Philippine Journal of Science
150 (S1): 165-171, Special Issue on Biodiversity
ISSN 0031 - 7683
Date Received: 29 Sep 2020

Taxonomy of the Gracilariaceae (Rhodophyta) from Western Macajalar Bay, Philippines

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Species of the marine red algal family Gracilariaceae are distributed in tropical and warm temperate waters of the world where they are harvested for their agar content. In the Philippines, the level of exploitation of these species is low despite their high diversity and potentially large harvestable biomass. A taxonomic survey of the Gracilariaceae was conducted along the western corridor of Macajalar Bay in northern Mindanao for the first time. Six common species were found in three sampling sites. The findings of this study will contribute to the baseline marine biodiversity information, can promote the use of the various species among coastal inhabitants, and provide guidance towards better policy formulation for more effective resource management.

Keywords: algae, agarophyte, biodiversity, Gracilariales, Mindanao, seaweed

INTRODUCTION

In the past several years, species of the marine red algal family Gracilariaceae have sparked global interest as a commercially important source of agar. They grow in tropical countries, particularly in Southeast Asia where high biomass is obtained from natural stocks and aquaculture facilities (Chen *et al*. 2009). In 2018, China was the world's top exporter of agar while Japan, the United States, and Russia were among the largest importers. The agar biopolymer has traditionally found its application in the microbiological (mainly as culture media) and food industries (gelatin products and food additives). Species of *Gracilaria* contain various chemicals with untapped bioactive and industrial potentials. But one of its latest and innovative applications is in the food packaging industry where agar-based films are developed to provide renewable, biodegradable, food-grade, and edible food packages (Mostafavi and Zaeim 2020) that can potentially help address the serious problem of plastics pollution and waste.

The tropical species of the Gracilariaceae have traditionally been recognized within the loosely defined genus *Gracilaria* with about 150 species until molecular data suggested recognizing them under segregate genera (Gurgel *et al*. 2018). The first major monographic work documenting the diversity of *Gracilaria sensu lato* in the Philippines was published by Trono *et al*. (1983), who described and reported eight species. Later, Silva *et al*. (1987) incorporated all previously published records, resulting in 24 species and two varieties of *Gracilaria* as occurring in the Philippines. The latest summary of Philippine marine algae documented 38 species, two

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varieties, and one form of the Gracilariaceae (Ang *et al*. 2013). Four new species of the Gracilariaceae have been described based on Philippine samples – namely, *Gracilaria salicornia* (C. Agardh) E.Y. Dawson and *G. manilaensis* Yamamoto & Trono both from Manila Bay, *G. sullivanii* Yamamoto & Trono from Batangas, and *Hydropuntia divergens* (B.M. Xia & I.A. Abbott) M.J. Wynne from Sorsogon (originally as *Polycavernosa divergens* B.M. Xia & I.A. Abbott).

Reports on the marine flora of the southern Philippine island of Mindanao are few and sporadic, compared to those emanating from the northern and central sections of the archipelago. The earliest report seems to be that of *Gracilaria dactyloides* Sonder by Dickie (1876), who studied the samples collected by the HMS Challenger Expedition that dropped anchor off Zamboanga. Other genera and species of the Gracilariaceae have also been reported from Mindanao, including undetermined species of *Polycavernosa* by Yamamoto (1989) and *Gracilariopsis* by Hurtado-Ponce and Liao (1998) – all from Zamboanga City.

The shores of Macajalar Bay in the northern section of Mindanao lie within the province of Misamis Oriental and are heavily impacted by industrial development and urbanization (Felisilda *et al*. 2019). Such impacts are mainly concentrated in the coastlines of the large city of Cagayan de Oro and suburban areas situated on the southern fringes of the Bay. A number of heavy industries have been built around Macajalar Bay and most of them very close to the coasts, thereby presenting potential sources of anthropogenic impact on the various marine ecosystems there – including mangrove forests, seagrass beds, and coral reefs. Very recently, marine pollution in the form of generated microplastics has been recently identified as a major concern (Kalnasa *et al*. 2019). In addition, algal blooms caused by a mixture of green macroalgae have been reported in the suburban areas and traceable to unregulated organic discharges to the sea from human settlements and industrial estates (Villaluz *et al*. 2016). The western corridor of Macajalar Bay is expected to see unabated economic and infrastructural development in the coming years as commercial traffic between the Cagayan de Oro metropolis and the new international airport in Laguindingan town to its west increases. Terrestrial and marine biota will expectedly be affected by further industrial development and anthropogenic outputs. Among the marine life there, seaweed communities that support fisheries and the marine food web will likely be impacted, if not already. This study accounts for the species of marine red algae belonging to the economically important Gracilariaceae along this corridor that is showing increased economic activity and development. The data may be useful in conserving these resources towards greater ecosystem stability and proper management and exploitation.

