

Gracilaria Species (Gracilariales, Rhodophyta) of Malaysia Including Two New Records

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ABSTRACT The west coast Peninsular Malaysia is dominated by mangrove swamps, which provide a natural habitat for *Gracilaria* species to grow. To date, twenty species of *Gracilaria* and one species of *Gracilariopsis* have been collected in Malaysia. There are many other areas yet to be surveyed for other *Gracilaria* species. Two new records of *Gracilaria*: *Gracilaria articulata* Chang et Xia and *Gracilaria manilaensis* Yamamoto et Trono collected for the first time in the west coast of Peninsular Malaysia, are described.

ABSTRAK Pantai Barat Semenanjung Malaysia kebanyakan kawasannya diliputi oleh kawasan paya bakau, yang mana ini memberikan habitat semulajadi untuk spesies *Gracilaria* untuk tumbuh. Sehingga kini, dua puluh satu spesies *Gracilaria* dan satu spesies *Gracilariopsis* telah direkodkan di Malaysia. Masih terdapat banyak kawasan masih belum diterokai untuk spesies yang lain. Dua rekod baru *Gracilaria* : *Gracilaria articulata* Chang et Xia and *Gracilaria manilaensis* Yamamoto et Trono yang terdapat di Pantai Barat Semenanjung Malaysia diuraikan.

(*Gracilaria articulata*, *Gracilaria manilaensis*, Malaysia, seaweeds)

INTRODUCTION

Gracilaria is one of the largest genera in the family of Gracilariaceae. There are about 100 species distributed widely from sub-boreal to tropical waters. *Gracilaria* is found in China, Japan, Taiwan, Hawaii, Chile, Micronesia, Pacific North, South America and some other countries including Malaysia. Twenty species of *Gracilaria* and one *Gracilariopsis* have been recorded in Malaysia. These are *Gracilaria cacalia* (Agardh) Dawson; *Gracilaria canaliculata* (Kutzing) Sonder; *Gracilaria changii* (Xia & Abbott) Abbott, Zhang & Xia; *Gracilaria confervoides* (Wiggers) Greville; *Gracilaria coronopifolia* J.Agardh; *Gracilaria crassa* Harvey ex J.Agardh; *Gracilaria cylindrica* Boergesen; *Gracilaria dura* Agardh, *Gracilaria edulis* (Gmelin) Silva; *Gracilaria firma* Chang et Xia; *Gracilaria foliifera* (Forskaal) Boergesen; *Gracilaria lichenoides* (Lamouroux) Greville; *Gracilaria minor* (Sonder) Chang & Xia; *Gracilaria multifurcata* Boergesen; *Gracilaria salicornia* (C.Agardh) Dawson; *Gracilaria*

subtilis (Xia & Abbott) Abbott, Zhang & Xia; *Gracilaria taenoides* Agardh; *Gracilaria tenuistipitata* Chang et Xia; *Gracilaria textorii* (Suringar) De Toni; *Gracilaria urvillei* (Montagne) Xia & Abbott; *Gracilariopsis bailinae* Zhang et Xia (Table 1) [1; 2; 3; 4; 5; 6; 7]. The authors have not personally collected *G. eucheumatoides* Harvey (Syn: *G. eucheumioides*), thus this species is not included in this paper.

Gracilaria species are one of the main sources of agar and agarose [7]. Chile is the largest supplier of the world's *Gracilaria* with an annual production of 137 000 wet tones in 2000 [8]. *Gracilaria* products are used in the food and confectionery industries, as well as in biotechnology and microbiology research.

MATERIALS AND METHODS

Specimens were collected from various localities in Malaysian waters. The specimens were processed to herbarium materials and deposited at

the University of Malaya Seaweeds and Seagrasses Herbarium.

Sections of specimens were made by hand using a razor blade. The sections were then stained with aniline blue and mounted in 50% glycerol on microscope slides and observations were done using a light microscope.

A short description of the *Gracilaria* species follows.

RESULTS

Description of Species

Gracilaria textorii (Suringar) De Toni, Sylloge algarum III, p. 27, 1895 [9]; Phang [4]

Distribution: Attached to the fish cages at Sungai Merbuk, Kedah; Batu Ferringhi, Sungai Batu in Pulau Pinang.

