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A new flattened species of *Gracilariopsis* (Gracilariales, Rhodophyta) from Japan

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RUNNING TITLE

Gracilariopsis mageshimensis sp. nov. from Japan

ABSTRACT

A new flattened species of *Gracilariopsis* from Mageshima Island, Japan is recognized based on morphological and molecular analyses of *rbc*L and *cox*1 DNA sequences. We name this new species *Gp*. *mageshimensis sp*. *nov*. The *rbc*L and *cox*1 analyses suggest that *Gp*. *mageshimensis* belongs to the *Gracilariopsis* clade, and that it is distinct from other *Gracilariopsis* species. *Gracilariopsis mageshimensis* is characterized by a flattened thallus, the lack of tubular nutritive cells, the lower gonimoblasts connect to the cystocarp floor cells via pit connections and small gonimoblasts filling the cystocarp cavity. The *Gp*. *mageshimensis* cystocarp is in accordance with the morphological concept of *Gracilariopsis*. *Gracilariopsis mageshimensis* is the second *Gracilariopsis* species with a flattened thallus to be described worldwide. *Gracilariopsis mageshimensis* is distinguished from *Gp*. *silvana*, the first flattened species by the rare production of lateral branches, a medulla consisting of fewer than five layers, and its deep-water habitat.

KEYWORDS

*cox*1; Gracilariaceae; *Gracilariopsis mageshimensis sp*. *nov*.; *rbc*L; Phylogeny; Taxonomy

INTRODUCTION

The marine red algal genus *Gracilariopsis* (= *Gp*.) in the family Gracilariaceae was originally described by Dawson (1949) based on *Gp*. *sjoestedtii* (Kylin) E.Y. Dawson. Several authors had considered *Gracilariopsis* indistinct from *Gracilaria* (= *G*.) (e.g. Papenfuss 1967; Yamamoto 1978; Gargiulo *et al*. 1992). More recent generic concepts of *Gracilariopsis* are based on morphological features and molecular data, and the distinctiveness of *Gracilariopsis* within

Gracilariaceae has been confirmed several times (e.g., Gurgel *et al*. 2003b, 2018; Lin 2008; Muangmai *et al*. 2014). *Gracilariopsis* is widely distributed in temperate, subtropical, and tropical waters worldwide. To date, 25 species of this genus have been described (Guiry & Guiry 2020), two of which, *Gp*. *chiangii* Showe M. Lin and *Gp*. *chorda* (Holmes) Ohmi, are known to occur in Japan (Yang & Kim 2015; Yoshida *et al*. 2015). Most *Gracilariopsis* species have cylindrical thalli, and *Gp*. *silvana* Gurgel, Fredericq & J.N. Norris described from Venezuela is the only known species of *Gracilariopsis* with a flattened thallus (Gurgel *et al*. 2003a). Since its description, *Gp*. *silvana* has been reported from Brazil (Soares *et al*. 2018). *Gracilariopsis silvana* seems to be confined to the tropical Western Atlantic Ocean.

We collected a gracilariacean species with flattened thalli from an offshore Japanese island, Mageshima. The species appeared to differ from known Japanese species, and we examined it molecularly and morphologically.

MATERIALS AND METHODS

The specimens were collected from the seafloor at a depth of 35 m offshore of Mageshima, Osumi Islands, Kagoshima Prefecture, southern Japan, by dredging (T/S *Nansei-Maru*, Faculty of Fisheries, Kagoshima University) (Table S1). For molecular analyses, specimens were quickly dried in silica gel. For anatomical observations, specimens were preserved in 5% formalin, and voucher herbarium specimens were deposited at the National Museum of Nature and Science, Japan (TNS-AL 213795–213800, 213913).

For molecular phylogenetic analyses, partial *rbc*L and *cox*1 genes were sequenced. Genomic DNA was extracted and sequenced as described previously (Suzuki *et al*. 2016). The *rbc*L and *cox*1 sequences of seven *Gracilariopsis* specimens were sequenced (Table S1). For *rbc*L analysis, we compiled sequence data available from GenBank for 15 *Gracilariopsis* species and four genera belonging to Gracilarioideae. For *cox*1 analysis, we compiled sequence data available from GenBank for 12 *Gracilariopsis* species and four genera belonging to Gracilarioideae. As Gracilarioideae has been resolved as a monophyletic group (Gurgel *et al*. 2018), three taxa belonging to Melanthalioideae were designated as outgroups in all analyses. The 40 *rbc*L and 48 *cox*1 sequences were aligned using ClustalW (Larkin *et al*. 2007). Samples with identical nucleotide sequences were removed, so that the data matrix included 33 *rbc*L taxa (1,257 bp) and 39 *cox*1 taxa (573 bp). The *rbc*L and *cox*1 sequences were subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. For both analyses, the alignment was partitioned by codon, and distinct models were applied to each partition. The substitution models for ML and BI are summarised in Table S2. ML analyses were performed using RAxML-NG v1.0.1 (Kozlov *et al*. 2019). Bootstrap values (BP) for the ML analysis were calculated based on 1,000 pseudoreplications. BI analyses were performed using MrBayes 3.2.7a (Ronquist *et al*. 2012), as described previously (Suzuki *et al*. 2012). The *p* distances for each pair of *Gracilariopsis* species were calculated using PAUP 4.0b10 (Swofford 2002).

For anatomical observations, specimens were sectioned by hand or using a freezing microtome (MA-101, Komatsu Electronics, Komatsu, Japan). Sections were stained with 1% cotton blue, acidified with 1% HCl, and mounted in 50% aqueous Karo syrup with 3% formalin to prevent microbial growth. Photomicrographs were taken using a BX51 microscope (Olympus, Tokyo, Japan) with an ATZ digital camera (Kenis, Tokyo, Japan). Drawings were made with the aid of a camera lucida.

RESULTS

All seven *rbc*L and *cox*1 DNA sequences of the samples from Mageshima were identical. Both *rbc*L and *cox*1 phylogenetic analyses generated using ML and BI analyses had similar topologies except for poorly supported branches (Figs 1, 2). In the *rbc*L analyses, the samples from Mageshima were positioned sister to the remaining *Gracilariopsis* species but without support (BP = 42%; PP = 0.66). In the *cox*1 analyses, the samples from Mageshima were sister to the clade that included *Gp*. *heteroclada* J.F. Zhang & B.M. Xia and *Gp*. *mclachlanii* Buriyo, Bellorin & M.C. Oliveira again without low support (BP = 68%; <0.5 PP). The *rbc*L sequence divergence between the samples from Mageshima and other *Gracilariopsis* species varied between 2.9 and 7.2%. The *cox*1 sequence divergence between the samples from Mageshima and other *Gracilariopsis* species varied between 8.1 and 12.2%. Although the tree topologies differed somewhat, both suggest that the samples from Mageshima are a distinct and new species of *Gracilariopsis*, which we propose as:

***Gracilariopsis mageshimensis* Mas. Suzuki & R. Terada *sp. nov*.**

Figs 3–14

DESCRIPTION: Thallus flattened, approaching 17 cm in height, brownish-red in colour. Blades irregularly dichotomously branched, rarely producing lateral branches, lacerated, approaching 4 cm in width. Thallus composed of cortex and medulla. The outer cortex composed of two to three cell layers. The medulla composed of two to three layers of large cells, with one to two layers of subcortical cells. Male thallus not observed. Cystocarps hemispherical, scattered over the blades. Carpogonial branch borne on an intercalary supporting cell and consisting of a carpogonium and hypogynous cell. The supporting cell bears a pair of two- to three-celled sterile filaments. The gonimoblast initials are cut off from the fusion cell. The lower gonimoblasts connect with the cystocarp floor cells via pit connections. Small gonimoblasts fill the cystocarp cavity. Tubular nutritive cells absent. Pericarp is 9–12 cell layers thick. Tetrasporangia scattered over both surfaces of blades, round to oblong, cruciately divided.

