oval opening, with the longitudinal axis antero-posteriorly. The
endostyle and a narrow zone along each side of the style look very whitish because
of the accumulation of nephrocytes. Nine stigmatal rows on the left and 8 or 9 rows
on the right side; the 9th row on either side may be rather irregular or rudimentary;
parastigmatic vessels are present. There are 3 inner longitudinal vessels on each side
of the branchial sac. The arrangement of stigmata between longitudinal vessels is:
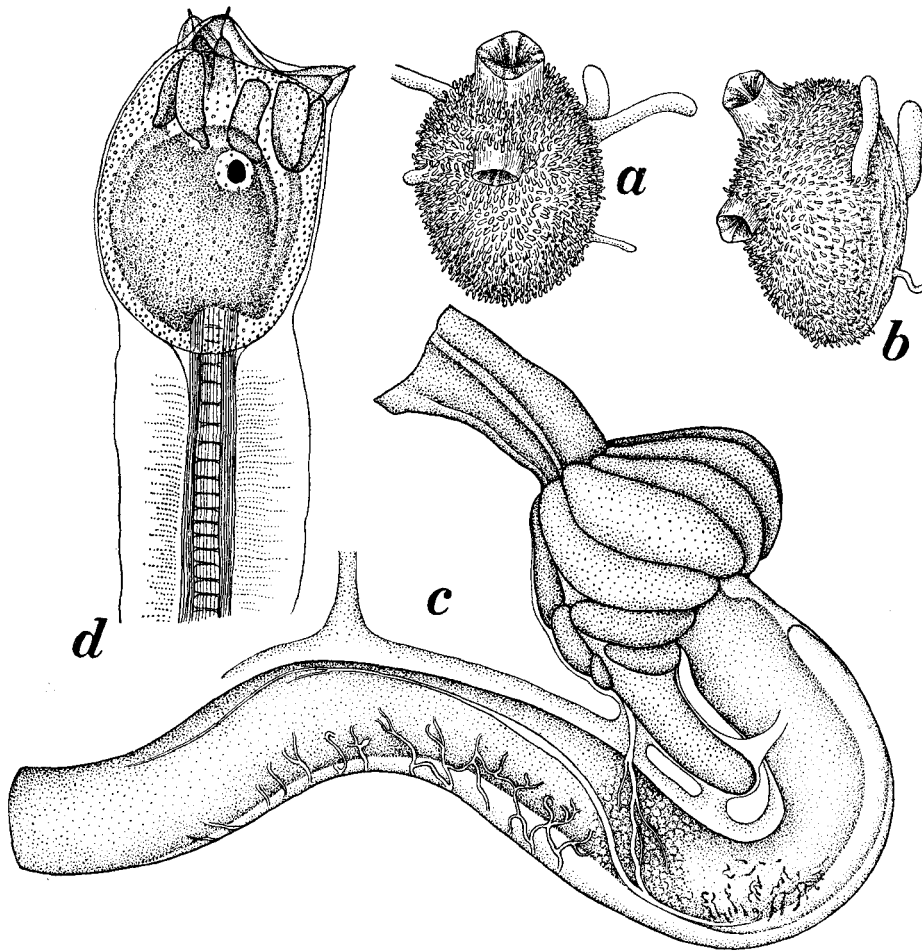
Fig. 1. *Polyzoa vesiculiphora* TOKIOKA, race 2*l*-5*r* nov. a, 4mm long zooid, dorsal. b, the same, right side. c, alimentary canal, left side, × 47. d, anterior half of larva, × 73.

A 4 mm long summer specimen, in the 2nd or 3rd row.

Left V 10 . 6 . 7 . 9 D 9 . 6 . 9 . 10 V Right

A 4 mm long winter specimen, in the 4th row.

Left V 15 . 8 . 8 . 10 D 10 . 10 . 9 . 19 V Right

The anterior edge of the first intestinal loop reaches anteriorly the middle of the 4th stigmatal row to the 3rd transverse vessel; the anus is plainly margined and situated at the 3rd transverse vessel or slightly posteriorly in the 4th stigmatal row. The stomach is seemingly slightly shorter than a half of the intestinal loop in examined specimens, with 12 to 14 plications on the surface and a very prominent elongate

pyloric coecum (Fig. 1 c). The proximal portion of the distal branch of the intestinal loop, just opposite the pyloric coecum, is glandular.

In every mature winter specimen examined by both authors, there are always 2 gonads on the left and 5 ones on the right side; the testis occupies the anterior side of respective gonads and the genital aperture opens generally dorso-posteriorly.

Larvae are found incubated in the peribranchial cavity of the winter specimens. The larval trunk is about 550 μ long and 390 μ wide, and the tail musculature about 950 μ long and 90 μ wide. Three attachment processes are arranged in a triangle and 8 ampullae are seen clearly in the anterior part of the trunk (Fig. 1 d). In very fresh specimens, the anterior part of the trunk inclusive of ampullae is light-yellow, while the posterior part is dark brownish.

Development of oozooid: This species is viviparous. Eggs are approximately 320 μ in diameter. The breeding season is limited to the period from the end of November to the middle of March, with a peak in January, and there is a certain rhythm in the liberation of larvae. The number of larvae released starts to increase at 07:00, reaches the maximum about 11:00, and declines till 13:00. The duration of the free-swimming life varies from 3 hours to 192 hours at 10 °C in a dish placed in the laboratory. Only a small part of larvae released, about 10%, will attach to the substratum. The tail
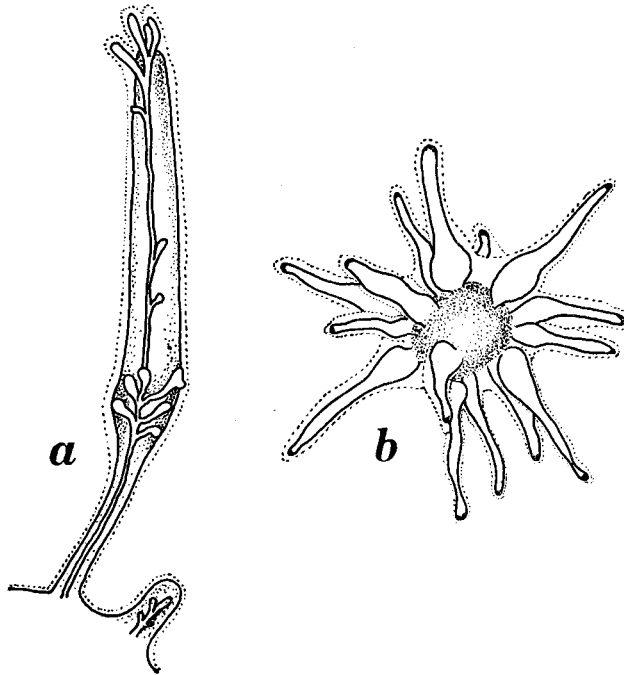