The plants range from brownish-red to yellowish red in colour, with a height from 5 to 20 cm. Thalli are coriaceous to membranous; the fronds are flattened with cylindrical stipes, and attach to the substratum by small discoid holdfasts. The fronds are irregularly dichotomous, with margins entire or with proliferations; apices blunt, bifurcate, or ligulate; branching in one plane, profuse, alternate or secund. The transition of cortical cells (9.5-16 μm x 6.5-11 μm) to medullary cells (200-310 μm x 150-270 μm) is abrupt. The cruciate-shaped tetrasporangia (40-50 μm x 23-30 μm) are scattered on almost entire surface of fronds. The conceptacles of spermatangia (*textorii* type) are shallow and cup-like with a depth of 20-30 μm . The cystocarps (1800 μm x 2000 μm) are globose, slightly rostrate and not constricted at the base.

Gracilaria changii (Xia & Abbott) Abbott, Zhang & Xia, Pac. Sci. 45 (1): 12-27. [10]; Phang [4].

Distribution: Middle Bank, Pulau Pinang; Sementa, Morib in Selangor; Pantai Dickson, Negeri Sembilan; Kukup, Sungai Pulai, Mersing in Johor.

The plants have discoid holdfasts with thalli length from 6 to 20 cm. Branching irregular, alternate or secund in two to four orders with an abrupt constriction at the base, forming a slender stipe; slightly swollen at distal end of stipe, tapering towards the tip. The transverse section of frond shows thick walled, large, rounded medullary cells (350 - 750 μm), two to three

layers of cortical cells, and the transition abrupt. The spermatangial conceptacles are *verrucosa* to *polycavernosa* type; however *verrucosa* type is more common in this study. Ovoid to elongated tetraspores are scattered around the frond surface. The cystocarps are conical or semiglobose, constricted at the base; gonimoblast consists of many small cells; absorbing filaments few, lateral and upper; the pericarp consists of two types of cells with rounded to oval cells at the outer, and compressed cells in the inner parts.

Gracilaria coronopifolia J. Agardh, Sp. gen. ordines algarum, vol. 2, p. 692, 1852 [11]; Phang [4].

Distribution: In intertidal pools at muddy beach of Pulau Tekong Besar, Singapore; Tanjung Bunga, Tanjung Tokong in Pulau Pinang.

The plants are generally very short ranging from 4-6 cm. The branching is irregular and subdichotomous, forming an entangled mass. Purplish-red when fresh and red when dry. The tertiary branches (up to 1.5 mm) are thicker than the main branches; ultimate branches have pointed apices. The transition from cortex to medulla is abrupt; one to two rows of small cortical cells with large medullary cells. Mature female plants were not collected; the young cystocarps are rostrate at ostiole and constricted at the base. No size variation in pericarp cells; gonimoblast cells are small, compressed, with upward-growing absorbing filaments.

Gracilaria edulis (Gmelin) Silva, Univ. Calif. Berkeley Publ. Bot. 25:293, 1952 [12]; Phang [4].

Distribution: Gelugor, Batu Ferringhi in Pulau Pinang; Sungai Merbuk, Kedah; Morib, Selangor; Pulau Besar, Melaka; Kukup, Sungai Pulai in Johor; Pulau Tekong Besar, Singapore.

The plants are much branched, wiry and form entangled masses, with maximum height of 27 cm tall, and attach by discoid holdfasts. Branching is dichotomous or trichotomous with up to a maximum of seven orders. The branches are cylindrical, with spinelike ultimate apices; diameter 1.0 -1.5 mm, constricted or slightly constricted or not at bases. The cell transition in the thallus is gradual, increasing in size from cortex to medulla (100-300 μm). The spermatangial conceptacles range from the *verrucosa* to *polycarvernosa* type. The cystocarps are constricted at bases; globose with rostrate tips. The pericarps consist of 9 to 14 rows of cells, with robust basal absorbing filaments

having many branches. The tetraspores (8 μm x 16 μm) are ovoid and cruciate, scattered over the surface of the thallus.

Gracilaria firma Chang & Xia, Stud. Mar. Sinica 11:143-144, 162-163, 1976 [13]; Phang [4].

Distribution: Attached to fish cages at Sungai Merbuk, Kedah; Tanjung Aru, Pulau Labuan.