MATERIALS AND METHODS

Samples were collected from three sites along the intertidal areas of western Macajalar Bay at low tide during the transition of the dry and wet season of the year (September–November 2012). These sites were selected as they show high diversity of marine flora as observed in an earlier assessment (Quiaoit *et al*. 2010). The three sites are briefly described below:

1. Barangay Moog, Laguindingan, Misamis Oriental (8°36’6”N, 124°28’19”E)
   The site is located approximately 1 km from the eastern end of the runway of the Laguindingan International Airport and the nearest human settlement is about 1 km. It is characterized by hard substrate and coral rubbles. There were few patches of seagrass, while seaweed communities are scarce throughout the site.

2. Barangay Tambaling, El Salvador, Misamis Oriental (8°34’15”N, 124°30’50”E)
   It is located about 100 m from the edge of the Butuan-Cagayan-Iligan highway and adjacent to human settlements. The substrate is essentially made up of coral rubbles and muddy-sandy bottom. Dense seagrass beds can be found while seaweed individuals are sparsely distributed. Groyne structures are also present in the site, which could have influenced the water movement resulting in low water clarity during collection.

3. Barangay Poblacion, Opol, Misamis Oriental (8°31’8”N, 124°34’45”E)
   The site is adjacent to the Butuan-Cagayan-Iligan highway and lined with restaurants and human settlements with their effluents discharged directly to the shore. The substrate is a mixture of silt, mud, and sand, and progresses to a hard substrate with shallow tide pools. Seagrass beds can be found in few patches, while seaweed communities are common on the seaward portion. Bloom-forming green algae are also common, leaving little substrate for other biota and even covering some seagrass and seaweeds.

In all sites, representative samples of the Gracilariaceae were collected along ten parallel transect walks per sampling site, each transect walk 100 m long and 5 m apart from each other. Samples were placed in plastic bags and taken to the laboratory for photography and preservation, following the method of Hurtado *et al*. (2006). Samples were dried into herbarium specimens, curated, and deposited in the Xavier University Department of Biology herbarium. Cross-sections were made by freehand sectioning using a double-edged razor blade, rehydrated and stained with aniline blue-phenol solution, mounted

in glycerine medium on a clean microscope glass slide, and topped with a coverslip, which is then sealed on all sides by colorless nail polish. Sections were examined under a compound microscope, cellular features were measured using a stage micrometer, and photomicrographs taken with a digital camera mounted on the microscope. Samples were identified using pictorial field guides and taxonomic references. An emphasis is made to use and cite taxonomic references that are supplied with color photos that are freely accessible on the Internet to allow easier identification and unrestricted access for the layperson.

RESULTS AND DISCUSSION

A survey of the species of Gracilariaceae from three sites along the western shore of Macajalar Bay revealed six species – namely, *Gracilaria arcuata* Zanardini, *G. canaliculata* Sonder, *G. coronopifolia* J. Agardh, *G. salicornia* (C. Agardh) Dawson, *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq, and *H. eucheumatoides* (Harvey) Gurgel & Fredericq. These species are considered common elements in tropical reef habitats that are moderately impacted by anthropogenic activities. Each species is described below.

***Gracilaria arcuata* Zanardini**
Type locality: Aqaba, Jordan off the shores of northernmost Red Sea

References: Cordero 1977: 124, Pl. XX, Fig. B; Trono *et al.* 1983: 32, Fig. 7 (as var. *snackeyi* Weber Bosse); Yamamoto 1989: 35, Fig. 2; Yamamoto 1990: 146, Figs. 1, 2; Hurtado *et al.* 2006: 10; Lin 2009: 10, Figs. 2a–c

Fronds usually terete to slightly compressed, forming loose clumps; main axis cartilaginous, thick, 6–10 cm long, 3–4 mm wide, frequently curved or arcuate, attenuating into acute apices; branching irregularly alternate or dichotomous, becoming secund, corymbose, ultimate branchlets slightly curved with acute tips, issued in random directions, without basal constrictions; transition from cortex to medullary region abrupt; medullary cells arranged in 5–6 layers, generally roundish, 150–350 µm in diameter; infracortex of 1–2 layered cells, roundish, 20–30 µm in diameter, filled with floridean starch grains, bounded by an outer cortex of small globular, pigmented cells mostly two-layered, of which the outermost cells are ovoid.