Fig. 2. *Polyzoa vesiculiphora* TOKIOKA, race 2*l*-5*r* nov.   a, two stolonic outgrowths from the right posterior side of a zooid, each together with the underlying vessel; larger one is about 2.5 mm in length, × 30. b, a planktonic bud just liberated from the parental zooid, × 37.
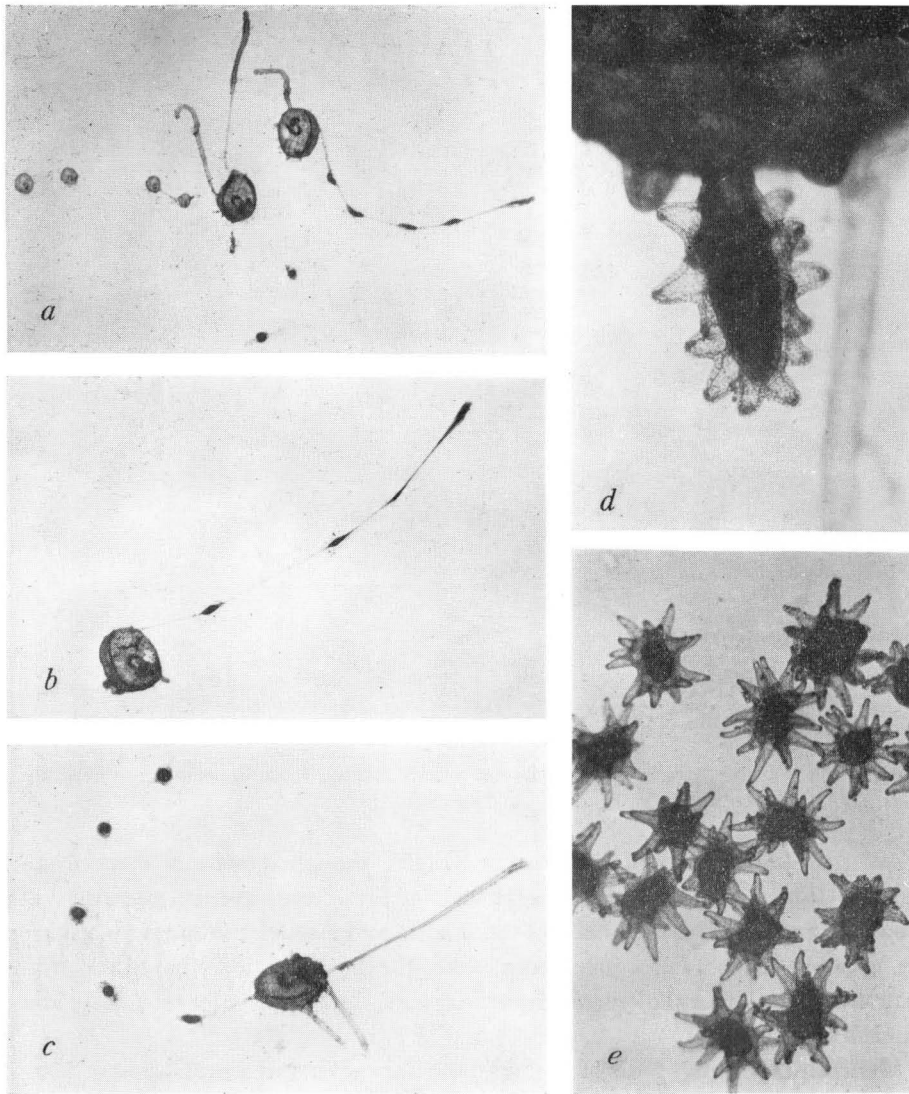
Fig. 3. *Polyzoa vesiculiphora* TOKIOKA, race 2*l*-5*r* nov.   a-c, some stages of stolonic budding. a, × 19. b, × 24. c, × 30. d, a planktonic bud in a later stage, but still connected to the parental zooid, × 36. e, planktonic buds one day after the liberation, × 14.

resorbs in about 15 minutes. The oozooid one day after settling will measure 450 μ in length and 380 μ in width, exclusive of 8 ampullae, and the metamorphosis is completed in 4 days after settling by beginning of heart beating. The attached individual becomes fully functional roughly on the next day of this stage, as the apertures open, about 6 rows of stigmata are definable on each side, and thus feeding becomes possible.

Asexual reproduction: The mode of budding in this species is quite strange as those in
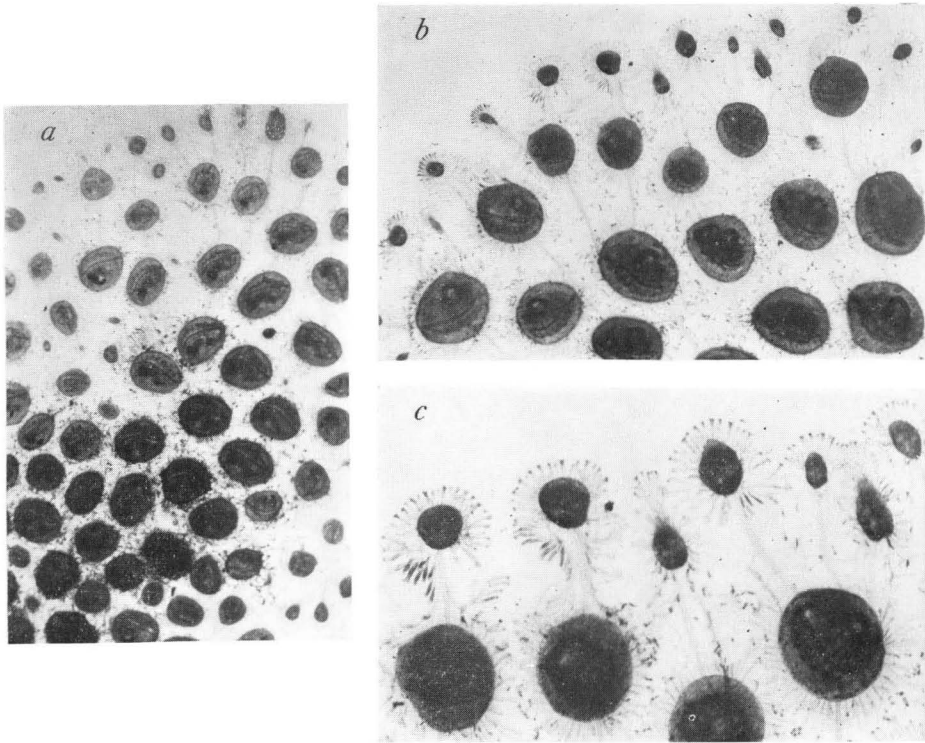
Fig. 4. *Metandrocarpa uedai* n. sp.   a, a part of a single colony, × 15. b, the same, × 27. c, the same, × 48.