The plants ranging from 4- 20 cm tall, with erect robust thalli arise from discoid holdfasts; thalli terete, caespitose or solitary. The branching is alternate or irregularly alternate, with marked constriction at the base; tertiary branches short, fine and hairlike. The cell size transition from medulla to cortex is gradual; medullary cells consist of seven layers of cells, 230-500 μm in diameter and with cell walls 10-20 μm thick; cortex consists of 1 to 2 layers only with dimensions of 7 μm length and 12 μm width. The ovoid to spherical tetrasporangia with diameter of 18-38 μm are scattered throughout the surface of the entire plants. The prominent conceptacles of spermatangia are *verrucosa* type (deep pot-shaped), 35-60 μm wide and 50-116 μm deep. The conical or semiglobose cystocarps are rostrate with occasional constriction at the base, 1- 1.3 mm tall, 0.8 - 1.2 mm wide; the gonimoblast filaments are abundant, consisting of small, dense and richly protoplasmic elongate cells, absorbing filaments scarce; the pericarp with a thickness of 83-95 μm consisting of 8-15 rows of undifferentiated cells with distinct walls.

Gracilaria multifurcata Børgesen, V.K. Danske Vidensk. Selks. Biol. Medd. 21(9): 1-62, 1953 [14]; Terada et al. 2000 [5]

Distribution : Sungai Batu, Batu Ferringhi, Pasir Pandak, Pasir Panjang, Teluk Kerachut in Pulau Pinang.

Several thalli of 4-16 cm length arise from a small discoid holdfast, with prostrate rhizomes. The thalli are cartilaginous, flattened and range from reddish brown to purplish brown in colour. Branching is dichotomous to trichotomous up to 4 times. Anastomoses between adjacent branches are frequent. The transition of cortical cells to medullary cells is gradual. The medullary cells are found up to 8 layers with the size increasing up to 250 μm in diameter, towards the centre. The cortex consists of 1-2 layers of globular cells. The spermatangial conceptacles are *polycavernosa* type with a depth of 120-160 μm and 100-200 μm width. The mature rostrate cystocarps are globose, up to 0.8 mm height and 1 mm width, ostiolate, with a slight constriction

at the base. Gonimoblasts consist of large cells 60-120 μm wide. The tetrasporangia are scattered throughout the surface of the frond and divide cruciately.

Gracilaria tenuistipitata Chang & Xia, Stud. Mar. Sinica. 11:91-163, 1976 [15]; Terada et al. 2000 [5]

Distribution: Batu Laut, Morib in Selangor; Kuah, Pulau Langkawi.

The plants are terete throughout, cartilaginous and brownish to deep purple in colour. The plants arise to 5-15 cm height from a discoid holdfast. The main axes are 0.2- 1.0 mm in diameter and bear densely and irregularly arranged branches. Branching can be up to a maximum of 16 orders. The medullary comprises up to 5 layers, with cells increasing in size towards the centre and reaching up to 350 μm in diameter. The cortex consists of 1-2 layers of cortical cells, 4 μm long and 8 μm wide. The cystocarps are prominently protruding, globose, rostrate, constricted at the base; gonimoblasts composed of large parenchymatous cells, connected with pericarps by very few nutritive filaments. The male plants have the *textorii* type of spermatangial conceptacles. The ovoid or oblong shaped tetrasporangia are scattered throughout the surface of the plants.

Gracilaria salicornia (C. Agardh) Dawson, *Gracilaria cacalia* (Agardh) Dawson; *Gracilaria canaliculata* (Kützting) Sonder; and *Gracilaria minor* (Sonder) Chang & Xia was combined by Xia in 1986 [16] into one species which is *Gracilaria salicornia* the oldest available name among the four variants. However from the molecular data [17], there are differences among the four variants, not including *G. minor*. In our survey over the last four years, *G. minor* was not found. Molecular studies show that the combination of the variants into one species is not justified. Thus, we retain the original names for the four variants.

Gracilaria salicornia (C. Agardh) Dawson, Bull. South. Calif. Acad. Sci. 53(1):1-7, figs.1-4, 1954 [18]; Phang [4].

Distribution: Sungai Pulai, Johor; Morib, Selangor; Pantai Dickson, Negeri Sembilan; Teluk Pelanduk, Negeri Sembilan; Kuala Merbuk, Kedah; Middle Bank, Pulau Pinang; Pulau Besar, Melaka.

