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Fredericqia (Phyllophoraceae, Rhodophyta) in the northwestern Pacific, with the description of *Fredericqia chiharae sp. nov*.

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RUNNING TITLE

Fredericqia chiharae sp. nov. from Japan

ABSTRACT

We describe a new species of *Fredericqia* (Phyllophoraceae, Rhodophyta) from the Choshi Peninsula, Japan (previously listed as '*Gymnogongrus incurvatus nom. nud.*') based on morphological and molecular analyses. We assign the binomial *Fredericqia chiharae sp. nov.* to this new entity. Our combined *rbc*L, *cox*1, and 28S rDNA analyses suggested that *F. chiharae* belongs to genus *Fredericqia*, and is distinct from other *Fredericqia* species. *Fredericqia chiharae* is characterized by a flattened incurved thallus, marginal proliferations, dioecious gametophytes and a procarp consisting of a three-celled carpogonial branch situated on a supporting cell and bearing a sterile lateral on the basal cell. The cystocarpic characteristics of *F. chiharae*, i.e. a cystocarp loosely immersed in the medulla, the absence of secondary medullary cells around the cystocarp and the presence of multiple carpostomes, are congruent with those of *Fredericqia*. *Fredericqia chiharae* is the first representative of the genus to be reported from the northwestern Pacific.

KEYWORDS

28S rDNA; *cox*1; Gigartinales; Phylogeny; *rbc*L; Taxonomy

INTRODUCTION

The marine red algal genus *Fredericqia* Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders, a member of the family Phyllophoraceae, was originally described by Maggs *et al*. (2013) with *F. deveauniensis* Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders as type species. Two additional species were recognized: *F. chiton* (M. Howe) Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders and *F. decewii* Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders (Maggs *et al*. 2013). *Fredericqia deveauniensis* had previously been misidentified as *Gymnogongrus devoniensis* (Greville) Schotter and as *G. crenulatus* (Turner) J. Agardh, in Ireland and the western Atlantic, respectively (Maggs *et al*. 1992, 2013). *Fredericqia chiton* was first described as *Actinococcus chiton* M. Howe, which was regarded as parasitic on *Gymnogongrus* species (Howe 1914). Doubt (1935) concluded that *A. chiton* is not a parasite but rather the tetrasporoblast produced by *G. platyphyllus* N.L. Gardner. Silva (1979) transferred *A. chiton* to *Gymnogongrus* as *G. chiton* (M. Howe) P.C. Silva & DeCew. McCandless & Vollmer (1984) treated *G. platyphyllus* as a heterotypic synonym of *G. chiton*. Maggs *et al.* (2013) transferred *G. chiton* from *Gymnogongrus* to *Fredericqia* based on *rbc*L and *cox*1 analyses. Silva (1979) noted that specimens previously identified as *Ahnfeltia gigartinoides* J. Agardh from the Pacific coast of North America belonged to a separate species, which he designated '*Ahnfeltiopsis pacifica* P.C. Silva & DeCew' (not validly published for lack of Latin diagnosis and type designation; Turland *et al.* 2018). Maggs *et al.* (2013) treated '*A. pacifica*' as a new species of *Fredericqia*, and described it under the name *F. decewii*.

A previously undescribed phyllophoracean species collected from the Choshi Peninsula, Japan, was recognized as a new entity by Dr Mitsuo Chihara; he labelled his collection with the binomial '*Gymnogongrus incurvatus* Chihara'. Fifty-two herbarium specimens of '*G. incurvatus*' prepared by Chihara are stored at the National Museum of Nature and Science, Japan (TNS). Yoshizaki (2008) cited '*G. incurvatus*' with a comment that Chihara's binomial had not been validly published. Since then, '*G. incurvatus*' has not been mentioned in the published literature and remains a *nomen nudum*. To clarify the taxonomy of '*G. incurvatus*', we collected specimens from the Choshi Peninsula, and sequenced *rbc*L and *cox*1 genes, and 28S rDNA, and examined their vegetative and reproductive morphology in detail. The molecular analyses indicated that '*G. incurvatus*' belongs to *Fredericqia*, and both molecular and morphological investigations suggested that '*G. incurvatus*' is distinct from other *Fredericqia* species.

MATERIAL AND METHODS

The specimens were collected from Tokawa, Choshi City, Chiba Prefecture, Japan (Table S1). For molecular analyses, specimens were quickly dried in silica gel. For anatomical observations, specimens were preserved in 5% formalin; voucher herbarium specimens were deposited at TNS. The TNS herbarium specimens collected from Cape Inubosaki, Choshi City, by Chihara were also studied.

For molecular phylogenetic analyses, partial *rbc*L and *cox*1 genes, and 28S rDNA were sequenced. Genomic DNA was extracted and sequenced as described previously (Suzuki *et al*. 2016). The *rbc*L, *cox*1 and 28S rDNA sequences of two specimens of *Fredericqia* from Japan were sequenced (Table S1). For combined *rbc*L, *cox*1 and 28S rDNA analysis we compiled sequence data available from GenBank for three *Fredericqia* species and eleven genera belonging to the Phyllophoraceae. As the Phyllophoraceae have been resolved as monophyletic (Maggs *et al*. 2013; Calderon & Boo 2016a, b, 2017; Calderon *et al*. 2016), three taxa belonging to Gigartinaceae were designated as outgroup (Table S1). The 18 *rbc*L, *cox*1 and 28S rDNA sequences were aligned using ClustalW (Larkin *et al*. 2007). The 28S rDNA sequence alignments were further refined by eye based on published secondary structure of the 28S rRNA of *Palmaria palmata* (Linnaeus) Kuntze (Wuyts *et al*. 2002) using SeaView 4.1 (Gouy *et al*. 2010). The ambiguous regions of the alignments were removed. The 18 *rbc*L (1275 bp), *cox*1 (645 bp) and 28S rDNA (2620 bp) taxa were included in the data matrix.