Stolonica and *Distomus* stated by Berrill (1949), though it belongs to peribranchial budding. Buds arisen from the lateral wall of the mantle consist essentially of epidermal and peribranchial epithelia of the mantle and a space containing many trophocytes between them. For convenience' sake, the buds asexually produced from the parent are divided morphologically into two types, though they are essentially identical with each other.

Generally speaking, buds of the one type appear first as a protrusion of 100–150 μ long bud-rudiment. In the summer season, the rudiment continues to grow very rapidly in length for 7 to 10 days, till in an extreme case, such a stolonic outgrowth may attain to 18 mm. One (or two) vessel derived from the parent extends between the substratum and the stolonic outgrowth, ending in a few ampullae usually situated beyond the tip of the outgrowth, which is contractile and with a slow movability in all directions, possibly due to the existence of some filamental structure in cells (Fig. 2 a). With the elongation of the stolonic outgrowth, usually 3 to 5 parts of the outgrowth in a series at certain intervals become fastened to the substratum with a few ampullae derived from the vessel underlying the outgrowth, meanwhile the trophocytes are gradually accumulated at these settled parts as well as near the terminal part.

Finally, when a certain mass of trophocytes is accumulated, these parts will be constricted off within 5 to 6 hours so that respective fragments will become to be an isolated bud, 300–550 μ in length (Fig. 3 a-c). The morphogenesis begins with the separation of bud, and in 2 to 3 days the apertures open, then feeding will start. The young zooids 7 to 11 days after the separation usually begin to bear new buds of either of two types on their lateral wall. The buds mentioned above may be termed the "stolonic" type.

Buds of the other type appear first as a 100–150 μ long protrusion just as in stolonic budding. The outgrowth, however, is extremely short as compared with that of the stolonic type and grows in 3 to 4 days to a nearly spherical mass surrounded all over the surface by ampullae varying in number from 11 to 21 and 15 on an average and connected to the parental zooid by only a thin thread of the test substance (Fig. 3 d). The connecting thread will break finally and some of such buds will frequently be carried away by water currents to some distance, generally not so far from the parental zooid, and will fall upon the substratum. The bud proper (Figs. 2 b, 3 e) measures 300–450 μ in diameter and ampullae are 500–600 μ in length. In 2 to 3 days after the attachment to the substratum by some of them, the ampullae begin to diminish gradually their length, the apertures open, and thus the attached individual becomes fully functional. The young zooids 10 to 14 days after the attachment bear usually on the lateral wall new buds of either type. It is proposed here to name this type of budding the "planktonic" one distinct from the "stolonic" type described just before. At present, it is quite unknown about the mechanism that makes a blastozooid take either of the two types of budding. Although every blastozooid gives rise to buds of the two types from any level of the lateral wall on each side, more buds arise on the right than on the left side, for the blastozooid is usually attached to the substratum by the left ventral side.

In budding, the oozooid will remain inactive at least for a few earlier weeks as seen later in *Polyandrocarpa*. About 40-days old oozooid, about 2.5 mm long and 2.0 mm wide and with 8 rows of definable stigmata, can give rise for the first time to an initial bud on the right side in the same manner as in budding blastozooids. Further details of the development of the oozooid and the budding process are now being extensively studied by the first author and his collaborator.

Remarks: The present *Polyzoa* resembles closely the two Japanese species, *P. vesiculiphora* TOKIOKA, 1951 and *P. pacifica* TOKIOKA, 1951, in the general morphology, especially to the former as it agrees in the number of its stigmatal rows, 8 to 9, more exactly with the former (with 9–10 rows) than with the latter (with 12 rows). In *P. vesiculiphora*, the gonads are 3–5 on the left and 6–9 on the right side, while in *P. pacifica* they are 3–5 on the left and 8–11 on the right side; anyhow the number is variable in both species. In the present specimens, however, the number of gonads is strictly fixed as 2 on the left and 5 on the right side. This might be attributed to the fact that all the specimens treated here are exactly the descent of a single clone.

In other words, the existence of 2 gonads on the left and 5 on the right side might be manifested genetically. Or, these specimens might belong to a species distinct from *vesiculiphora* and *pacifica*. However, this seems rather improbable, because the number of gonads varies more or less in some ranges generally in most forms of social styelids exclusive of such special forms as some botryllids, *Symplegma*, *Kükenthalia* and *Gynandrocarpa*, in which there is only a single gonad on one or each side. Thus, it is proposed here to define in *P. vesiculiphora* a distinct race, 2*l*-5*r*, to include the present specimens constantly with 2 gonads on the left and 5 on the right side.

Metandrocarpa uedai n. sp.

(Figs. 4-6)

A colony which was collected in 1970 by Mr. Hifumi UEDA of the Shimoda Marine Biological Station of Tokyo Kyoiku University from the southern rocky coast of Arasidomari Cove, east of Simoda, has since been reared continuously by the first author on glass slides at the Shimoda Marine Biological Station, and a slide of the summer stock was examined for identification.

Morphology: Zooids increasing by successive budding cover the whole surface of the slide, but adult zooids are usually separated, though some ones are connected one another by interzooidal tracts of the test-vessel surrounded by very thin sheet of test substance, extending from the periphery of respective zooids. They are oval, rather flattened dorso-ventrally and attached to the substratum by whole ventral side; generally up to 5 mm in length and 3.6 mm in width, somewhat less high than wide. Both apertures open on the dorsal side; the branchial at the anterior fourth and the atrial at the posterior third, the former is a little larger; both siphons short but distinct (Fig. 5 e). The test is cartilaginous rather than leathery, not so thick and extremely thin on the ventral side of attachment, where the arrangement of gonads is seen through. It looks superficially reddish orange as the mantle colour is seen through, especially more deeply at apertures; the surface is covered by colonies of small hydrozoans and usually finely wrinkled in preserved specimens. The mantle is brilliant reddish orange throughout, but extremely thin on the ventral side. There are several, about 5, small endocarps on the inner surface of the mantle on each side, mainly in the dorsal half. Atrial tentacles were undefinable in preserved specimens.

