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Amphiroa itonoi[1] (Corallinales, Rhodophyta), a new species of marine algae from Japan

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Amphiroa itonoi (Corallinales, Rhodophyta), a new species of articulated corallines, is described from Japan. The plants are minute and semi-endophytic on *Amphiroa dilatata* LAMX., *Amphiroa misakiensis* YENDO and *Amphiroa rigida* LAMX. by crustose holdfasts with processes from the lower surfaces projecting into host tissue. Genicula are made up of two tiers of cells which are unequal in length and join each other by oblique cross-walls. Reproductive structures in tetrasporangial, male and female plants are typical of *Amphiroa*. *Amphiroa itonoi* bears a resemblance *A. rigida*, especially as regards genicula and holdfasts.

Key Index Words: Amphiroa; Amphiroa itonoi; *Corallinales*; *Rhodoyhyta*; *semi-endophyte*; *taxonomy*.

While studying articulated corallines in southern Japan, a small species of *Amphiroa* was discovered growing as a semi-endophyte[2] in *Amphiroa dilatata* LAMX., *Amphiroa misakiensis* YENDO and *Amphiroa rigida* LAMX. This is somewhat unusual, since most species of *Amphiroa* are epilithic. However, some species are epiphytic, such as *Amphiroa currae* GANES. on *Gelidium serrulatum* J. Ag. (GANESAN 1971)., and some are semi-endophytic, such as *Amphiroa verruculosa* KÜTZ., *A. rigida* LAMX. and *Amphiroa* sp., which grow in crustose corallines (CABIOCH 1969, 1972). Some non-articulated corallines, such as *Clathromorphum parcum* (SETCH. et FOSL.) ADEY in *Calliarthron* sp. (ADEY and JOHANSEN 1972) and *Choreonema thuretii* (BORNET) SCHMITZ (WOELKERLING, in press) in species of *Jania*, *Haliptilon* and *Cheilosporum* are also semi-endophytic in other corallines. Most species that grow in other corallines are anchored to the host by peg-like structures that have developed in conjunction with growing host tissue. On close examination, the small plants from southern Japan are recognized as constituting an undescribed species of *Amphiroa*. The purpose of this paper is to describe the vegetative structures and reproductive organs in a new species of coralline algae, *Amphiroa itonoi*.

Materials and Methods

The observations were based on materials from: (1) Ushinohama, Kagoshima Pref. (the type locality), 1 June, 1984 and 26 February 1985; (2) Shiraiwazaki, Reihoku Town, and Tsujishima, Itsuwa Town, Kumamoto Pref., 2-3 March, 1985; (3) Akasaki, Yoronjima, Kagoshima Pref., 25 May, 1983; (4) Okitsu, Kubokawa Town, Kochi Pref., 28 March, 1978 and (5) Nabeta Bay, Shimoda City, Shizuoka Pref.,

1) The species is named for Dr. H. ITONO of Kagoshima University, who is a specialist on the Ceramiaceae of southern Japan, and has for more than five years encouraged the first author in studying coralline algae.

2) The term semi-endophyte is used according to CABIOCH (1972) and applies to plants which are partially embedded in host tissue, but without cellular connections with the host.

19 April, 1981. Freshly collected specimens were preserved in 10% formalin-seawater. The holotype of *Amphiroa crustiformis* DAWS. in AHFH (herbarium of the Allan Hancock Foundation, University of Southern California) was also examined.

Materials for sectioning were decalcified in Pérényi's solution (MASON 1953) and embedded in paraffin. Sections were cut 6–8 μm thick and stained either in Delafield's hematoxylin and counterstained with 2% aqueous eosin, or in phosphotungstic acid hematoxylin.

Diagnosis

Amphiroa itonoi sp. nov. (Figs 1–19)