HOLOTYPE: TNS-AL 213799, female gametophyte, collected 30 May 2019 by R. Terada, deposited in the National Museum of Nature and Science, Japan (TNS). DDBJ Accession numbers: *cox*1: LC589294, *rbc*L: LC589283.

ISOTYPE: TNS-AL 213800, tetrasporophyte, collected 30 May 2019 by R. Terada, deposited in TNS. DDBJ Accession numbers: *cox*1: LC589295, *rbc*L: LC589284.

OTHER SPECIMENS EXAMINED: TNS-AL 213795-213798 and 213913, deposited in TNS (Table S1).

TYPE LOCALITY: 30°43′N, 130°49′E, offshore of Mageshima Island, Kagoshima Prefecture, Japan.

ETYMOLOGY: The specific epithet refers to the collection locality, Mageshima Island.

JAPANESE NAME: Magekabanori (new name).

Erect thalli arising from a discoidal holdfast, flattened throughout, approaching 17 cm in height, brownish-red in colour. Blades irregularly dichotomously branched, approaching 4 cm in width, rarely producing lateral branches, and often irregularly lacerated (Figs 3, 4). Thallus solid, comprising a cortex and medulla. Outer cortex composed of two to three cell layers. Cortical cells ovoid, pigmented, 3–9 × 4–18 µm. Medulla composed of two to three layers of large cells, with one to two layers of subcortical cells (Figs 5, 6, S1). Medullary cells compressed laterally, 85–457 × 126–670 µm.

Cystocarps hemispherical, scattered over the blades (Fig. 3). Carpogonial branches borne on an intercalary supporting cell and consisting of a carpogonium and hypogynous cell. The supporting cell bears a pair of two- to three-celled sterile filaments (Figs 7, S3, S4). After presumed fertilization, the hypogynous cell fuses to an adjacent cell (Figs 8, S5). Gonimoblast initials are cut off from the resulting fusion cell (Figs 9, S6). The fusion cell cuts off more gonimoblast initials bilaterally (Fig. S7). As the carposporophyte grows, the pericarp becomes thicker (Figs 10, 11). During the early stages of gonimoblast development, lower gonimoblasts connect with the floor cells of cystocarp via pit connections (Figs 12, S8). In the nearly mature stage, dense, small gonimoblasts fill the cystocarp cavity (Fig. 13). The base of cystocarp is not constricted (Fig. 13). Tubular nutritive cells are absent. The pericarp is 9–12 cell layers thick. Mature cystocarp and carposporangia were not observed. Male thalli were also not observed. Tetrasporangia were scattered over both surfaces of blades, round to oblong, and cruciately divided (Figs 14, S2). Mature tetrasporangia 39–48 µm long and 29–33 µm in transverse diameter.

DISCUSSION

Our morphological and molecular analyses clearly indicate that these specimens are a new species of *Gracilariopsis* in Japan. *Gracilariopsis* is characterized morphologically by superficial spermatangia, the absence of tubular nutritive cells, lower gonimoblast filaments linked to the cystocarp base through secondary pit connections, and a gonimoblast mass composed of small cells (Gurgel *et al*. 2003b, 2018; Lin 2008; Muangmai *et al*. 2014). Although the male reproductive organ and fully mature cystocarp were not observed, the characteristics of the *Gp*. *mageshimensis* cystocarp, such as the absence of tubular nutritive cells within the cystocarp, the attachment between gonimoblasts and cystocarp floor cells, and small gonimoblasts filling the nearly mature cystocarp cavity, are in accordance with the morphological concept of *Gracilariopsis*.

Both the *rbc*L and *cox*1 analyses suggest that *Gp*. *mageshimensis* is distinct from other *Gracilariopsis* species. *Gracilariopsis mageshimensis* is the second *Gracilariopsis* species with a flattened thallus. *Gracilariopsis silvana*, the first flattened species, is recorded from Venezuela and Brazil and characterized by flattened thalli, that are sometimes slightly undulated and subdichotomously or irregularly branched, with branches decreasing in width distally, lateral branches arising mostly from the thallus margin, a medulla consisting of 5–6(9) layers, and a constriction at the base of the cystocarp (Gurgel *et al*. 2003a; Soares *et al*. 2018). *Gracilariopsis mageshimensis* is distinguished from *Gp*. *silvana* by the rare production of lateral branches and a medulla consisting of fewer than five layers (including subcortical cells) (Table 1). Furthermore, the constriction at the cystocarp base was not observed at least in nearly mature cystocarps of *Gp*. *mageshimensis*. Gurgel *et al*. (2003a) and Soares *et al*. (2018) collected *Gp*. *silvana* specimens by hand in the shallow subtidal zone, or in tidal pools, whereas *Gp*. *mageshimensis* seems to be a deep-water species.

Gracilariopsis mageshimensis was collected from the seafloor offshore of Mageshima Island at a depth of 35 m. Although *Gp*. *mageshimensis* has not been collected from other regions in Japan, it has probably been misidentified before as a flattened *Gracilaria* species growing in the sublittoral zone due to superficial similarities. Tanaka (1963) recorded *G*. *sublittoralis* Yamada & Segawa *ex* H. Yamamoto, a flattened species of *Gracilaria*, from offshore Mageshima Island, and it is reportedly widely distributed in the sublittoral zone from middle to southern Japan (Yamamoto 1978, 1994). *Gracilaria sublittoralis* has deep cortical spermatangial conceptacles and tubular nutritive cells within the cystocarp (Yamamoto 1978, 1994). Although the reproductive structures clearly show that *G*. *sublittoralis* is a member of *Gracilaria*, the outer appearance of *G*. *sublittoralis* resembles that of *Gp*. *mageshimensis* (Table

1). Vegetative *Gp. mageshimensis* could be misidentified as *G. sublittoralis*. Further investigations based on molecular and reproductive characteristics of more specimens of *G. sublittoralis* and other flattened species of Gracilariaceae are required to clarify the occurrence and diversity of *Gp. mageshimensis* in Japan and the north-western Pacific.

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FIGURES

Figs 1-2. Phylogenetic trees.

Fig. 1. Maximum Likelihood (ML) phylogeny based on *rbc*L DNA sequences. Numbers at the branches indicate the bootstrap values (BP) / Bayesian posterior probabilities (PP), respectively. Only the BP (≥ 50%) and PP (≥ 0.95) are shown. * = 100% BP and 1.00 PP.

Fig. 2. ML phylogeny based on *cox*1 DNA sequences. Numbers at the branches indicate the BP / PP, respectively. Only the BP (≥ 50%) and PP (≥ 0.95) are shown. * = 100% BP and 1.00 PP.

Figs 3–4. Habits of *Gracilariopsis mageshimensis sp. nov.* from Mageshima Island, Japan.

Fig. 3. Holotype, female gametophyte (TNS-AL 213799). Scale bar = 1 cm.

Fig. 4. Tetrasporophyte (TNS-AL 213796). Scale bar = 1 cm.

Figs 5–14. Vegetative and reproductive structures of *Gracilariopsis mageshimensis sp. nov.*

Fig. 5. Transverse section of thallus (TNS-AL 213799). Scale bar = 100 µm.

Fig. 6. Close-up of cortex (TNS-AL 213799). Scale bar = 10 µm.