Branchial tentacles are arranged as 8 larger and 8 smaller alternating. The ciliated groove is a small oval orifice. Perfect stigmatal rows are 8–9 on either side, in addition several imperfect or rudimentary rows; for instance, 9 perfect and 5 rudimentary rows on the left and 8 perfect and 4 imperfect (anterior 1 or 2) or rudimentary (posterior 3 or 2) rows on the right side in an examined 4 mm long zooid. There are 6 inner longitudinal vessels on each side, parastigmatic vessels are present, and stigmata are distributed between the longitudinal vessels as follows:

Left V 6 . 4 . 4 . 3 . 3 . 3 . 4 D 7 . 2 . 3 . 3 . 3 . 4 . 6 V Right.
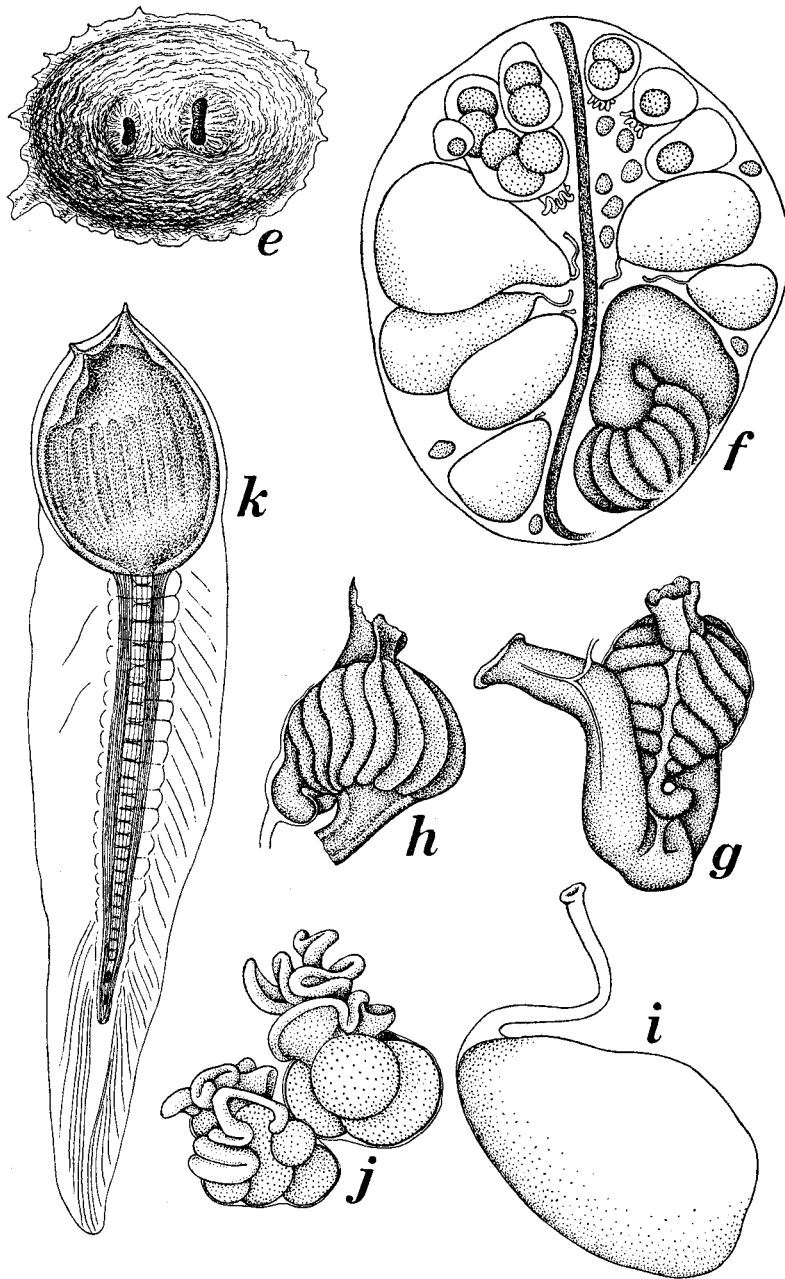
Fig. 5. *Metandrocarpa uedai* n. sp. e, 4 mm long zooid, dorsal. f, the same, ventral side of mantle body. g, alimentary canal. h, stomach. i, testis, × 47. j, two smaller ovaries, × 47. k, larva, left side, × 73.

The anterior edge of the intestinal loop reaches about the middle of the 4th stigmatal row, the anal margin is a little thickened and plain and is attached to the 5th transverse vessel. The stomach is roughly half as long as the intestinal loop (Fig. 5 f) and furnished with 15, inclusive of 3–4 shorter ones ending on the typhlosole, longitudinal plications on the surface and a curved coecum at the inner pyloric end (Fig. 5 h).

There are a few anterior ovaries on each side and a few (on the left side) or several (on the right side) posterior testes; for instance, 3 ovaries and 2 testes on the left and 3 ovaries and 4 testes on the right side in an examined zooid (Fig. 5 f), thus in all up to 5–6 gonads on the left and about 8 gonads on the right side. The vas deferens is thin and very elongate (Fig. 5 i), the ovarian aperture is membranous and complicatedly lobated (Fig. 5 j).

Up to 15–20 fertilized eggs of various stages of development to larvae, all coloured dark red orange, are found in the peribranchial cavity. The larval trunk is ellipsoidal in shape, 450–500 μ in length and 350–360 μ in width, the chorda is 800–900 μ long, and the tail fin is 1200–1300 μ long (Fig. 5 k). Three attachment processes are arranged in a triangle, two smaller on the dorsal and one larger on the ventral side. Ampullae are 21–27, rather difficult to count exactly as the trunk is made very opaque by dense yolk, but easily definable in the stage developing to the oozooid after attachment.

Development of oozooid: This species is viviparous, too, embryos being incubated within the peribranchial cavity till they are ready to be released as active tadpoles. The periodical release of mature tadpoles is seen throughout the year as in *M. taylori* stated by HAVEN (1971).