The plants range from greenish to brownish red in colour and have no main axis. The segments

are constricted throughout the thallus with 2-4 branches per node, fronds cylindrical or slightly compressed, brittle and fleshy. The holdfast is disc-like. The branching is irregular to dichotomous, trichotomous, tetrachotomous and divaricate.

Gracilaria cacalia (Agardh) Dawson, Agardh, Leipzig. 3 (1): i-vii +676, 677-724, 1876 [19]; Dawson, Bull. S. Calif. Acad. Sci. 53:2, 1954 [20]

Distribution: Pantai Dickson, Negeri Sembilan; Pulau Jerejak, Pulau Pinang.

The plants have main axis and thalli are not constricted into nodes and internodes. The plant colour ranges from brownish to orange red, with a maximum height up to 6 cm. Branching is irregular from main axes; bases of branches not constricted or slightly constricted, branches constricted into nodes. This species normally grows on corals, rocks and muddy areas.

Gracilaria canaliculata (Kützinger) Sonder, Naturwissenschaftlicher Verien, Abhandlungen & C. 5(2): 35-74, t. 1-6, 1871 [21]

Distribution: Pantai Dickson, Teluk Pelanduk at Negeri Sembilan; Kuala Merbuk, Kedah; Middle Bank, Pulau Jerejak in Pulau Pinang; Pulau Merambong, Johor.

The main axis of the plant is inconspicuous (absent), segments not constricted throughout the plant (if present only slightly articulated at the upper part). The branching is dichotomous or sometimes irregular; fronds cylindrical or slightly compressed, growing on rock or coral and forming a mat. It is generally very small in size with maximum height up to 2-3 cm only. The colour ranges from brownish to reddish. The three variants show similarity in their anatomical aspect. The transition of the medullary cells to cortical cells is gradual to abrupt. The diameter of medullary cells is 250-600(-720) μm , with cell walls 2-15 μm thick. The cortical cells have dimensions of 10 -15 μm x 20-30 μm , are irregular shaped or oblong, ovate, rounded and arranged in 2-3 layers. The tetrasporangia are spherical to oblong, with 30 -35 μm diameter, cruciately divided and scattered throughout the entire plant. The conceptacles of the spermatangia are the deep *verrucosa* type, with dimensions of 26-50 μm x 85 μm in longitudinal section. Cystocarps are prominently protruding, globose, constricted at base or forming a neck, medullary cells are large and the transition of the cells abrupt, the maximum diameter of the cell up

narrow at the base, composed of conspicuously vacuolate cells; pericarps 180-270 μm thick, connected with gonimoblast by numerous nutritive filaments.

Gracilariopsis bailinae (as *bailinea*) Zhang & Xia, Flora Algarum Marinarum Sinicarum 2:31-33 [22]; Terada et al. 2000 [5].

Distribution : Middle Bank, Pulau Pinang; Pulau Bai, Sandakan in Sabah

Plants with discoid holdfasts and several thalli arise from holdfasts up to a height of 10-20 cm. The thalli are terete throughout, fleshy and range from green to dark brown in colour. The main axes range from 0.8-2.0 mm in width and the branching is irregular, alternate or secund. Branching is up to 4 four orders. The 5 layers of medullary cells which are unpigmented, abundantly vacuolated and spherical, increase in size towards the centre and reach up to 500 μm in diameter. The cortex consists of 2 layers of globular cells up to 10 x 6 μm . The spermatangia are superficial. The mature cystocarps are prominently protruding, globose with a dimension of up to 1.5 mm height and 2.0 mm width, and have a slight constriction at the base. The cruciate tetrasporangia are up to 30 μm high and 20 μm wide.

Gracilaria articulata Chang et Xia , 1976: 11:113, fig. 16-17 [23].

Distribution : Hainan Province, China [13; 24]; Vietnam [24]

Specimens examined: Johor: Sungai Pulai (PSM 6159-6163; 6171-6174)