Fig. 7. Carpogonial branch apparatus (TNS-AL 213799) showing sterile cells (arrowheads),

supporting cell (sc), hypogynous cell (hy) and carpogonium (cp) with trichogyne (tr). Scale bar = 5 µm.

Fig. 8. An early post-fertilization stage (TNS-AL 213799) showing the hypogynous cell (hy) fusing with an adjacent cell (arrow). Scale bar = 10 µm.

Fig. 9. An early post-fertilization stage (TNS-AL 213799) showing forming pericarp and a gonimoblast initial (gi) cut off from a fusion cell (fc). Scale bar = 20 µm.

Fig. 10. Transverse section of young cystocarp (TNS-AL 213799) showing a young carposporophyte and the cavity of cystocarp. Scale bar = 50 µm.

Fig. 11. Transverse section of immature cystocarp (TNS-AL 213799) showing fusion cell (fc). Scale bar = 50 µm.

Fig. 12. Close-up of immature carposporophyte (TNS-AL 213799) showing pit-connections (arrowheads) between lower gonimoblast cells and cystocarp floor cells. Scale bar = 20 µm.

Fig. 13. Transverse section of nearly mature cystocarp (TNS-AL 213797) showing dense, small-sized gonimoblast filaments. Scale bar = 200 µm.

Fig. 14. Transverse section of tetrasporophyte (TNS-AL 213796) showing a mature tetrasporangium. Scale bar = 20 µm.

Figs S1–S2. Hand drawings of vegetative and reproductive structures of *Gracilariopsis mageshimensis sp. nov.*

Fig. S1. Transverse section of thallus (TNS-AL 213799) showing cortical, subcortical and medullary cells. Scale bar = 50 µm.

Fig. S2. Transverse section of tetrasporophyte (TNS-AL 213796) showing cruciately divided tetrasporangia. Scale bar = 10 µm.

Figs S3–S8. Hand drawings of carposporophyte development of *Gracilariopsis mageshimensis sp. nov.*

Fig. S3. Carpogonial branch apparatus (TNS-AL 213799) showing sterile cells (arrowheads), supporting cell (sc), hypogynous cell (hy) and carpogonium (cp) with trichogyne (tr). Scale bar = 5 µm.

Fig. S4. Carpogonial branch apparatus (TNS-AL 213799) showing sterile cells (arrowheads), supporting cell (sc), hypogynous cell (hy) and carpogonium (cp) with trichogyne (tr). Scale bar = 5 µm. Note that one of the sterile cell branches is composed of three sterile cells.

Fig. S5. An early post-fertilization stage (TNS-AL 213799) showing a hypogenous cell (hy) fusing with an adjacent cell (arrow). Scale bar = 10 µm.

Fig. S6. An early post-fertilization stage (TNS-AL 213799) showing pericarp and a gonimoblast initial (gi) cut off from a fusion cell (fc). Scale bar = 10 µm.

Fig. S7. A young carposporophyte (TNS-AL 213799) showing a fusion cell (fc) bearing gonimoblast cells. Scale bar = 10 µm.

Fig. S8. A carposporophyte at a later stage (TNS-AL 213797) showing a fusion cell (fc) bearing more gonimoblast cells. Note that lower gonimoblast cells and cystocarp floor cells are connected by pit-connections (arrowheads). Scale bar = 10 µm.

Table S1. Collection locations and details, and INSD (DDBJ/EMBL/GenBank) accession numbers of samples used in the *rbc*L and *cox*1 gene sequence analyses. Accession numbers in bold were determined for this study.

Table S2. Substitution models for the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses on the basis of *rbc*L and *cox*1 sequences.

Table 1. Comparison of diagnostic characteristics among *Gracilariopsis mageshimensis, Gp. silvana* and *Gracilaria sublittoralis*.

Species	*Gp. mageshimensis*	*Gp. silvana*	*G. sublittoralis*
Habitat	The seafloor at a depth of 35 m	Intertidal, just below intertidal, tidal pool	The seafloor at a depth of 10–40 m
Height	17 cm	14–20 cm	15–25 cm
Blade	Flattened, lacerated	Flattened, slightly undulated	Flattened
Width of blade	4 cm	1–3 cm	3–5 cm
Branching pattern	Dichotomously, irregularly	Dichotomously, subdichotomously or irregularly	Dichotomously, trichotomously
Lateral branches	Rarely present	Often present	Rarely present
Cortex	2–3 layers	1–3 layers	1–2 layers
Medulla	4–5 layers [1]	5–6 (9) layers	3–5 layers
Spermatangia	N/A [2]	N/A [2]	Confine to deep cortical conceptacles
Size of gonimoblasts in the cystocarp cavity	Small	Small	Rather small and elongated
Connection between lower gonimoblasts	Present	Present	N/A [3]

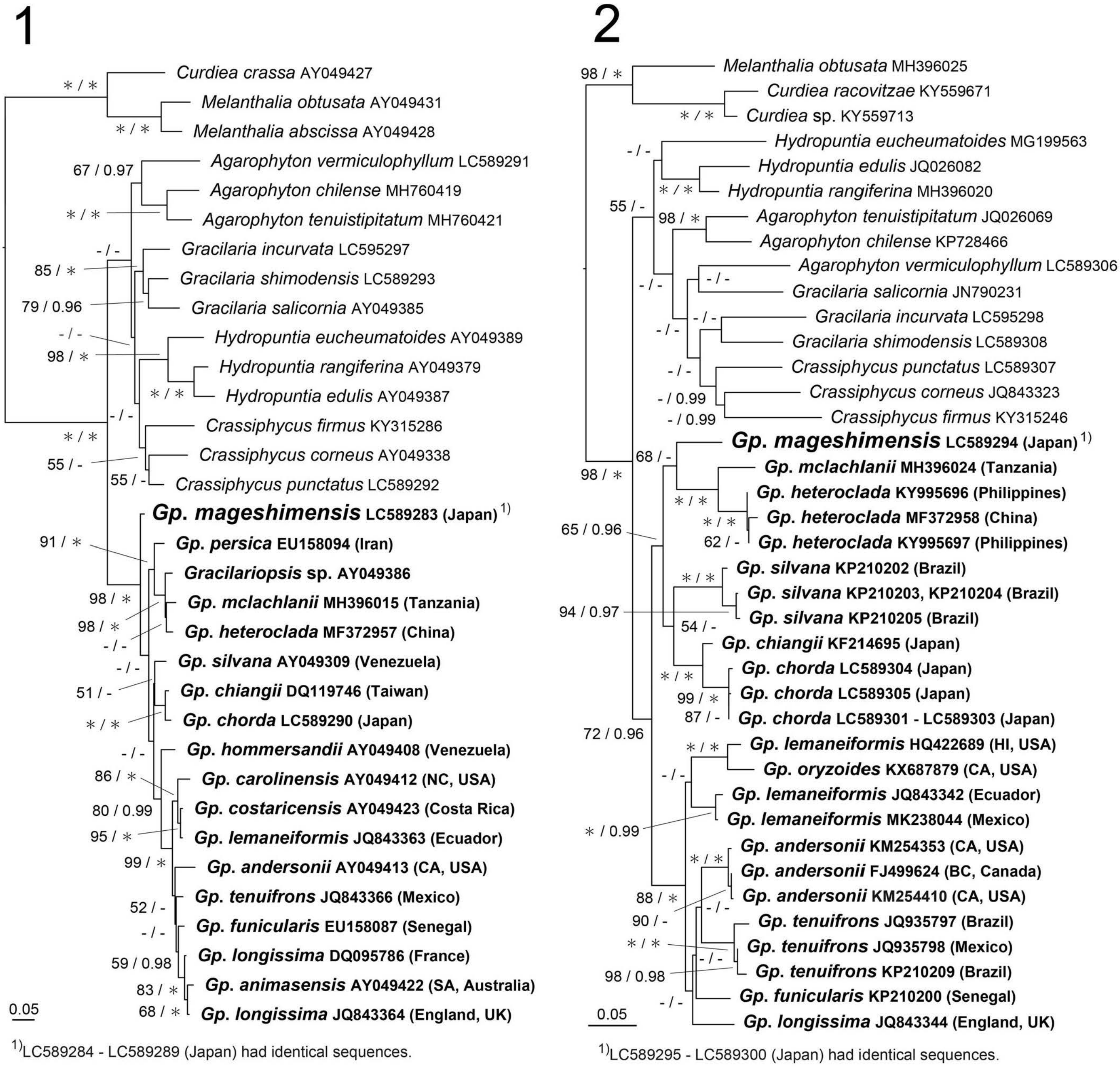
and the floor cells of cystocarp			
Tubular nutritive cell	Absent	Absent	Present
Constriction at the base of cystocarp	N/A [4]	Present	Present
Thick of pericarp	9–12 cell layers	12–14 cell layers	N/A [3]
References	This study	Gurgel *et al*. (2003a), Soares *et al*. (2018)	Tanaka (1963), Yamamoto (1978, 1994)