Immediately after the attachment, the distal tip of epidermal ampullae which are numerous, with a mean of 24.8, and completely encircling the middle part of the larval trunk, begins to extend radially, giving the oozooid an appearance something like a corona. In the winter season, a greater part of active swimming larvae, about 85%, is found attached to slide glasses on the next day of the liberation and these newly settled individuals are 404.7 μ long and 378.1 μ wide on an average, excluding the ampullae. The endostyle becomes visible in 2 days after settling. The heart begins to beat and the digestive organ is formed in 3 to 4 days after the attachment. In about 6 days after the attachment, the apertures open, feeding starts, and thus the attached individual becomes fully functional. On the other hand, remarkably elongated ampullae always enter the period of regression, lasting for 7 to 10 days and resulting in the extreme decrease in number. Thus, in 15 to 16 days after the attachment ampullae are nearly reduced to zero. The 15-days old oozooids are provided with approximately 8 protostigmata on each side and 4–5 small endocarps on the inner surface of the mantle on either side. After this regressive stage, the ampullae return to the progressive stage, gradually increasing their number, till they attain the maximum, about 30, shortly before the appearance of the initial bud. The life span of oozooids remains still uncleared. The development of gonads has not yet been recog-

nized at least in 50 days, then at present, it is unknown whether or not the gonads will develop in the oozooid. Thus, so many things are left for future investigations about the development of the oozooid.

In blastozooids belonging to the first generation, on the other hand, the gonads will develop in about 50 days after their appearance, even a few embryos of various developmental stages will be found in their peribranchial cavity.

Asexual reproduction: The budding manner of this species is peribranchial and fundamentally the same as in *M. taylori* fully described by ABBOTT (1953) and NEWBERRY (1965). In the normal development of blastozooids, buds usually appear as a protrusion from the mantle wall of parental zooids. The pallial buds consist essentially of epidermal and peribranchial epithelia of the mantle and a space containing mesenchymal tissues and blood corpuscles between them. The vascular ampullae usually extend fully to the test-margin in advance at the sites of bud-protrusion. Simultaneously, the mantle behind these vascular ampullae migrating to the periphery bulges out, this is the first sign of the pallial bud.

In 3 to 4 days after the beginning of protrusion, the bud becomes physically isolated by breakage of the stalk connecting the bud to the parent. The blood circulation between the parent and the bud is, however, soon reestablished by secondary fusion of the epidermal vesicle of the bud with test vessels running out from the parent. The morphogenesis in the bud usually begins strictly after the isolation as in *M. taylori* described by ABBOTT. In 5 to 7 days after the isolation, the apertures begin to open. Thus, the development from the bud appearance to the state of functional feeding takes about 2 weeks, and this is considerably shorter than in *M. taylori* as is observed by the first author at the Friday Harbor Laboratories (unpublished). At present, however, it is not clear whether this shortness is attributed to the higher water temperature (24–26 °C) in the summer season in Japan, or to the intrinsic specific property. Despite some exceptions, buds of the following generation appear most frequently a few days before or after the commencement of feeding. As shown in Fig. 4, buds are seen densely along the periphery of the colony. In general, young blastozooids have already born 3 to 4 buds within a few days after the commencement of feeding.

As stated already by ABBOTT, the endostyle appears first, as one of the first structures, in the form of an elongate, midventral placode running nearly or wholly throughout the length of the body. In the present species, the endostyle, namely the anteroposterior axis, of the bud arisen from any point of the parental blastozooid is always placed tangentially to the mantle wall of the parent. Although some buds may be sometimes rotated by about 180°, most buds are arranged with tangential orientation. Therefore, there is seemingly a tendency to localization of budding areas as seen clearly in Fig. 6, there the young buds arisen from the right (or left) side of the parent tend to produce their new buds similarly on their right (or left) side.

In oozooids, there is no sign of budding at least during two earlier weeks even in the summer season. First budding from oozooids occurs in 15 to 25 days after the
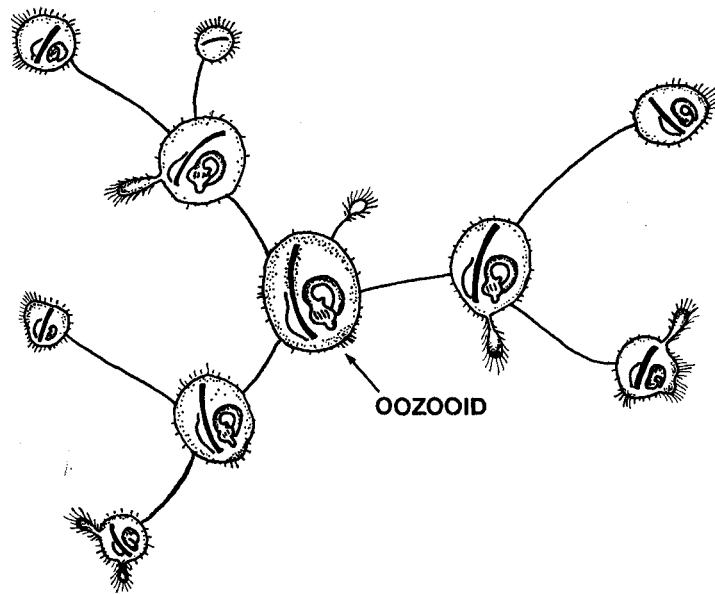
Fig. 6. *Metandrocarpa uedai* n. sp. A small colony derived from an oozooid in 38 days, × 12.5.

attachment in the same manner as in blastozooids, when they measure 1.5–1.8 mm in length and 1.4–1.7 mm in width. The buds generally can arise from any point along the basal margin of the mantle wall, though there may be some unbalanced bud issues; for instance, 17 of 24 buds from the oozooids observed were derived from the posterior half of the basal margin. The oozooid generally gives off 2 or 3 buds simultaneously or in rapid succession.

The complete life history of the animal is not yet fully known. However, in rearing colonies it has been noted that the animal is in its most active state in the autumn and winter rather than in the summer, possibly the lower water temperature may be a favourable condition for the present new species.

Remarks: In 1926, A. OKA described a social styelid found attached to the outer surface of an abalone empty shell from unknown locality as a new species of a new genus, *Dictyostyela depressa*. The superficial morphology and the mode of budding resemble closely those of the present specimens. Very unfortunately, however, OKA's description lacks the structure of gonads and the number of inner longitudinal vessels (internal longitudinal bars in his description), thus at present it is impossible to the present authors to guess the generic and specific situations of "*Dictyostyela depressa*" exactly. The general morphology of the present specimens conforms well to that through the North American species *M. dura* (RITTER), 1896, *M. taylori* HUNTSMAN, 1912 and *M. michaelseni* RITTER and FORSYTH, 1917. However, those American species are all provided with 5 inner longitudinal vessels on each side of the branchial sac,

instead of 6 in the present specimens. The present specimens seem to form a new species endemic to the Japanese waters, and the species is named *uedai* in memory of the collector.