Representative specimen examined: Escoro2012_01, Moog, Laguindingan, 04 Nov 2012

Remarks: The samples on hand may be referrable to *Gracilaria arcuata* var. *snackeyi* Weber Bosse (type locality: Makassar, Indonesia) due to their dense and corymbose habit with frequent upcurved distal portions (Trono 2004), different morphologically from the type variety from the Red Sea with arcuate thalli and secund branching. This variety is also the most frequently reported form throughout Southeast Asia.

***Gracilaria canaliculata* Sonder**
Type locality: Wagap, New Caledonia in the western Pacific

References: Calumpong and Meñez 1997: 179; Trono 2004: 81, Fig. 134; Hurtado *et al*. 2006: 10; Lin 2009: 14, Figs. 4a, b

Thallus forming stubby erect to sometimes slightly decumbent clumps, robust, cartilaginous, greenish to reddish throughout, erect, up to 20 mm high, attached by a discoid holdfast from where a single main axis arises; distinct stipe short, terete, tapering towards the base, to 6 mm long, constricted at base; branching mostly subdichotomous up to four times, with lowermost branches slightly constricted at the base and gradually enlarging distally; upper branches cylindrical with unconstricted bases and obtuse apices; transition of cell size from cortex to medulla abrupt; peripheral medullary cells up to 18 µm wide, polygonal to mostly spherical, increasing in size towards the center, up to 370 µm wide; cortex of 1–2 cells thick, each cortical cell up to 16 µm wide.

Representative specimens examined: Escoro2012_02, Escoro2012_03, Escoro2012_3, all from Poblacion, Opol, 25 Sep 2012

Remarks: This species was for some time confused with another closely similar species, *Gracilaria salicornia* (C. Agardh) Dawson due to their cylindrical and smooth thalli. The former is sometimes constricted especially in the more mature segments while the latter is consistently deeply constricted in all parts of the thallus, more frequently so in younger portions, producing narrowly clavate segments.

***Gracilaria coronopifolia* J. Agardh**
Type locality: O'ahu, Hawaiian Islands

References: Cordero 1977: 127, Pl. XX, Fig. A; Trono *et al*. 1983: 20, Figs. 2a–c; Yamamoto 1989: 36, Fig. 4; Yamamoto *et al*. 1999: 95, Figs. 1–11

Frond forming loosely caespitose clumps, brownish, bushy, softly cartilaginous in substance, arising from a small discoid holdfast, usually 10–30 cm tall; main axes cylindrical, slender to filiform, up to 2.5 mm in diameter, gradually attenuating distally to a sharp apex, repeatedly branched a few times in a divaricate manner, at times

adhering to one another; branchlets faintly constricted at the base, branching irregularly to subdichotomously many times, resulting to a corymbose outline of crowded, finely filiform branchlets; transition from cortical to medullary region abrupt throughout, consisting of 4–5 layers of large roundish and polygonal medullary cells, 20–200 µm in diameter; cortical layer consisting of 1–2 layers of cuboid to roundish cells, 6–10 µm in diameter.

Representative specimen examined: Escoro2012_07, Poblacion, Opol, 25 Sep 2012

Remarks: This identification is tentative due to the absence of fertile materials. Although most of the observed vegetative features compare well with the original description, authentic materials show "verrucosa" type of spermatangial conceptacles as a distinctive character. Yamamoto *et al*. (1999) and Lin (2009) had suggested that this species may not occur in the western Pacific Ocean and may be restricted to the Hawaiian Islands and that records of this commonly reported species from Southeast Asia should be reexamined. This species is superficially similar to *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq, with which it is usually confused. The latter, however, has "polycavernosa" type of spermatangial conceptacles.