Plantae parvae, usque ad 6 mm alt. e 3–6 frondibus erectes e hapteris crustosis enascentibus constitutis; haptera circularia, 4–7 mm diam., superficies superiores convexae, superficies inferiores in telis intergenicularibus aliarum specierum *Amphiroae*, protrusionibus claviformibus, ex parte inclusae; frondes dichotome semel ad bis ramosae; intergenicula prorsus cylindrica, 0.2–0.3 mm diam., aut supra compressa facta et in partibus superioribus 0.4–0.5 mm lat.; medullae intergeniculares multizonales, strata cellularum plerumque altitudines duas differentes praebentia; strata brevia 10–30(–37) μm alt. atque strata longa (27)35–100 μm alt.; genicula e duobus stratis cellularum altitudine inaequalibus constituta, strata superiora 15–35 μm alt., strata inferiora 75–155 μm alt., dissepimentis transversis inter strata plerumque obliquis; conceptacula tetrasporangialia 100–125 μm diametro interiore, tetrasporangia in periferiis centrisque conceptacularum reperta; conceptacula mascula 80–145 μm diametro interiore; conceptacula feminea 65–87 μm diametro interiore; conceptacula carposporangialia 100–155 μm diametro interiore, cellulae coalescentes 5–7 μm crass., 63–83 μm lat., filamenta gonimoblasti e marginibus atque superficiebus superioribus cellularum coalescentium enascentia, regiones centrales cellularum coalescentium sine filamentis; carposporangia 10–12 μm diam.; omnia conceptacula conspicue protrudentia.

Holotypus: Plantae semi-endophyticae in *Amphiroa misakiensis* YENDO in saxis 1 m infra planitiam mediam maris colentes; in loco Ushinohama, 6 km south of Akune City, Kagoshima Pref., Japan dicto. 26 m Feb. 1985. In loco Laboratory of Marine Botany, Faculty of Fisheries, Hokkaido University, Hakodate, Japan dicto depositae.

Plants small, up to 6 mm high, consisting of 3–6 erect fronds from crustose holdfasts; holdfasts circular, 4–7 mm in diameter, upper surfaces convex, lower surface partially embedded in intergenicular tissues of other species of *Amphiroa* by peg-like protrusions; fronds branching dichotomously 1–2 times; intergenicula cylindrical throughout, 0.2–0.3 mm in diameter, or becoming compressed above and 0.4–0.5 mm broad in the upper parts; intergenicular medullae multizonal with cell tiers mostly two different heights, short tiers 10–30(–37) μm high, long tiers (27) 35–100 μm high; genicula of two cell-tiers of unequal height, upper tiers 15–35 μm high, lower tiers 75–155 μm high, transverse walls between tiers mostly oblique; tetrasporangial conceptacles 100–125 μm in inner diameter, tetrasporangia in the peripheries and centers of the conceptacles; male conceptacles 80–145 μm in inner diameter; female conceptacles 65–87 μm in inner diameter; carposporangial conceptacles 100–155 μm in inner diameter, fusion cells 5–7 μm thick, 63–83 μm broad, gonimoblast filaments arising from the edges and upper surfaces of the fusion cells, central regions of fusion cells lacking filaments, carposporangia 10–12 μm in diameter; all conceptacles protruding prominently.

Japanese name: Itokagari

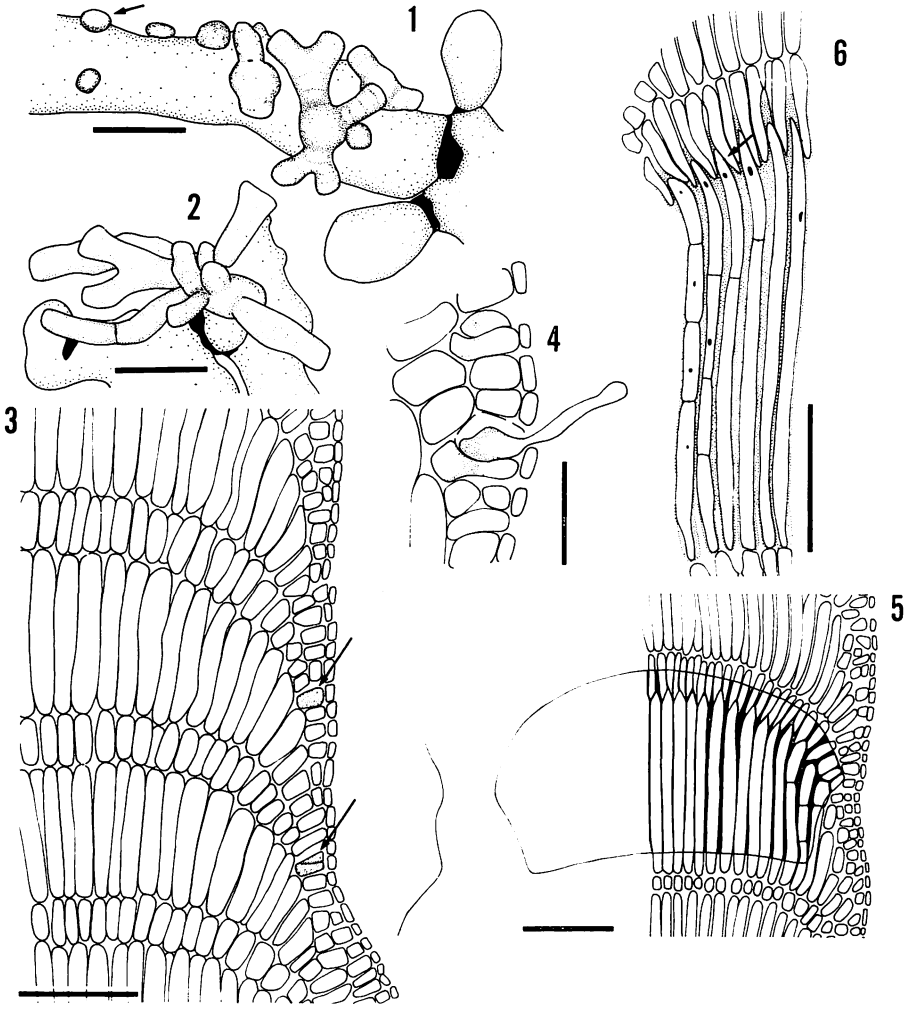
Holotype: Plants semi-endophytic on *Amphiroa misakiensis* YENDO growing on rock, 1.5 m below M.S.L., Ushinohama, 6 km south of Akune City, Kagoshima