Polyandrocarpa (*Eusynstyela*) *misakiensis* n. sp.

(Figs. 7-10)

A colony collected in 1970 by Dr. Masashi YAMAGUCHI at Misaki has been maintained alive by the first author on glass slides at Simoda. A slide from the summer stock was examined for identification.

Morphology: Zooids are elongate oval in outline, slightly narrowed anteriorly (Fig. 7 l), and up to 7 mm in length, 4.2 mm in width and as high as wide. They are attached to the substratum by whole ventral side and connected to some others by very thin test substance extended from the periphery of respective zooids; many budding individuals are found in various degrees of development. Both apertures open each on a short siphon situated roughly at each third, though the interval between siphons is seemingly slightly larger than one-third. The branchial aperture is a little larger and 4-lobed at least distinctly in living specimens, while the atrial is smaller and somewhat roundish in outline. The test is rather leathery, wrinkled irregularly in preserved specimens, and covered on the dorsal surface with colonies of small hydrozoans as in the preceding two species; but much thinner on the ventral side. It is dark reddish vermilion, somewhat whitish and with reddish radial striations on siphons, but coloured darkly around apertures, and provided in adults with a white longitudinal band between siphons, which is widest nearer to the branchial aperture than to the atrial. This white band is gradually formed in adults by the accumulation of nephrocytes and undefinable in juveniles which are uniformly orange in colouration. The mantle is also deep reddish orange, very thin on the ventral side. There are some endocarps on the inner surface of the mantle on the dorsal side.

Branchial tentacles are 12; 3 large, 3 median and 6 small ones are defined and arranged regularly (Fig. 7 q), though this arrangement becomes indistinct in preserved specimens. The ciliated groove (Fig. 7 n) is represented by a longitudinal slit. The branchial sac is coloured reddish, too, and provided with 4 folds on each side and up to about 25 stigmatal rows, posterior ones of which are very narrow and imperfect. Inner longitudinal vessels are arranged in an examined adult as follows:

Left side D 0 (6) 1 (3) 1 (6) 3 (4) 1 V
Right side D 0 (9) 0 (4) 1 (9) 0 (3) 0 V.

Three to 5 stigmata in a mesh, they may be up to 6–8 by the endostyle.

The anterior end of the intestinal loop attains to the middle of the body and the stomach occupies the posterior half of the loop, with the pyloric end at about the middle of the loop. There are 12 plications on the surface and a distinct coecum at
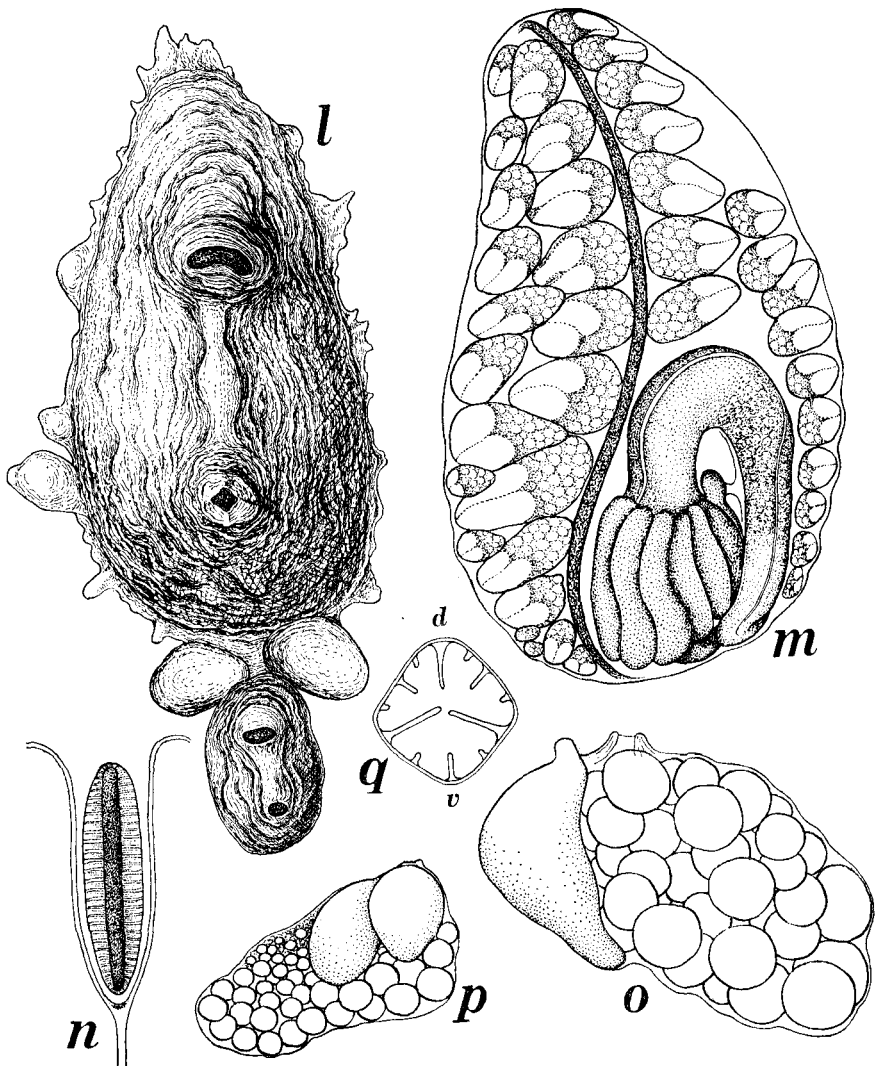
Fig. 7. *Polyandrocarpa* (*Eusynstyela*) *misakiensis* n. sp.   l, an adult, dorsal. m, the same, ventral
side of mantle body. n, ciliated groove, magnified. o, gonad, lateral; showing apertures,
× 73. p, gonad, side of attachment; showing two testicular follicles. q, tentacular ring, *d*
dorsal, *v* ventral.

the inner pyloric corner of the stomach.   The anus is bilobed and open at the level
of the middle of the stomach.

Hermaphroditic gonads are numerous, roughly arranged in one to two rows
on either side along the endostyle (on the right) or along the endostyle and the distal
branch of the first intestinal loop (on the left) on the ventral side.   For instance, in
an examined adult, 17 gonads were found on the left and 24 ones on the right side.