[1] Including subcortical cells.

[2] Male gametophyte and spermatangia were not observed.

[3] Tanaka (1963) and Yamamoto (1978, 1994) did not mention these characteristics.

[4] Mature cystocarp was not observed.


1
Curdiea crassa AY049427
Melanthalia obtusata AY049431
Melanthalia abscissa AY049428
Agarophyton vermiculophyllum LC589291
Agarophyton chilense MH760419
Agarophyton tenuistipitatum MH760421
Gracilaria incurvata LC595297
Gracilaria shimodensis LC589293
Gracilaria salicornia AY049385
Hydropuntia eucheumatoides AY049389
Hydropuntia rangiferina AY049379
Hydropuntia edulis AY049387
Crassiphycus firmus KY315286
Crassiphycus corneus AY049338
Crassiphycus punctatus LC589292
Gp. mageshimensis LC589283 (Japan) 1)
Gp. persica EU158094 (Iran)
Gracilariopsis sp. AY049386
Gp. mclachlanii MH396015 (Tanzania)
Gp. heteroclada MF372957 (China)
Gp. silvana AY049309 (Venezuela)
Gp. chiangii DQ119746 (Taiwan)
Gp. chorda LC589290 (Japan)
Gp. hommersandii AY049408 (Venezuela)
Gp. carolinensis AY049412 (NC, USA)
Gp. costaricensis AY049423 (Costa Rica)
Gp. lemaneiformis JQ843363 (Ecuador)
Gp. andersonii AY049413 (CA, USA)
Gp. tenuifrons JQ843366 (Mexico)
Gp. funicularis EU158087 (Senegal)
Gp. longissima DQ095786 (France)
Gp. animasensis AY049422 (SA, Australia)
Gp. longissima JQ843364 (England, UK)
0.05
1)LC589284 - LC589289 (Japan) had identical sequences.
2
Melanthalia obtusata MH396025
Curdiea racovitzae KY559671
Curdiea sp. KY559713
Hydropuntia eucheumatoides MG199563
Hydropuntia edulis JQ026082