***Gracilaria salicornia* (C. Agardh) E.Y. Dawson**
Type locality: seawall off Manila Bay, Philippines

References: Cordero 1977: 132, Figs. 116–118, Pl. XIX, Fig. C; Cordero 1980: 57, Pl. 38; Yamamoto 1989: 38, Figs. 12–14; Calumpong and Meñez 1997: 177; Liao and Hommersand 2003: 1212, Figs. 3a–h, 4a–I; Hurtado *et al*. 2006: 12; Lin 2009: 24, Figs. 8a, b

Frond loosely caespitose, cartilaginous, greenish to yellowish, attached to rocky substrate, 6–10 cm high, producing 1–3 fronds from a small basal disc; main axes terete, occurring in segments demarcated by distinct constrictions, each segment becoming elongated, clavate, proximal section 2–2.5 mm in diameter, becoming slightly inflated distally, giving off 2–5 branchlets issued from depressed apices, alternately or irregularly and repeatedly dichotomo-divaricately branched; branchlets conspicuously articulato-constricted at base when young, becoming less conspicuous in older parts; transition from cortical to medullary region gradual; medullary cells large, isodiametric, 100–400 µm in diameter; cortex consisting of two layers of small, anticlinally arranged pigmented cells, 30–50 µm long; cystocarps globose, prominently protruding, up to 1.8 mm in diameter, slightly constricted at the base; outer pericarp 240 µm in thickness, connected to gonimoblasts by numerous nutritive filaments.

Representative specimen examined: Escoro2012_06, Poblacion, Opol, 25 Sep 2012

Remarks: This species could be polymorphic depending upon different ecological conditions, as pointed out by Trono *et al*. (1983). This morphological variability and the lack of appreciation thereof could explain the variety of names applied to this species, all of which have been listed as synonyms by Xia (1986). Originally described based on materials collected from Manila Bay, the species has been reported across the wide expanse of the Indo-Pacific, from the Red Sea in the west to the Hawaiian Islands to the east where it is considered an invasive species to this day.

***Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq**
Type locality: 'India orientalis," likely Ambon in Indonesia

References: Cordero 1977: 128, Fig. 111 (as *Gracilaria edulis*); Yamamoto 1989: 39, Figs. 8, 9 (as *Polycavernosa* sp.); Liao and Hommersand 2003: 1213, Figs. 5a–g, 6a–i (as *G. edulis*); Trono 2004: 82, Fig. 135 (as *G. fastigiata*); Hurtado *et al*. 2006: 11 (as *G. edulis*); Lin 2009: 33, Figs. 11a–d

Frond frequently entangled with other algae, forming soft caespitose clumps, brownish to light straw colored, to 30 cm tall, attached to the substrate by a single discoidal holdfast; main axis percurrent, ca. 2.5 mm in diameter, repeatedly branching a few times forming short internodes every 2–5 mm, with frequently subdichotomously branchlets, basal constrictions absent, gradually attenuating upwards terminating in cervicorn apices, forming loosely corymbose distal parts; transition between medullary and cortical regions abrupt; consisting of 4–5 layers of medullary cells, ovoid to sometimes polygonal, thin-walled, variable in size, from 20–200 µm in diameter; cortical region consists of 1–2 layers of cuboid to roundish cortical cells, 6–10 µm in diameter

Representative specimen examined: Escoro2012_08, Tambaling, El Salvador, 12 Oct 2012

Remarks: This species may show some polymorphism in the branching pattern of its generally filiform branches which makes it easily confused with species of similar morphology. Years back, most of the Philippine reports of *Gracilaria* with fine branches are instantly referred to as the once-cosmopolitan *G. verrucosa* (Hudson) Papenfuss. This name is now applied in a limited sense to a species found in Western Europe under the earlier name *Gracilariopsis longissima* (S.G. Gmelin) Steentoft, L.M. Irvine & Farnham. Previous reports of *G. verrucosa* have been reexamined and given other names, among them, *H. edulis*.

***Hydropuntia eucheumatoides* (Harvey) Gurgel & Fredericq**
Type locality: Ryukyu Islands, in southwestern Japan

References: Cordero 1977: 129, Pl. XX, Fig. C (as *Gracilaria eucheumioides*); Cordero 1980: 57, pl. 37, Figs. A, B (*as G. eucheumioides*); Trono *et al*. 1983: 27, Figs. 5a–d (as *G. eucheumoides*); Yamamoto 1989: 36, Fig. 5 (as *G. eucheumoides*); Liao and Hommersand 2003: 1217, Figs. 9a–h (as *G. eucheumatoides*); Hurtado *et al*. 2006: 12 (as *G. eucheumatoides*); Lin 2009: 35, Figs. 11a, b