Respective gonads are up to 1 mm in length, the ovary occupies the exposed side of the gonad and is marked on the surface with reddish orange pigment spots, while the testis occupies the distal half of the side of attachment and always consists of two oval follicles (Fig. 7 o, p).

Development of oozooid: This species is oviparous. The spawning season extends from July through October and the periodical discharge of eggs anteceded by sperm shedding is done regularly in the afternoon around 16:00 in September. Eggs measure about 160 μ in diameter including follicle cells (Fig. 9 a) and seem to be fertilized immediately after they are shed into the water.

The development is relatively fast at 23–24 °C. Gastrulation begins about 3 hours after and the chorion ruptures in 10 to 12 hours after spawning. The free-swimming life lasts for 15 to 60 hours. The larval trunk is a little slender and about 200 μ in length; the chorda is 460–500 μ long and the tail fin is 600–750 μ in length. Three attachment processes are arranged in a triangle and the anterior part of the trunk is light-orange in colour. Except for the sense organ including a large photolith typical to styelids, no other permanent organs are developed (Fig. 9 c). In 24 to 30 hours after hatching, the tadpole becomes attached by the extension of 8 ampullae arranged circularly; such a newly settled oozooid is about 200 μ in diameter. Usually the apertures open and feeding starts in 3 to 4 days after the attachment, the oozooid in this stage measures about 260 μ in length and 240 μ in width.

Asexual reproduction: The budding of this species is peribranchial and fundamentally the same as in other genera of so-called polystyelids reproducing asexually to form colonies. Buds arise each as a protrusion from the basal margin of parental zooids and consist essentially of epidermal and peribranchial epithelia and a space containing many trophocytes (?) between them.

In large colonies extending on the glass plate, about 8 cm × 10.5 cm in size, young buds in different stages of development issued from the anterior side of zooids are clearly found densely in the periphery of the colony (Fig. 10). There is, thus, seemingly a distinct tendency to the localization of budding areas. In order to learn whether or not such a localization of budding areas is regular, zooids, 2.6–4.1 mm in length and 1.5–2.3 mm in width, were fixed separately together with some test substance on the glass-plate. As shown in Fig. 8, isolated blastozooids give rise each to 6–8 bud-protrusions almost at a time from any point along the basal margin in 7 to 13 days after the treatment. Generally speaking, vascular ampullae will usually extend fully to the test-margin at the sites of bud-protrusion, just as in *Metandrocarpa* stated before. The bud-rudiment becomes definable when it has grown to a protrusion of 200 μ long and 400 μ wide. The maximal size of bud-protrusion before isolation, ever observed by the first author, is 3.8 mm in length and 1.4 mm in width. Then, such protrusions become completely isolated by breakage of the stalk connecting the bud to the parent in 5 to 8 days after their appearance. No differentiation occurs in them till they become roundish in shape after the isolation. The apertures usually
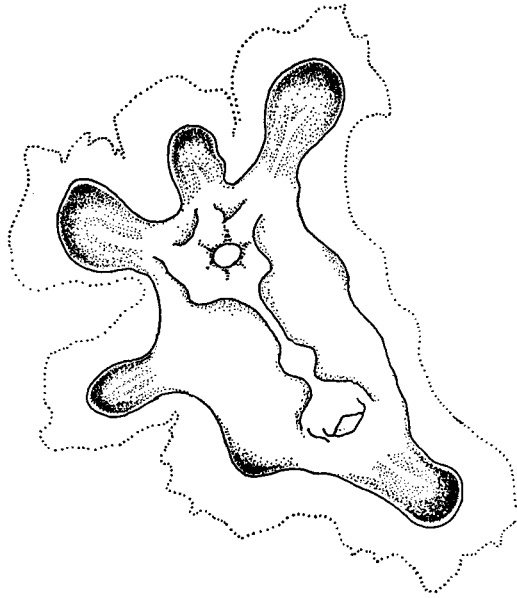
Fig. 8. *Polyandrocarpa (Eusynstyela) misakiensis* n. sp. A zooid experimentally separated from the colony 10 days before, with 6 bud-protrusions issued randomly from the basal margin, × 7.2.

open and feeding starts in about 4 days after the isolation. There is seemingly a trend that further buds are issued from the same sites where the preceding buds were protruded out and at the time when the apertures begin to open in them. Thus in the result, successive budding tends to be restricted to certain places of the basal margin of the parent. The buds continue to grow and will bear their own buds in about 15 days after the isolation. Thus, in 85 to 100 days after the isolation of their first bud, parent zooids will give rise successively to 28.5 buds on an average. Although there is no connection between individuals in young colonies, sheets of test substance extend gradually between individuals with further growth and at last in fully grown up colonies, zooids tend to be in a close contact with one another. Then, it may happen that small buds arisen from mature zooids crowded at the colony centre sometimes escape the parental colonial mass and are occasionally carried away by water currents to some distant places.

In oozooids, there is no sign of budding at least in earlier 40 days of their functional life. In 43 to 45 days after their settling, when oozooids measure about 3.0 mm in length and 1.8 mm in width, vascular ampullae will extend to the test margin and the epidermal layer at the basal margin sometimes becomes irregular in appearance. Then, the initial buds are ready to appear at any point of the basal margin in the same manner as in blastozooids. The number of buds is indefinite, though most often they are 2–4. The development and budding process of the oozooid are still being
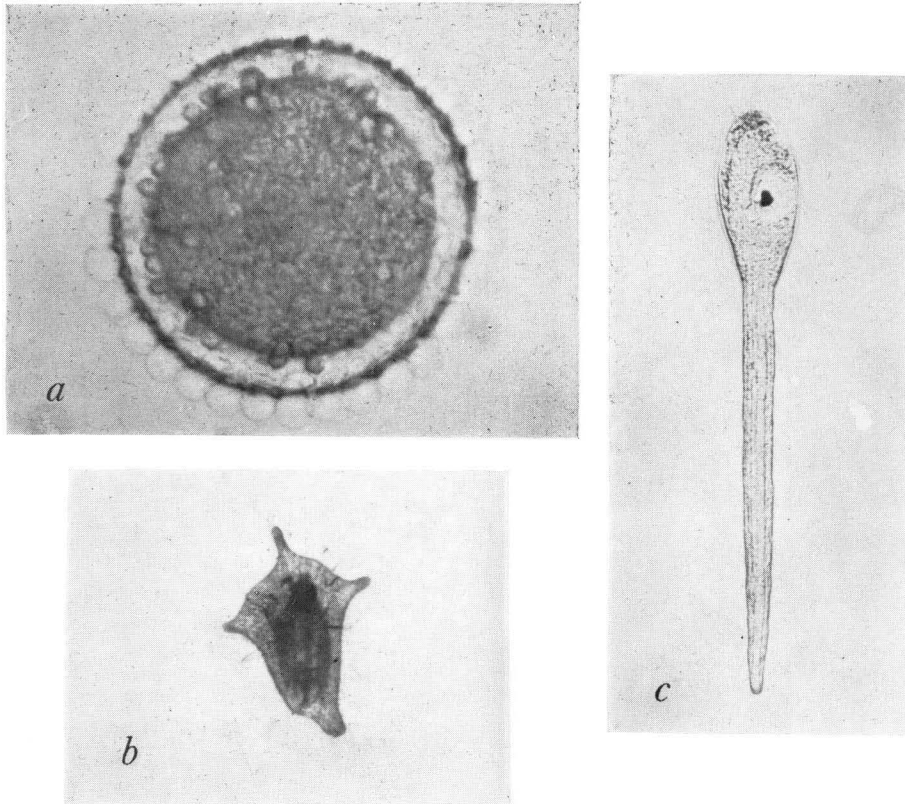
Fig. 9. *Polyandrocarpa (Eusynstyela) misakiensis* n. sp. a, fertilized egg, ×350. b, a 41-days old oozooid bearing 4 bud-protrusions, × 62. c, a swimming larva, × 110.