This is a new record for Malaysia. Plant is terete, succulent and fleshy. In general, the plant height ranges from 8.0 – 19.0 cm. When fresh it is red in colour. The thallus is cylindrical and the branching is lateral, opposite, alternate or irregular dichotomous (Figure 1). The thallus is constricted 1-3 times or more and forms many nodes along the entire length. The diameter of the thallus at the point of constriction ranges from 1.26 – 2.38mm. New branches are observed to grow at the tip of the thallus as well as from any part of the node. Tips of thallus are normally rounded. The thallus is multi-axial, consisting of cortical and medullary cells. The outermost cells are thick with diameter of around 10 μm . The cortical cells are arranged in 1-2 rows, with dimensions ranging from 7-11 μm X 3-6 μm . The

to 1050 μm . The cystocarp is semiglobose with some constricted at the base (Figure 2). The

diameter of the constriction ranges from 0.9 – 1.5 mm. The height ranges from 1.0 – 1.7 mm, and the width from 1.0 – 1.6 mm. The pericarp layer ranges from 65 – 92 μm . The pericarp cells are oblong, cell wall is prominent and arranged orderly. Nutritive filament was not observed. Gonimoblasts gather in the center of the cystocarp. There is an ostiole on the top of the cystocarp (Figure 3). The spermatangia are in oval conceptacles belonging to the *verucosa* type with depth to 38 μm and up to 34.8 μm wide (Figure 4). The surrounding vegetative outermost cells are different from the normal vegetative

cell. The tetrasporangia are scattered throughout the surface of tetrasprophytes except at the basal and apical portions (Figure 5). The cells are cruciately divided. The cells are up to a height of 40 μm and width of 32 μm . *Gracilaria articulata* grow at seagrass beds where there is an inflow of freshwater river water to the sea. The sediment is muddy or sandy. This species was collected in 1999, however was not found again over the last two years. This could be due to the fact that the new port of Tanjung Pelepas recently built at the collection site might have disturbed the habitat causing the loss of the species.

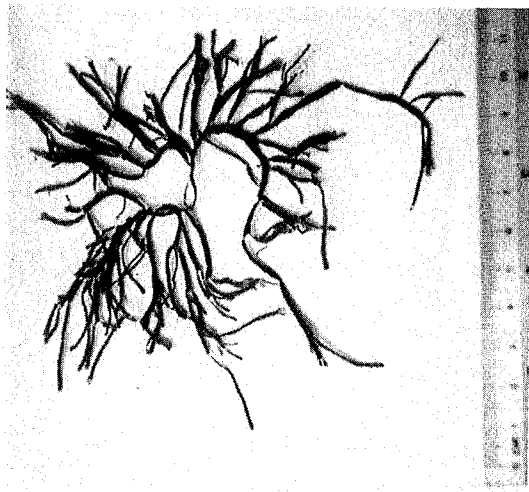


Figure 1. Herbarium specimen of *Gracilaria articulata*

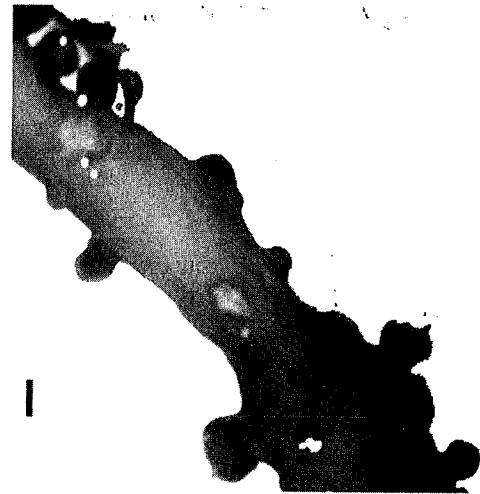


Figure 2. The surface view of cystocarp of *Gracilaria articulata*. The scale bar = 1.0 mm

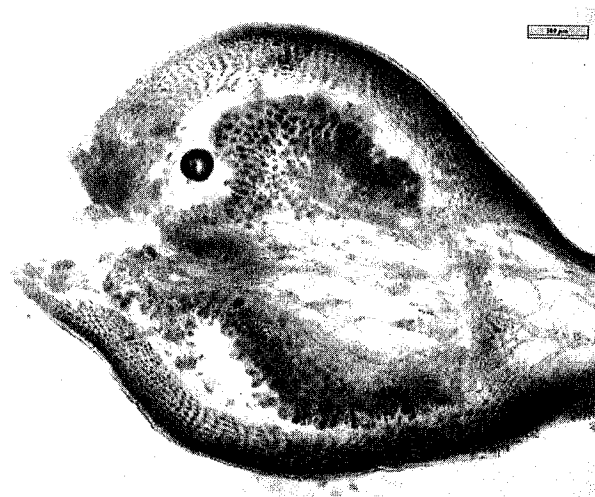


Figure 3. Cross-section (XS) of cystocarp of *Gracilaria articulata*. The scale bar = 1.00 μm