Pref., Japan, 26 February, 1985. Housed in the Laboratory of Marine Botany, Faculty of Fisheries, Hokkaido University, Hakodate. Isotype in the National Science Museum, Tokyo.

Distribution: From the central part of western Honshu south along the Pacific coast, south coast of Shikoku, south and west coasts of Kyushu and Yoronjima of the Ryukyu Islands.

Observations

Vegetative anatomy: Each plant of *Amphiroa itonoi* is embedded in the cortex of the host plant, *Amphiroa dilatata*, *Amphiroa misakiensis* or *Amphiroa rigida*, by a wedge-shaped projection extending downward from the cushion-like crustose holdfast (Figs 1, 2, 14). As seen in vertical sections through the holdfasts, the filaments are



Figs 1-6. Habit and anatomy of *Amphiroa itonoi* sp. nov. Camera lucida drawings. Fig. 1. Habit of young plants on *A. misakiensis* YENDO and young holdfast (arrow) before producing erect fronds. Scale bar=1 mm. Fig. 2. Habit of the holotype element on *A. misakiensis*. Scale bar=1 mm. Fig. 3. Longitudinal section of an intergeniculum, showing medullary cell arrangement and trichocytes (arrows). Scale bar=50 μm. Fig. 4. Detail of trichocyte with its hair. Scale bar=20 μm. Fig. 5. Longitudinal section of a geniculum. Scale bar=50 μm. Fig. 6. part of vertical section of a geniculum, showing oblique cross-walls between upper and lower cells. Note the presence of primary pit connections (arrow). Scale bar=50 μm.

anticlinally organized, spreading out radially in a fan-like fashion from the bottom of the wedge. A hypothallium and a perithallium are indistinguishable. The cells divide more or less synchronously; they are 7–48 μm high and 7–12 μm wide. The holdfasts are covered by single layers of epithellial cells (Figs 12, 16).