investigated and will be discussed in detail in a future paper.

Remarks: Of the Indo-Pacific species of *Polyandrocarpa (Eusynstyela)*, *monotestis* TOKIOKA, 1953 has only a single testicular follicle in each gonad and *transversalis* TOKIOKA, 1963 is unique in having transverse stigmata. In *hartmeyeri* MICHAELSEN, 1904 from the Red Sea and the Indian Ocean, the stomach is furnished with 18 plications and gonads are found only on the left side. In *latericius* (SLUITER), 1904, inner longitudinal vessels of the branchial sac are more numerous and gonads arranged in a single row on each side are provided each with a pair of testicular follicles which are always elongate and extending nearly the whole length of the gonad instead of being pear-shaped and occupying only the distal part of the gonad in the present specimens. Of the species reported from the West Indies and probably living on the Pacific coast of Central America, *floridana* VAN NAME, 1921 seems to be related most closely with the present specimens in that zooids lie some distance apart and are connected one another by thin sheet of irregular strands of test substance just as in the latter, while in *tincta* (VAN NAME), 1902 and *gravei* VAN NAME, 1931, especially in the former, the
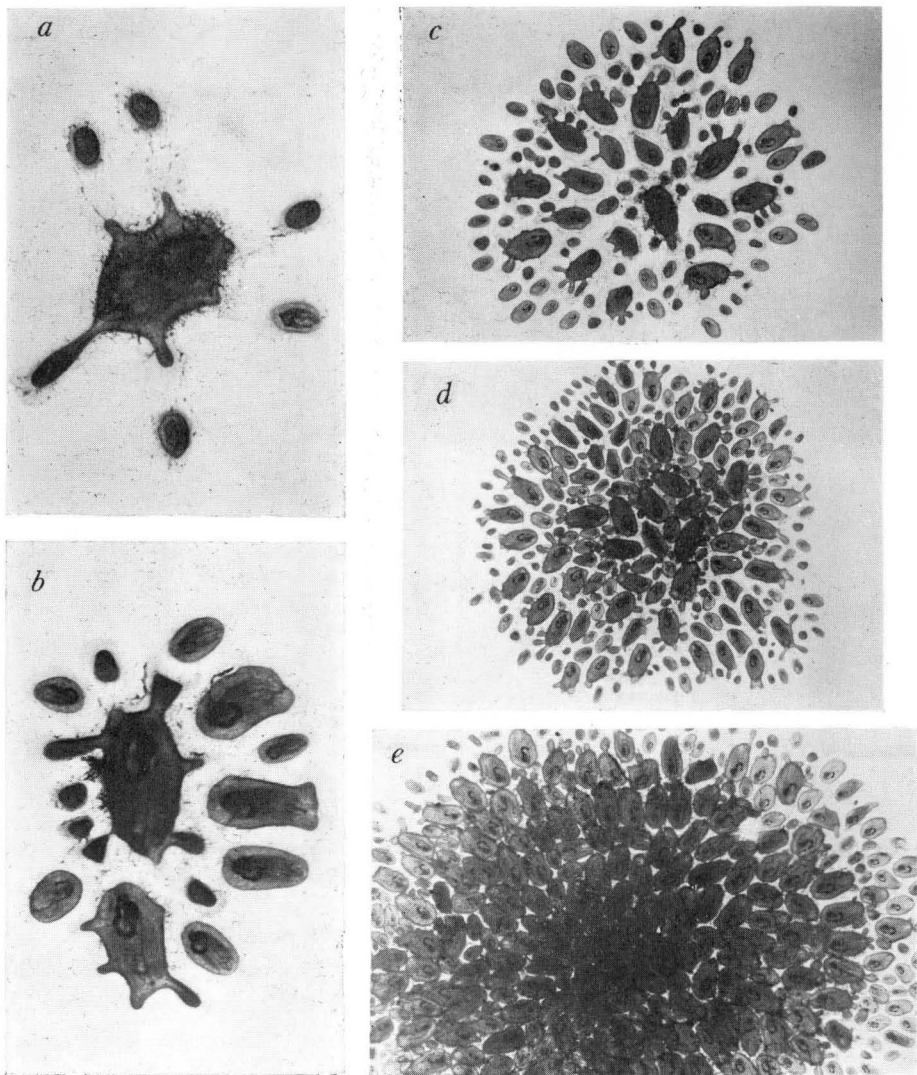
Fig. 10.   *Polyandrocarpa (Eusynstyela) misakiensis* n. sp.   a, a colony formed from an oozooid in 59 days, × 33. b, a colony formed in 47 days from a blastozooid separated experimentally from the colony, × 30. c, the same in 78 days after the separation, × 11. d, the same in 96 days after the separation, × 8.7. e, the same in 192 days after the separation, × 11.

outline of respective zooids is quite obscure as they are wholly embedded in the thick common test. In *gravei*, the stomach is very elongate (cit. Van Name 1945, Fig. 150 E), and in *tincta* and *floridana* two testicular follicles are elongate instead of being pear-shaped. Moreover, in *floridana* inner longitudinal vessels of the branchial sac are about two times as numerous as in the present specimens. Then, most probably the present specimens represent a new species, which is here named after its original locality.

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