Hydropuntia rangiferina MH396020
Agarophyton tenuistipitatum JQ026069
Agarophyton chilense KP728466
Agarophyton vermiculophyllum LC589306
Gracilaria salicornia JN790231
Gracilaria incurvata LC595298
Gracilaria shimodensis LC589308
Crassiphycus punctatus LC589307
Crassiphycus corneus JQ843323
Crassiphycus firmus KY315246
Gp. mageshimensis LC589294 (Japan) 1)
Gp. mclachlanii MH396024 (Tanzania)
Gp. heteroclada KY995696 (Philippines)
Gp. heteroclada MF372958 (China)
Gp. heteroclada KY995697 (Philippines)
Gp. silvana KP210202 (Brazil)
Gp. silvana KP210203, KP210204 (Brazil)
Gp. silvana KP210205 (Brazil)
Gp. chiangii KF214695 (Japan)
Gp. chorda LC589304 (Japan)
Gp. chorda LC589305 (Japan)
Gp. chorda LC589301 - LC589303 (Japan)
Gp. lemaneiformis HQ422689 (HI, USA)
Gp. oryzoides KX687879 (CA, USA)
Gp. lemaneiformis JQ843342 (Ecuador)
Gp. lemaneiformis MK238044 (Mexico)
Gp. andersonii KM254353 (CA, USA)
Gp. andersonii FJ499624 (BC, Canada)
Gp. andersonii KM254410 (CA, USA)
Gp. tenuifrons JQ935797 (Brazil)
Gp. tenuifrons JQ935798 (Mexico)
Gp. tenuifrons KP210209 (Brazil)
Gp. funicularis KP210200 (Senegal)
Gp. longissima JQ843344 (England, UK)
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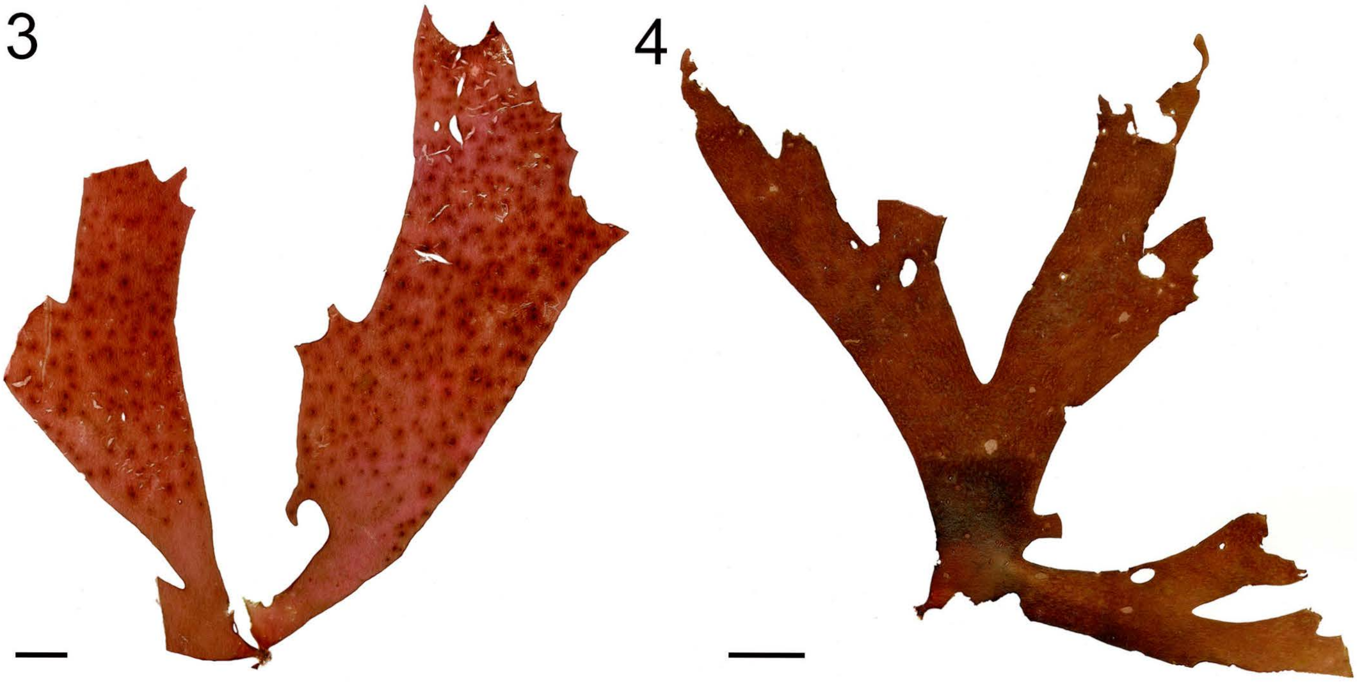
1)LC589295 - LC589300 (Japan) had identical sequences.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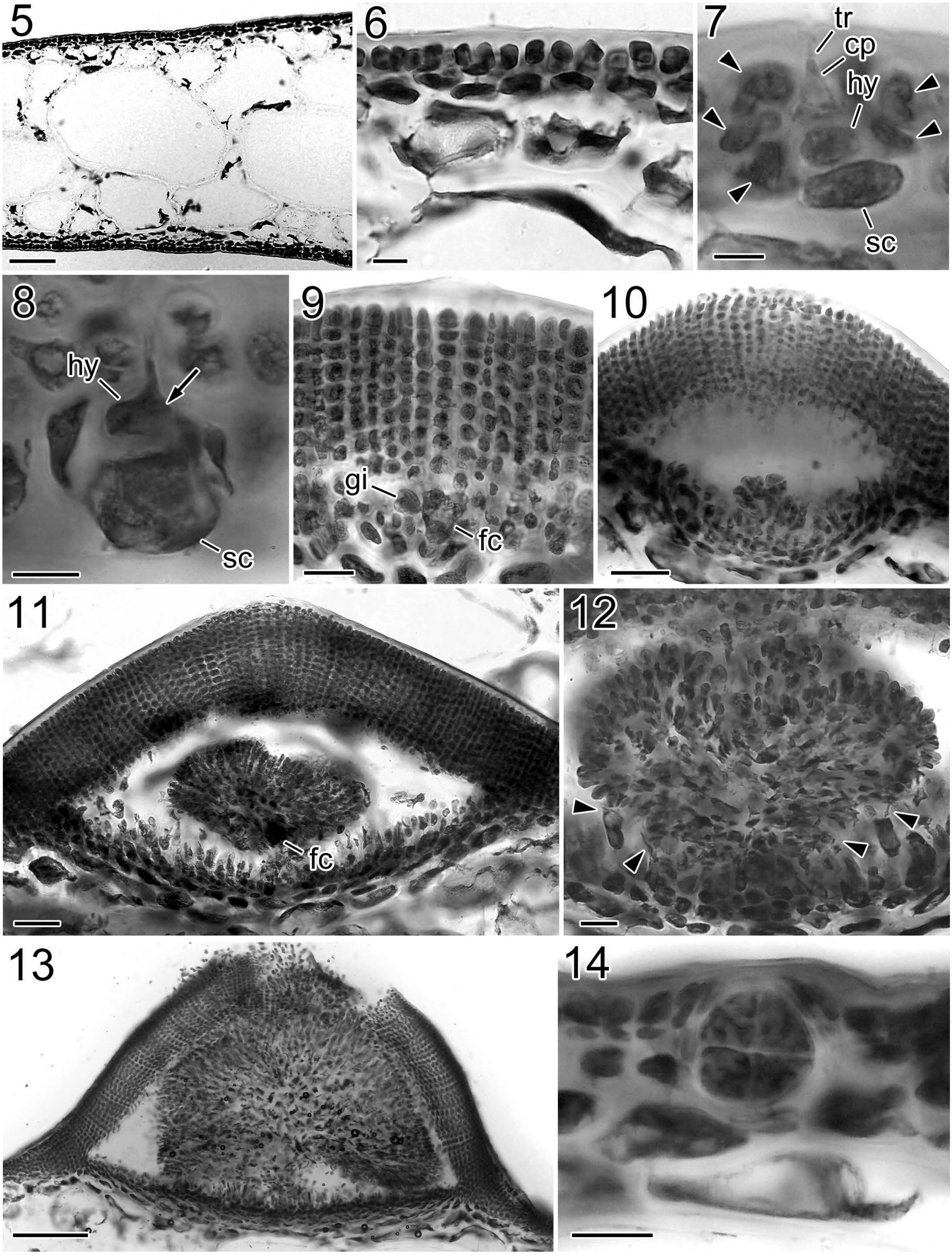
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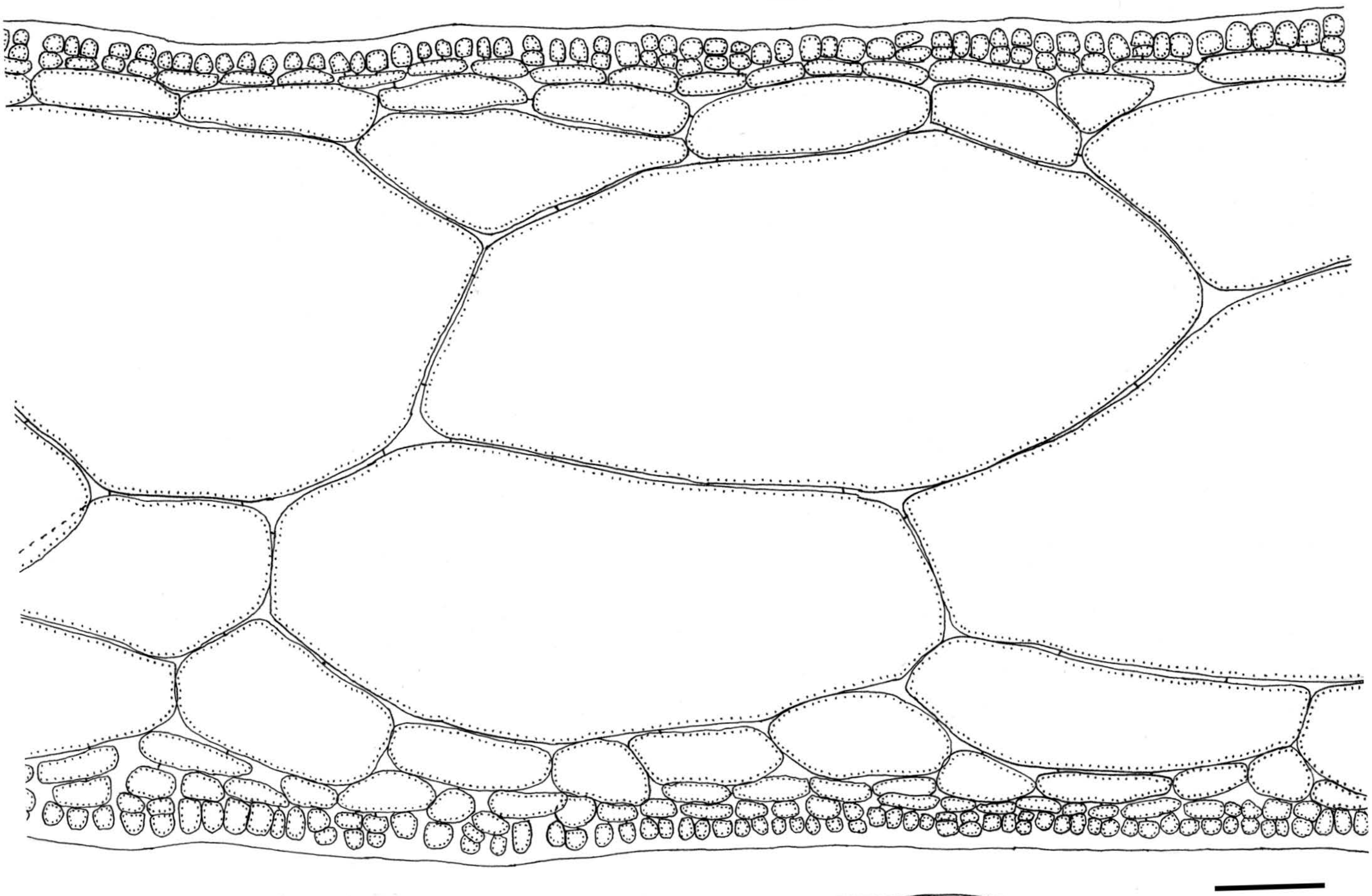