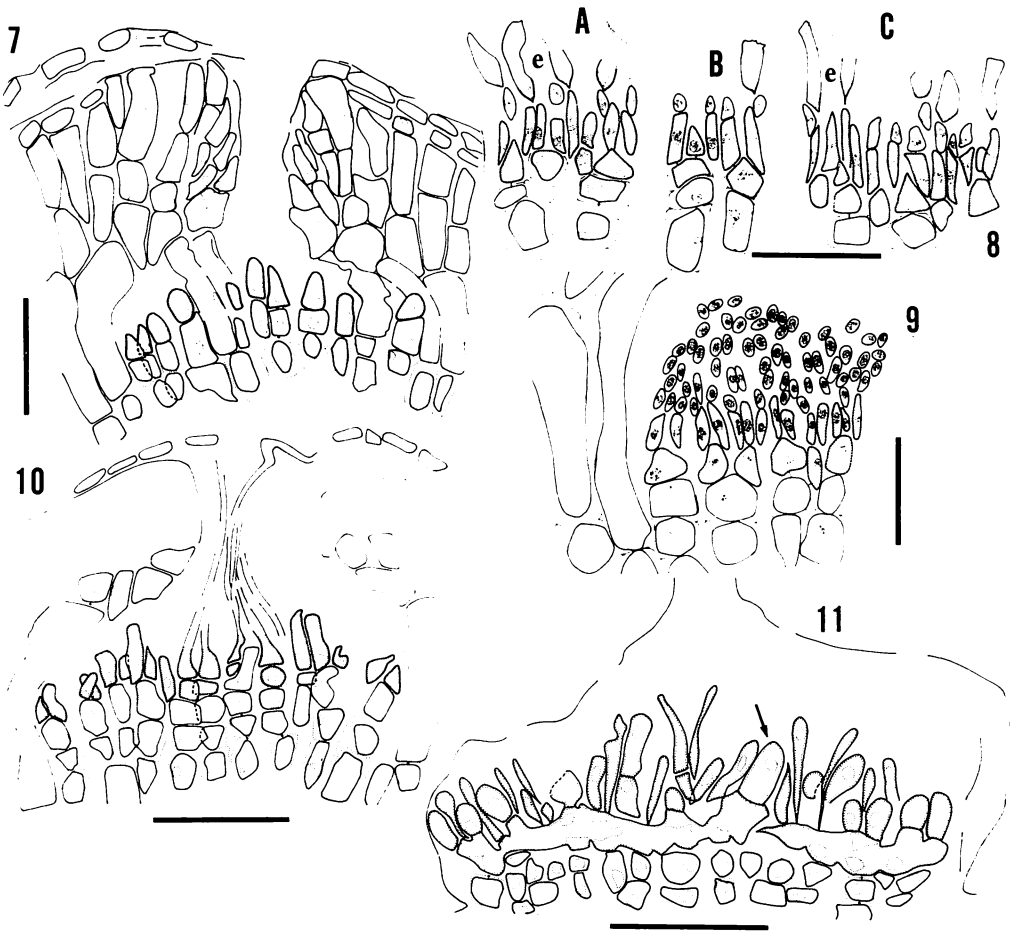
The intergenicular medulla consists of alternating tiers of long and short cells 7–11 μm in diameter (Figs 3, 14; Table 1). The intergenicular cortex is thin, consisting only of one or two layers of cells near the branch tips and three or four layers in older parts (Fig. 3). Trichocytes are present in the cortex (Fig. 4). Secondary pit-connections occur between adjacent cells in medulla and cortex. The epithallia are single-layered, as in the holdfast. Each geniculum consists of two tiers of cells, with those in the lower tier much longer than those in the upper (Figs 5, 13). Each cell in the lower tier connects with two cells of the upper tier by oblique walls containing primary pit connections (Fig. 6). The heights of genicular tiers are given in Table 1. Cortical tissue covers the genicula in early stages of their development, and, unlike the medullary cells, it does not become decalcified during development (Fig. 5). Eventually these genicular cortices are mostly sloughed, a process possibly facilitated by wave action or animal activity; occasionally fragments of cortical tissue remain attached to the mature genicula (Figs 13, 14). Cross walls are sometimes secondarily formed in genicular cells, especially in those near the periphery of the genicula (Figs 5, 13). Secondary pit-connections are present between adjacent genicular cells.

In order to compare *A. itonoi* with *A. crustiformis,* vegetative features of the holotype of *A. crustiformis* were studied. The crust has a massive hypothallium of upward and downward curving filaments consisting of 1–2(–3) rows of cells 45–100 μm long alternating with single rows of cells 8–35 μm long. A perithallium of several layers of subquadrate or elongate cells 10–35 μm high and a monostromatic epithallium of cells 3–5 μm high are also present. The intergenicular medulla contains repeating sequences of one or two tiers of long cells 40–125 μm long alternating with single tiers of short cells 8–35 μm high. The cortex is up to 16 or more layers of cells thick in lower parts of the fronds. The epithallium consists of a single layer of cells 2–5 μm high and 5–10 μm in diameter. The genicula are made up of 7–8 tiers of alternating long and short cells the same size and arrangement as in the intergenicular medulla.