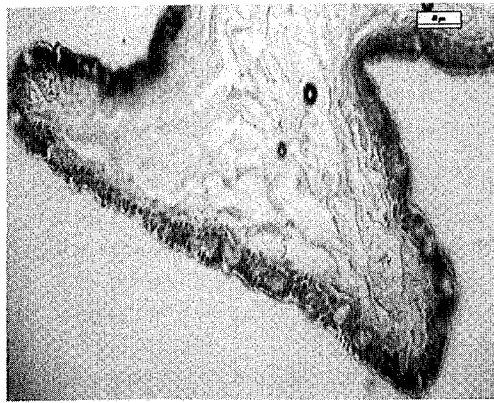


Figure 4. Longitudinal section (LS) of male Gametophyte showing spermatangial cavities of *Gracilaria articulata*.

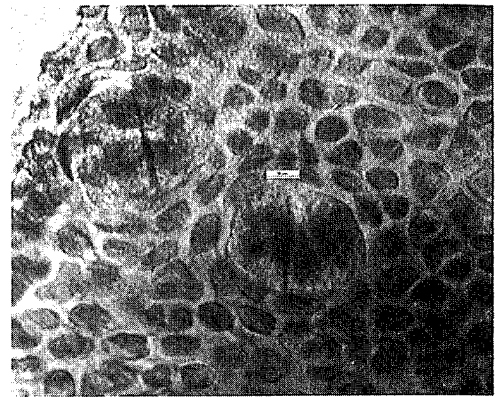


Figure 5. The tetrasporangia are scattered throughout the surface of the thallus in *Gracilaria articulata*.

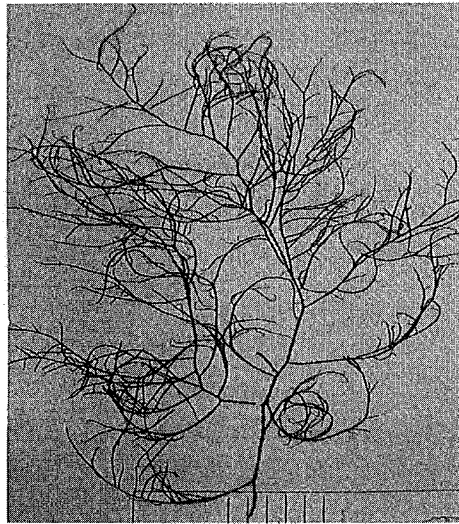


Figure 6. Herbarium specimen of *Gracilaria manilaensis*

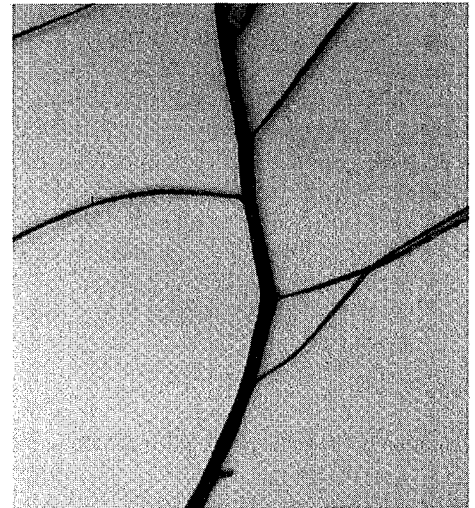


Figure 7. Closer view of the thallus of *Gracilaria manilaensis* showing the constriction at the base of each branch.

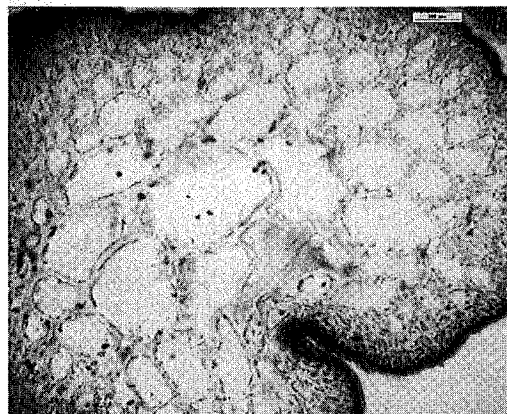


Figure 8. X.S. of thallus showing medullary cells of *Gracilaria manilaensis* increasing in size towards the centre.