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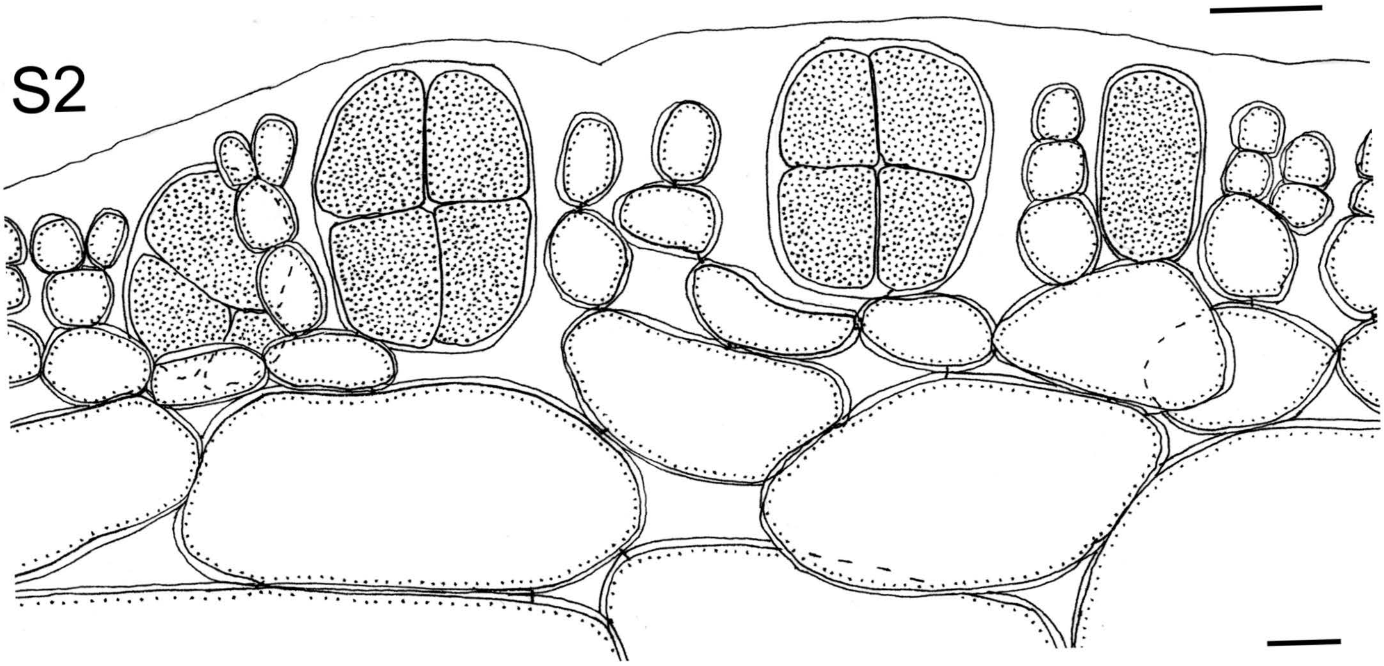

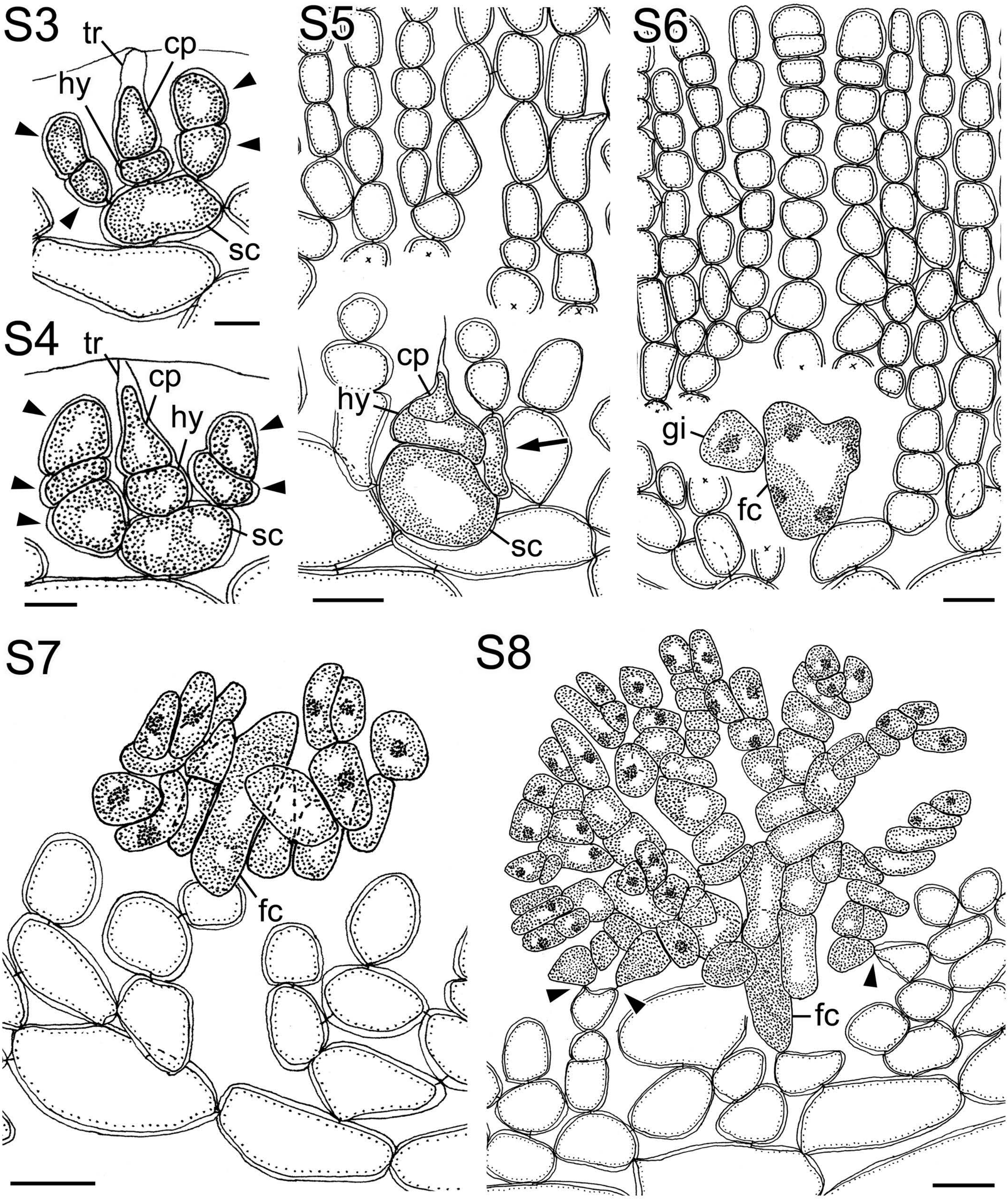

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Table S1. Collection locations and details, and INSD (DDBJ/EMBL/GenBank) accession numbers of samples used in the *rbc*L and *cox*1 gene sequence analyses. Accession numbers in bold were determined for this study.