Reproduction: *Amphiroa itonoi* produces conceptacles on the intergenicula as well as on the holdfasts (Fig. 16). In young tetrasporangial conceptacles, sporangial initials are produced at the center and periphery of the conceptacle floor. Cavity cells (JOHANSEN 1968) occupy the rest of the incipient chamber. Some of the initials divide transversely into a premeiotic sporangium and a stalk cell; meanwhile

Table 1. Quantitative data (μm) on various structures of *Amphiroa itonoi*

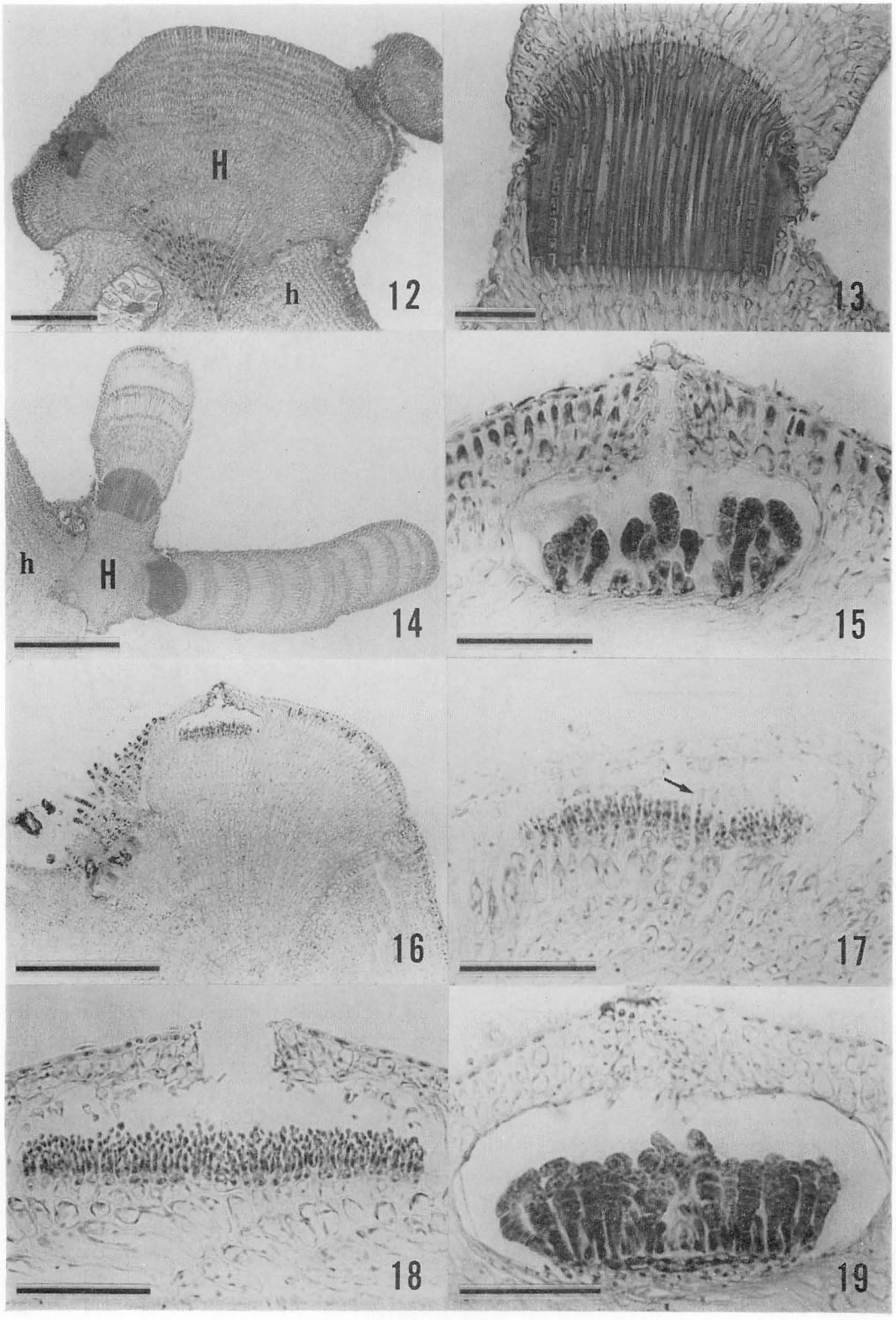
	Range	Mean	S.D.	No. of measurements
Length of intergenicular medullary cells (short tiers)	10–30 (–37)	19.6	6.2	84
Length of intergenicular medullary cells (long tiers)	(27) 35–100	63.6	17.2	84
Length of genicular cells (short tiers)	15–35	24.1	4.5	22
Length of genicular cells (long tiers)	75–155	105.6	18.7	22
Inner diameter of tetrasporangial conceptacles	100–125	110.1	8.7	18