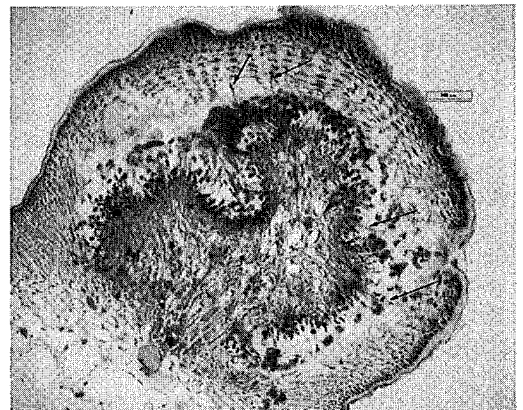


Figure 9. L.S. of *Gracilaria manilaensis* cystocarp. Arrows indicate the nutritive filaments.

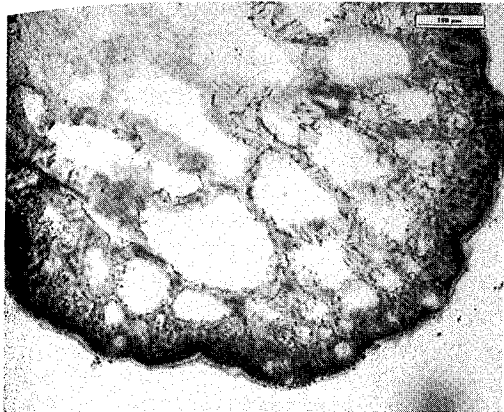


Figure 10. L.S. of the *verrucosa* type of conceptacles in *Gracilaria manilaensis*.

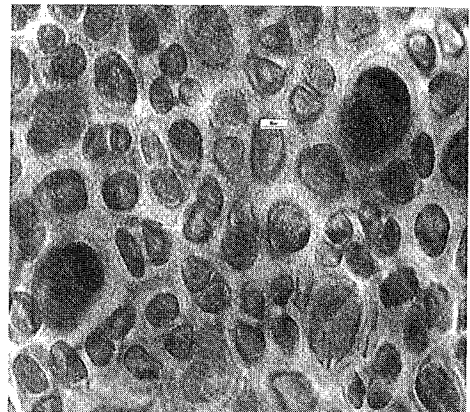


Figure 11. Surface view of male gametophyte showing tetrasporangia in *Gracilaria manilaensis*.

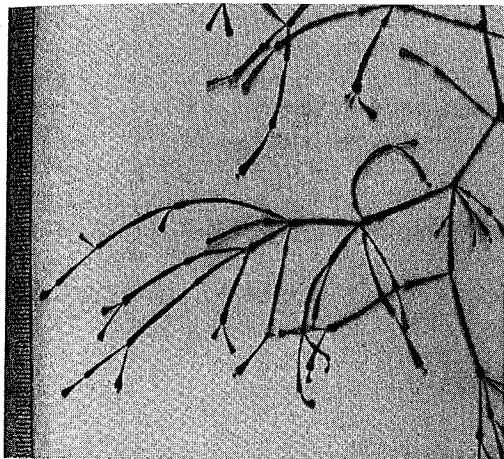


Figure 12. New branches of *Gracilaria salicornia* form at the end of each node.

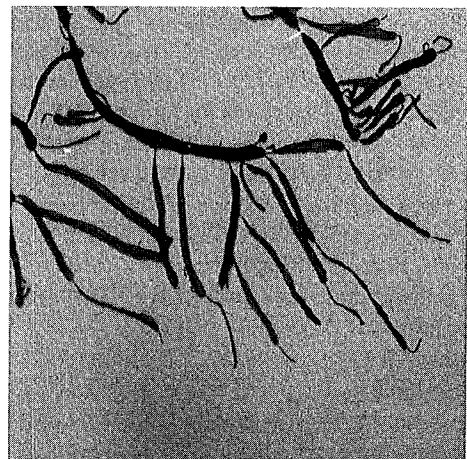


Figure 13. New branches form at any part of the node for *Gracilaria articulata*.

Gracilaria manilaensis Yamamoto et Trono, 1994[25]

Distribution : Philippines [25]

Specimens examined (PSM 6039-6044, PSM 6340-6343): Pantai Merdeka, Kedah; Gelang Patah, Johor.