Species	Collection information (locality; date; collector, voucher; reference)	*rbc*L	*cox*1
Gracilarioideae			
Agarophyton chilense (C.J. Bird, McLachlan & E.C. Oliveira) Gurgel, J.N. Norris & Fredericq	Valdivia, Isla Mancera, Chile; S. Fredericq; Gurgel *et al*. (2018)	MH760419	
	Ancud, Chile; 1 Feb. 2003; CNU050183; Lee *et al*. (2015)		KP728466
Agarophyton tenuistipitatum (C.F. Chang & B.-M. Xia) Gurgel, J.N. Norris & Fredericq	Hue, Viet Nam; 5 Jun. 2006; H.N. Le; LAF-719; Gurgel *et al*. (2018)	MH760421	
	Kok Tay yoaw, Songkhla, Thailand; 17 Jul. 2009; GF0601; Yang & Kim (2015)		JQ026069
Agarophyton vermiculophyllum (Ohmi) Gurgel, J.N. Norris & Fredericq	Uranohama Beach (39°26' N, 141°58' E), Funakoshi, Yamada Town, Shimohei Co., Iwate Pref., Japan; 29 Aug. 2014; M. Suzuki; TNS-AL 195933; this study	**LC589291**	**LC589306**
Crassiphycus corneus (J.Agardh) Gurgel, J.N. Norris & Fredericq	Guajiru, Trairi, CE, Brazil; Feb. 1992; E. Plastino; BG0115; Costa *et al*. (2012); as *G. cornea*.	JQ843357	JQ843323
Crassiphycus firmus (C.F. Chang & B.-M. Xia) Gurgel, J.N. Norris & Fredericq	Kouhu Township, Yunlin County, Taiwan; 20 Aug. 2015; NTOU-KH20viii2015-3; Ng *et al*. (2017); as *G. firma*.	KY315286	KY315246
Crassiphycus punctatus (Okamura) Gurgel, J.N. Norris & Fredericq	Offshore Mageshima I. (30°43' N, 130°49' E), Kagoshima Pref., Japan; 18 May 2016; R. Terada; TNS-AL 209781; this study	**LC589292**	**LC589307**
Gracilaria incurvata Okamura	Araihama Beach (35°09' N, 139°36' E), Koajiro, Misaki Town, Miura City, Kanagawa Pref., Japan; 18 May 2014; M. Suzuki; TNS-AL 188608; this study	**LC595297**	**LC595298**
Gracilaria salicornia (C. Agardh) E.Y. Dawson	Sulpa, Cebu, Philippines; 19 Apr. 1998; S.-M. Lin; Gurgel & Fredericq (2004)	AY049385	
	Zamboanga City, Philippines; 19 Apr. 2010; G410; Yang *et al*. (2013)		JN790231
Gracilaria shimodensis R. Terada & H. Yamamoto	Koura Fishing Port (35°04' N, 139°50' E), Minamiboso City, Chiba Pref., Japan; 9 Aug. 2013; M. Suzuki; TNS-AL 182124; this study	**LC589293**	**LC589308**
Gracilariopsis andersonii (Grunow) E.Y. Dawson	Pigeon Point, San Mateo Co., CA, USA; 20 May 1992; M.H. & F.H. Hommersand; Gurgel *et al*. (2003); as *Gp. sjoestedtii*.	AY049413	
	Subtidal, 6 m depth, on cobble in sand; Tahsis, Rosa Harbour, BC, Canada; 24 May 2008; G.W. Saunders & B. Clarkston; GWS010205; Saunders (2009)		FJ499624

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; reference)	*rbc*L	*cox*1
	Bird Rock, Pacific Grove, CA, USA; 22 May 2010; GWS022306; Saunders (2014)		KM254353
	Santa Cruz, CA, USA; 19 May 2010; GWS021892; Saunders (2014)		KM254410
Gracilariopsis animasensis Gurgel & J.N. Norris	Lake Butler, Robe, SA, Australia; 3 Mar. 1995; H.B.S. Womersley; Gurgel *et al*. (2003); as *Gp. megaspora*.	AY049422	
Gracilariopsis carolinensis L.M. Liao & Hommersand	Kure Beach, Fort Fisher, NC, USA; 14 Apr. 1991; D.W. Freshwater; Gurgel *et al*. (2003)	AY049412	
Gracilariopsis chiangii S.-M. Lin	Sail Rock, Kending National Park, Taiwan; 2 Oct. 2002; S.-M. Lin; Lin (2008)	DQ119746	
	Okinawa Pref., Japan; 20 Apr. 2011; GR008; Yang & Kim (2015)		KF214695
Gracilariopsis chorda (Holmes) Ohmi	Chojagasaki (35°15' N, 139°34' E), Shimoyamaguchi, Hayama Town, Miura Co., Kanagawa Pref., Japan; 30 Apr. 2014; M. Suzuki; TNS-AL 186966; this study	**LC589290**	**LC589301**
	Okinoshima (34°59' N, 139°49' E), Tateyama City, Chiba Pref., Japan; 1 Jan. 2006; M. Suzuki; TNS-AL 182568; this study		**LC589302**
	Nabeta Beach (34°40' N, 138°56' E), Shimoda City, Shizuoka Pref., Japan; 9 May 2005; M. Suzuki; TNS-AL 190022, this study		**LC589303**
	Chiba Port Park (35°36' N, 140°05' E), Chuoko, Chuo Ward, Chiba City, Chiba Pref., Japan; 5 Apr. 2014; M. Suzuki; TNS-AL 184208; this study		**LC589304**
	Kuzuma (35°24' N, 139°54' E), Kisarazu City, Chiba Pref., Japan; 17 Jan. 2003; M. Suzuki; TNS-AL 194970; this study		**LC589305**
Gracilariopsis costaricensis E.Y. Dawson	South end, Playa Tamarindo, Nicoya Peninsula, Guanacaste, Costa Rica; 17 Mar. 1999; D.T. Talbot & D.W. Freshwater; Gurgel *et al*. (2003)	AY049423	
Gracilariopsis funicularis Iyer, Bolton & Coyne	Pta. Serène, Senegal; 2005; Mr. Boudian; Bellorin *et al*. (2008)	EU158087	
	Mousa Boudian, Senegal; Sega07; Lyra *et al*. (unpublished)		KP210200
Gracilariopsis heteroclada J.-F. Zhang & B.-M. Xia	Shantou, Guangdong Province, China; 2016050140; Liu *et al*. (2019), Zhang & Liu (unpublished)	MF372957	MF372958