Figs 7–11. Microscopic details of *Amphiroa ilonoi* sp. nov. Camera lucida drawings. Fig. 7. Tetrasporangial conceptacle at an early stage of development. Note the premeiotic sporangia at the center and peiiphery of conceptacle. Scale bar=25 μm. Fig. 8. Three stages (A, B, and C) in the development of spermatangia, taken from three conceptacles. Note the elongated cells (e). Scale bar=15 μm. Fig. 9. Part of mature male conceptacle. Scale bar=15 μm. Fig. 10. Female conceptacle. Scale bar=25 μm. Fig. 11. Detail of young cystocarpic conceptacle floor with gonimoblast filament initials (arrow). Scale bar=25 μm.

the cavity cells elongate and successively divide at their distal ends thus contributing to the formation of the roof (Fig. 7). Finally the cavity cells atrophy and a chamber forms (Fig. 15).

Female and spermatangial conceptacles occur in separate plants. Prior to fertilization, a supporting cell typically bears a two-celled carpogonial filament composed of a hypogynous cell and a carpogonium; occasionally a sterile cell may also be present. The central fertile cells in the female conceptacle reach maturity whereas the peripheral ones only give rise to slightly elongate cells in which further cell divisions do not take place (Fig. 10). Early stages in the formation of carposporophytes were seldom encountered. Presumably following fertilization, a disk-shaped fusion cell is formed at the bottom of the conceptacle (Fig. 11). Gonimoblast filaments are produced from the edges and the upper surfaces of the fusion cells except in the center (Fig. 19). Ripe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gonimoblast filaments consist of eight to twelve cells. In male conceptacles, the fertile areas are limited to the floor (Fig. 18). Two or three spermatangial mother cells are formed on each basal cell and spermatangia are cut off successively from their mother cells. The fully developed spermatangia are elliptic or rounded (Fig. 9). Weakly staining elongate cells (6–12 μm long) that may be paraphyses are present in the early stages of spermatangium production. A precise interpretation of the initiation of these cells can not be made at present; they seem to be cut off from the mother cells before the formation of the spermatangia, but they subsequently disappear (Figs 8, 17).

Discussion

Superficially *Amphiroa itonoi* resembles *Amphiroa currae*. However, they are markedly different in habitat and internal morphology. Plants of *A. currae* are attached to *Gelidium serrulatum* by crustose holdfasts complete with hypothallia and perithallia; they are not embedded in host tissues. In addition, the intergenicular medullae consist of repeating sequences of 2–3 tiers of long cells separated by single tiers of short cells. The genicula consist of two or three tiers of cells. These features are clearly different from *A. itonoi* as described above.

The erect fronds of *Amphiroa crustiformis* are only 4–7 mm high and, except for the extended crusts, they strongly resemble *A. itonoi*. However, the holotype of *A. crustiformis* has a basal crust anatomically similar to that of *A. currae* and its genicula are made up of 7–8 tiers of cells. Thus *A. crustiformis* can by no means be confused with the present species.

On the other hand, *Amphiroa rigida* and *Amphiroa verruculosa* appear to be more closely related to *A. itonoi*, especially as regards genicula and holdfasts. The genicula of *A. itonoi* and *A. rigida* are very similar. They are always made up of two tiers of cells with oblique cross-walls between them. However, they differ from each other in that the tiers differ in height in the former whereas they are the same in the latter. YENDO (1904 p. 17) stated that "In *Amp. rigida* the genicula ...always formed of a single zone, the cell being often interwined at the equatorial points...". This is a misinterpretation; it was shown later that the genicula of *A. rigida* are made up of two tiers of cells (SUNESON 1937, SEGAWA 1940, HAMEL and LEMOINE 1953 p. 41, NORRIS and JOHANSEN 1981). The genicula of *A. verruculosa* KÜTZ., which has been treated as a synonym of *Amphiroa cryptarthrodia* ZAN. by certain authors, also consists of two tiers of cells (SOLMS-LAUBACH 1881 p. 28), and the fine illustration in WEBER-VAN BOSSE (1904 pl. 16, fig. 14) shows that the cell-tiers are unequal and meet each other by horizontal cross-walls. On the contrary, HAMEL and LEMOINE (1953 p. 43) described the genicula of *A. cryptarthrodia* as made up of only one tier of cells jointed with the upper intergenicular cells by oblique cross-walls. The genicula of this species, especially the type, need to be reexamined.