This is a new record for Malaysia. The dark brownish red plant has cylindrical thalli reaching heights of up to 48 cm or longer. A main axis with a diameter up to 2.34 mm is present. The branches are abundant (Figure 6). The lateral branches are arranged in opposite, alternate or irregular order. The branching is mainly dichotomously but sometimes is trichotomous or irregular. The branches are constricted and form very fine thalli (Figure 7). The specimen sticks

to the herbarium paper upon drying. The cortical cells consist of one to two dense rows of protoplasmic cells which are arranged orderly. The medullary cells are polygonal, increasing in size towards the center (Figure 8). The maximum diameter of the cell is up to 450 μ m. The cystocarp is globose and seldom constricted at the base. The diameter of the cystocarp is up to 1025 μ m and the height is up to 800 μ m. There is a presence of nutritive filaments connecting the gonimoblast to the pericarp (Figure 9). The spermatangia are formed in conceptacles of *verrucosa* type (Figure 10). Conceptacles range from oval, roundish to cup-shaped with the depth up to 58.0 μ m and width up to 42.1 μ m and crowded by vegetative cells. The tetrasporangia

are scattered throughout the surface of the thallus except at the basal and apical portions of the plant (Figure 11). The cells are cruciately divided up to a maximum of 38 µm height and 27 µm width.

DISCUSSION

We agree with Plastino & Oliveira [26] that the identification of *Gracilaria* species which are terete, freely branched, and have deep subcortical spermatangial conceptacles is very difficult. Initially we identified *Gracilaria articulata* wrongly as *Gracilaria salicornia* as it has very distinct constrictions throughout the plant. Although it is slightly different from the type species of *Gracilaria salicornia*, we thought it could possibly be morphological differences due to environmental factors. It is different from *Gracilaria salicornia* in terms of the colour. *Gracilaria aarticulata* has bright red colour compared to *G. salicornia* which normally ranges from brownish to greenish red in colour. The bases of the thalli at points of constriction are much thinner in comparison to *G. salicornia*. Another major difference between the two species is that in *G. salicornia*, new branches will only be formed at the ends (Figure 12), however in *G. articulata*; new branches can grow from any part of the node (Figure 13). We also confirmed that *G. articulata* is different from *G. salicornia* by doing gene sequencing on *rbcL* (the large-subunit of *rubisco* gene) and the Genebank accession number is AY769259. The conceptacles of the spermatangia from both species belong to the *verrucosa* type. In this case the type of conceptacles cannot be used to differentiate the two species. Careful observations on the branching pattern and shape of thallus is very important for identification of the species. The slight morphological differences whether due to the plasticity of the species or are genetic-based, can only be confirmed through molecular analysis.

We also compared the specimens with the type species of *Gracilaria articulata* at the Seaweed Herbarium of the Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China. They are similar in terms of the gross morphology and the reproductive structures.

Gracilaria manilaensis has very similar gross morphology to *G. changii*. The major difference is the proliferous branching in *G. manilaensis*.

Yamamoto and Trono [25] also pointed out that, *G. manilaensis* has been misidentified as *G. verrucosa*, *G. lemaneiformis*, *G. tenuistipitata*, *G. blodgettii*, and *G. changii*. According to them, *G. manilaensis* is different from the above mentioned species due to the different type of conceptacles. However, we would like to point out that although *G. changii* is considered as having *verrucosa-polycavernosa* type of conceptacles, according to our observations, the *polycavernosa* type of conceptacles is very rare. Furthermore, Abbott *et al.* [2] showed that of the species which were placed under *Polycavernosa* [27; 28] not all of the specimens showed the variation of spermatangial arrangement from *verrucosa* to *polycavernosa* type, as was reported for *G. mixta*. Evidence is not strong enough to support the statement that the *polycavernosa* type of spermatangia is aggregated from the *verrucosa* type. Thus in this case, we strongly proposed that gene sequencing using *rbcL* gene should be conducted on *G. manilaensis* to further confirm its taxonomic position.

CONCLUSION

The identification of *Gracilaria* species, based on traditional approaches which are based on morphological structures, vegetative cells, reproductive structures, is a very tedious job and only trained phycologists can differentiate the species that are very similar in gross morphology. We propose that the identification of *Gracilaria* species should be based on a combination of morphological and molecular analysis. In many instances, the identification of morphologically similar species of *Gracilaria* that are also widely distributed geographically has been made possible using molecular analysis [6; 29-32].

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