Thalli forming decumbent clumps, attached to the substrate at many points, compressed to flattened, dark purplish, cartilaginous, 3–5 mm thick, 7–10 mm wide; branching irregular or somewhat pinnate in the opposite manner; branches compressed, with distinct dorsal and ventral surfaces, somewhat subcylindrical at some portions, with short club-shaped, proliferous or dentate margins on opposite sides or sometimes on the surface; transition of cortical region to medullary tissues gradual; cortical layer consisting of 1–2 rows of roundish cells, 3–5 µm in diameter; medulla consisting of about 15 layers of roundish cells, 10–20 µm in diameter, except in the outer apical region of the medulla where meristematic cells are elongated vertically outward to the surface, 10–35 µm long

Representative specimen examined: Escoro2012_05, Poblacion, Opol, 25 Sep 2012

Remarks: This is one of the most distinctive species in the field because of its decumbent habit and slightly compressed branches showing some degree of dorsiventrality and lined with small marginal teeth. Due to its creeping habit, it is often covered with a thin layer of sand with the dorsal surface often epiphytized by crustose coralline algae, appearing lighter than the dark purple ventral surface.

Six species of the economically important marine red algal family Gracilariaceae have been documented from the western shores of Macajalar Bay, including four species of *Gracilaria* and two species of *Hydropuntia*. Most of them have economic importance as sources of agar and have in fact been subject to commercial exploitation in many tropical countries where they occur (Chen *et al*. 2009). In the Philippines, the exploitation of the Gracilariaceae has been minimal owing to the more popular and more highly valued carrageenophytes such as *Eucheuma* and *Kappaphycus* species. The local agar market, while very small compared to the demand for the carrageenan-bearing species, is largely fulfilled by harvesting natural stocks of the agarophyte, *Gelidiella acerosa* (Forsskål) Feldmann & Hamel (Abanil *et al*. 1984). In Macajalar Bay, the potential harvestable biomass of the Gracilariaceae is unknown but data from the current study should encourage further surveys, biomass measurements, and seasonality studies as a matter of priority especially in areas experiencing rapid economic and infrastructural development. Overwhelming evidence available in the literature outlines the many deleterious impacts of urbanization on seaweed communities and, indeed, on the entire benthic ecosystems (Fowles *et al*. 2018).

This report appears to be among the first to recognize the genus *Hydropuntia* Montagne as applied to species occurring in the Philippines. The genus *Polycavernosa* Chang & Xia was erected to accommodate species of *Gracilaria*, which show a polycavernous configuration of the spermatangial conceptacles (Chang and Xia 1963). The nature of the male sexual structure was first recognized for use in the subgeneric classification within the big genus by Yamamoto (1975), who at that time was not aware of the paper by Chang and Xia (1963) as scientific information coming out of China was restricted. The multicavitied spermatangial structure was first documented in *G. henriquesiana* Hariot from Ghana, hence the name "henriquesiana" type spermatangia. Unfortunately, the name *Polycavernosa* published in 1963 was preempted by the name *Hydropuntia* proposed in 1842. Molecular evidence was subsequently used to justify the recognition of *Hydropuntia* as an independent genus by Lin (2009), Gurgel *et al*. (2018), and others, although there are those with contrasting taxonomic interpretations such as Abbott *et al*. (1989), Lyra *et al*. (2015), to name a few.

Compared to similar habitats around the Philippines for which seaweed species have been surveyed, the shores of western Macajalar Bay may be said to be comparable in terms of species number and diversity. All species encountered in this study are common elements for which their potential commercial value has not been assessed in detail pending a greater market demand nationally, among other factors. Due to the rapid urbanization witnessed in portions of the western Macajalar Bay, the need to further survey the seaweed and other marine resources become more urgent to provide baseline information for better resource management and policy formulation. For instance, it will be useful to know the present level of seaweed use among the population residing in this corridor and consider introducing more locally abundant species like those of the Gracilariaceae for food and other applications. The academe, fisheries extension, and economic policy offices have important roles to play and must cooperate to promote seaweeds as food and/or aquaculture ventures for income generation, poverty alleviation, and greater food security within the coastal communities, which are known among the poorest sectors of Philippine society.

ACKNOWLEDGMENTS

This work was submitted by the first author to Xavier University – Ateneo de Cagayan as part of an undergraduate thesis. The comments from two anonymous reviewers are much appreciated. The authors declare no conflict of interest in the conduct of this study.

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