The peg-like holdfasts of *A. itonoi* closely resemble those of *A. rigida* and *A. verru-*

Figs. 12–19. Photomicrographs of sections of *Amphiroa itonoi* sp. nov. Fig. 12. Vertical section of holdfast (H). Note the fan-shaped outline of lower surface in host tissue (h). Scale bar=150 μm. Fig. 13. Median section of a geniculum. Scale bar=50 μm. Fig. 14. Vertical section of a whole plant through branches and holdfast (H) in host tissue (h). Scale bar=250 μm. Fig. 15. Median section of mature tetrasporangial conceptacle. Scale bar=50 μm. Fig. 16. Vertical section of basal crust through male conceptacle. Scale bar=200 μm. Fig. 17. Median section of young male conceptacle. Note the presence of elongated cells (arrow). Scale bar=50 μm. Fig. 18. Median section of mature male conceptacle. Scale bar=50 μm. Fig. 19. Median section of carposporangial conceptacle, showing gonimoblast filaments developing on upper surface of fusion cell. Scale bar=50 μm.

Table 2. Some important characters of *Amphiroa itonoi*, *A. crustiformis*, *A. currae*, *A. rigida* and *A. verruculosa*

Characters	*A. itonoi*	*A. crustiformis*	*A. currae*	*A. rigida*	*A. verruculosa* (*A. cryptarthrodia*)
Host plant	*A. dilatata* *A. misakiensis* *A. rigida*	—	*Gelidium serrulatum*	*Neogoniolithon notarisii*	*Pseudolithophyllum expensum*
Holdfast outline in vertical section	Wedge-shaped	Not wedge-shaped	Not wedge-shaped	Wedge-shaped	Wedge-shaped
Height of fronds (mm)	6	4–7	1–5	10–30	20–40
Number of intergenicular medullary tiers per series*	2	2–3	3–4	2–3	2
Number of tiers per geniculum	2	7–8	2–3	2	2 (1)
Height of genicular tiers	Unequal	Unequal	Unequal	Equal	Unequal

* A series is a set of medullary cell tiers repeatedly expressed in a branch.

culosa, which grow on the crustose corallines *Neogoniolithon notarisii* (DUFOUR) HAM. et LEM. and *Pseudolithophyllum expansum* (PHIL.) LEM. respectively (CABIOCH 1969, 1972). However, *A. itonoi* may readily be distinguished from *A. rigida* (cf. SEGAWA 1965, NORRIS and JOHANSEN 1981) and *A. verruculosa* (cf. KÜTZING 1858, pl. 39, fig. 2, FUNK 1927 pl. 9, fig. 3, pl. 10, fig. 1) by of the smaller habitat and different internal characteristics of *A. itonoi* (Table 2).

The development of reproductive organs is basically the same as in other species of *Amphiroa*. Tetrasporangial conceptacles are the same as in *A. rigida* (SUNESON 1937) and *A. zonata* YENDO (MURATA and MASAKI 1978), but slightly different from those of *A. ephedraea* (LAM.) DEC. (JOHANSEN 1968) in that in *A. itonoi* the mature tetrasporangia are located both at the center and periphery of the chambers. Paraphyses in male conceptacles are common in the tribe Corallineae JOHANSEN 1969, MURATA and MASAKI 1978) but this is the first report of their presence in *Amphiroa*.

Conceptacles of all reproductive types occur in both the holdfasts and fronds in *A. itonoi*, *A. currea* (GANESAN 1971) and *A. rigida* (personal observations), and tetrasporangial conceptacles occur in each structure in *A. crustiformis* (DAWSON 1963). This supports the contention that articulated corallines, at least *Amphiroa*, evolved from crustose corallines.

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スリマノーパス，V・正置富太郎：日本産紅藻カニノテ属の1新種

鹿児島県牛ノ浜産有節サンゴモ，カニノテ属の標本に基づいて *Amphiroa itonoi* イトカガリを初めて記載した。本種は藻体が矮小でカニノテ，ヒメカニノテ，イソハリに着生し，殻状の基部の一部が楔形になり寄主に嵌入している。体組織及び生殖器官も調べ，近縁種との比較も試みた。(041 函館市港町3丁目1-1 北海道大学水産学部植物学講座)