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; reference)	*rbc*L	*cox*1
	Aklan, Batan, Sungkulan, Philippines; 2 Dec. 2015; J.S. Mondragon; SKH2; Ferrer *et al*. (unpublished)		KY995696
	Zambales, Masinloc, Bamban, Philippines; 28 Aug. 2015; M.S.R. Ferrer; BMH1; Ferrer *et al*. (unpublished)		KY995697
Gracilariopsis hommersandii Gurgel, Fredericq & J.N. Norris	Los Francisky Is., Los Roques Archipelago, Venezuela; 4 Jul. 1999; C.F.D. Gurgel; Gurgel *et al*. (2003)	AY049408	
Gracilariopsis lemaneiformis (Bory de Saint-Vincent) E.Y. Dawson, Acleto & Foldvik	Ecuador; E. Oliveira; BG0078; Costa *et al*. (2012)	JQ843363	JQ843342
	HI, USA; ARS00947; Sherwood *et al*. (2010)		HQ422689
	Boca del Cielo, Chiapas, Mexico; 25 Mar. 2018; UAMIZ1349#1; Hernandez *et al*. (2020)		MK238044
Gracilariopsis longissima (S.G. Gmelin) Steentoft, L.M. Irvine & Farnham	Roscoff, France; 4 Apr. 2000; G48; Kim *et al*. (2006)	DQ095786	
	England, UK; BG0052; Costa *et al*. (2012)	JQ843364	JQ843344
	Roscoff, France; 4 Apr. 2000; G48; Kim *et al*. (2006)	DQ095786	
Gracilariopsis mageshimensis Mas. Suzuki & R. Terada *sp*. *nov*.	Offshore of Mageshima I. (30°43’ N, 130°49’ E), Kagoshima Pref., Japan; 30 May 2019; R. Terada; TNS-AL 213799 *Holotype; this study	**LC589283**	**LC589294**
	Offshore of Mageshima I. (30°43’ N, 130°49’ E), Kagoshima Pref., Japan; 30 May 2019; R. Terada; TNS-AL 213800 *Isotype; this study	**LC589284**	**LC589295**
	Offshore of Mageshima I. (30°43’ N, 130°49’ E), Kagoshima Pref., Japan; 20 Jul. 2017; R. Terada; TNS-AL 213795; this study	**LC589285**	**LC589296**
	Offshore of Mageshima I. (30°43’ N, 130°49’ E), Kagoshima Pref., Japan; 20 Jul. 2017; R. Terada; TNS-AL 213796; this study	**LC589286**	**LC589297**
	Offshore of Mageshima I. (30°43’ N, 130°49’ E), Kagoshima Pref., Japan; 20 Jul. 2017; R. Terada; TNS-AL 213797; this study	**LC589287**	**LC589298**

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; reference)	*rbc*L	*cox*1
	Offshore of Mageshima I. (30°43' N, 130°49' E), Kagoshima Pref., Japan; 20 Jul. 2017; R. Terada; TNS-AL 213798; this study	**LC589288**	**LC589299**
	Offshore of Mageshima I. (30°43' N, 130°49' E), Kagoshima Pref., Japan; 26 May 2018; R. Terada & M. Suzuki; TNS-AL 213913; this study	**LC589289**	**LC589300**
Gracilariopsis mclachlanii Buriyo, Bellorin & M.C. Oliveira	Zanzibar, Tanzania; Jun. 1992; E. Oliveira; BG0072; Iha *et al*. (2018)	MH396015	MH396024
Gracilariopsis persica Bellorin, Sohrabipour & E.C. Oliveira	Bandarabbas, Iran; 29 Feb. 2004; E. C. Oliveira; Bellorin *et al*. (2008)	EU158094	
Gracilariopsis oryzoides (Setchell & H.L.Wilson) Gurgel, J.N. Norris & Fredericq	Pigeon Point, Pescadero, CA, USA; Salomaki & Lane (2017); as *Gracilariophila oryzoides*.		KX687879
Gracilariopsis silvana Gurgel, Fredericq & J.N. Norris	La Vela de Coro, Falcon State, Venezuela; 14 Jul. 1999; C.F.D. Gurgel, J. E. Conde & C. Carmona; Gurgel *et al*. (2003)	AY049309	
	RS, Brazil; W. Oliveira; 57185 (SPF); Lyra *et al*. (2015)		KP210202
	Castelhanos, ES, Brazil; 6 May 2012; F.N. Silva; BRA103; Lyra *et al*. (2015)		KP210203
	Castelhanos, ES, Brazil; 6 May 2012; F.N. Silva; BRA102; Lyra *et al*. (2015)		KP210204
	Meaipe, ES, Brazil; 9 May 2012; M.C. Oliveira; BRA100; Lyra *et al*. (2015)		KP210205
Gracilariopsis tenuifrons (C.J. Bird & E.C. Oliveira) Fredericq & Hommersand	Punta Banda, Baja California, Mexico; 7 Jun. 1989; E. Oliveira; BG0050; Costa *et al*. (2012)	JQ843366	JQ935798
	Pajucara, Maceio, AL, Brazil; 21 Jan. 1993; E. Oliveira; BG0085; Costa *et al*. (2012)		JQ935797
	Lagoa de Mundau, AL, Brazil; 3 Mar. 1994; E. Plastino; BG0086; Lyra *et al*. (2015)		KP210209
Gracilariopsis sp.	Gurgel & Fredericq (unpublished)	AY049386	
Hydropuntia edulis (S.G. Gmelin) Gurgel & Fredericq	Little Santa Cruz, Philippines; 28 Apr. 1998; L.M. Liao; Gurgel & Fredericq (2004); as *G. edulis*.	AY049387	
	Currimao, Ilocos Norte, Philippines; 26 May 2010; G320; Yang & Kim (2015)		JQ026082
Hydropuntia eucheumatoides (Harvey) Gurgel & Fredericq	Tambuli, Cebu, Philippines; 18 Apr. 1998; S.-M. Lin; Gurgel & Fredericq (2004)	AY049389	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; reference)	*rbc*L	*cox*1
	General Santos City, Bula, Philippines; 5 Aug. 2016; R.N. Gomez; Gomez *et al*. (unpublished)		MG199563
Hydropuntia rangiferina (Kützing) Gurgel & Fredericq	Guajiru, Trairi, CE, Brazil; 8 Apr. 1993; E. Plastino; BG0092; Costa *et al.* (2012)	JQ843362	
	Trairi, CE, Brazil; 16 Sep. 1989; E.C. Oliveira; Iha *et al*. (2018)		MH396020
Melanthalioideae			
Curdiea crassa A.J.K. Millar	Bongin Bay, North of Sydney, NSW, Australia; 18 Feb. 1994; A. Millar & P. Richards; Gurgel & Fredericq (2004)	AY049427	
Curdiea racovitzae Hariot	Punta Lacaze, Paradise Bay, Antarctic Peninsula; Guillemin *et al*. (2018)		KY559671
Curdiea sp.	Artigas, King George Island, South Shetland Islands; Guillemin *et al*. (2018)		KY559713
Melanthalia abscissa (Turner) J.D. Hooker & Harvey	New Zealand; 25 Apr. 2020; W. Nelson; Gurgel & Fredericq (2004)	AY049428	
Melanthalia obtusata (Labillardière) J.Agardh	Warrnambool, VIC, Australia; 13 Jul. 1995; M.H. Hommersand; Gurgel & Fredericq (2004), Iha *et al*. (2018)	AY049431	MH396025

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Table S2. Substitution models for the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses on the basis of *rbc*L and *cox*1 sequences.

	*rbc*L	*cox*1
Number of taxa	33 (40)[1)]	39 (48)[1)]
Number of nucleotides (bp)	1257	573
Substitution model for ML analyses[2)]	1st codons (GTR+I+G4), 2nd codons (GTR+I), 3rd codons (TPM1uf+I+G4)	1st codons (TIM2+I+G4), 2nd codons (F81), 3rd codons (TIM1+I+G4)
Substitution model for BI analyses[3)]	1st codons (GTR+I+G), 2nd codons (GTR+I), 3rd codons (GTR+I+G)	1st codons (GTR+I+G), 2nd codons (F81), 3rd codons (GTR+I+G)

[1)] The numbers within parentheses indicate original number of taxa including the samples with identical nucleotide sequences.

[2)] Each substitution model was selected by Akaike's information criterion using ModelTest-NG 0.1.6 (Darriba *et al*. 2019).

[3)] Each substitution model was selected by Akaike's information criterion using MrModeltest 2.3 (Nylander 2004).

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