The *rbc*L, *cox*1 and 28S rDNA analyses were subjected to maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. For both analyses, the alignment was partitioned by region and codon, and distinct models were applied to each partition. The substitution models for ML and BI are summarized in Table S2. The ML analysis was 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 analysis was performed using MrBayes 3.2.7a (Ronquist *et al*. 2012), as described previously (Suzuki *et al*. 2016).

Heights and widths of thalli were measured in 56 specimens. 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

Molecular analyses

The ML tree based on combined *rbc*L, *cox*1 and 28S rDNA sequences and the BI analyses had similar topologies, only the phylogenetic positions of *Archestenogramma profundum* C.W. Schneider, Chengsupanimit & G.W. Saunders and *Schottera koreana* M.S. Calderon, T.H. Seo & S.M. Boo differed, but with no statistical support (BP = 30%; PP <0.5) (Fig. 1). The three *Fredericqia* species and the new Japanese specimens formed a monophyletic group with full statistical support (BP = 100%; PP = 1.00). Within this *Fredericqia* clade, the Japanese sample was sister to the other *Fredericqia* species but this relationship had moderate support (BP = 83%; PP = 0.99). Molecular analyses suggest that the Japanese sample is a distinct *Fredericqia* species, which we describe here.

Fredericqia chiharae Mas. Suzuki & Kitayama *sp. nov.*

Figs 2–14

DESCRIPTION: Thalli linear, flattened, slightly to moderately incurved, 5–12 cm high and < 3 mm wide, and arising in groups from a discoid holdfast. Thalli dichotomously branched three to five times, often producing marginal proliferations. Thallus solid, with an outer cortex of compact anticlinal filaments composed of three to four small ellipsoidal cells, and a medulla composed of 12–14 layers of large ovoid cells, with two to three layers of smaller subcortical ovoid to rounded cells present between the cortex and medulla. Gametophytes dioecious. Spermatangia developing in surface sori on apical parts of the thalli. Cystocarps scattered over the middle and upper parts of the thallus surface, except in the terminal portion. Procarp consisting of a three-celled carpogonial branch situated on a supporting cell and bearing a sterile lateral on the basal cell. Auxiliary cell forming gonimoblast initials radially. Mature cystocarps loosely immersed within the medulla, slightly protruding equally on both sides of the thallus, and bearing a thick pericarp penetrated by multiple carpostomes.

HOLOTYPE: TNS-AL 209185, female gametophyte, collected 12 May 2017 by M. Suzuki, deposited in the National Museum of Nature and Science, Japan (TNS). DDBJ Accession numbers: *cox*1, LC589700; *rbc*L, LC589696.

ISOTYPE: TNS-AL 209186, female gametophyte, collected 12 May 2017 by M. Suzuki, deposited in TNS.

ADDITIONAL SPECIMENS EXAMINED. 1) **Japan:** Cape Inubosaki, Choshi City, Chiba Prefecture, *M. Chihara*, 11 June 1972, TNS-AL 150323–150365; 4 May 1969, TNS-AL 150366–150370; 25 April 1970, TNS-AL 150371–150374. 2) **Japan:** Tokawa, Choshi City, Chiba Prefecture, *M. Suzuki*, 22 June 2008, female gametophyte, TNS-AL 207116 (DDBJ Accession Numbers: 28S rDNA, LC589692; *cox*1, LC589699; *rbc*L, LC589695); 23 June 2013, female gametophyte, TNS-AL 213791; 23 June 2013, male gametophyte, TNS-AL 213914.

TYPE LOCALITY: Tokawa, Choshi City, Chiba Prefecture, Japan, 35°41′N, 140°51′E.

ETYMOLOGY: The species is named for Dr Mitsuo Chihara (1927–2016), who first recognized it as a distinct species.

JAPANESE NAME: Mizo-okitsunori.

Gametophytes grow on rocks in lower intertidal zones with strong wave action. Thalli are linear, flattened, slightly to moderately incurved, 5–12 cm high and < 3 mm wide, rose-red in colour, and arising in groups from a discoid holdfast (Figs 2, 3). Thalli are dichotomously branched three to five times, and often produce marginal proliferations (Fig. 3, arrows). The thallus is solid, with an outer cortex of compact anticlinal filaments composed of three to four small ellipsoidal cells, and a medulla composed of 12–14 layers of large ovoid cells, with two to three layers of smaller subcortical ovoid to rounded cells present between the cortex and medulla (Figs 4, 5, S1, S2). Subcortical and medullary cells are secondarily pit-connected.

Gametophytes are dioecious. Spermatangia develop in surface sori on apical parts of the thallus. One to two spermatangia are produced terminally on parental cells. Mature spermatangia are ellipsoidal, 5–6 µm long and 2–3 µm in diameter (Figs 6, 7). Cystocarps are scattered over the middle to upper parts of the thallus surface, except in the terminal portion (Fig. 8). The procarp consists of a three-celled carpogonial branch situated on a supporting cell and bearing a sterile lateral on the basal cell (Figs 9, 10). The auxiliary cell forms gonimoblast initials radially (Figs 11, S3, S4). The medullary cells close to the auxiliary cell are connected by secondary pit connections and produce cells that grow into filaments similar to gonimoblast filaments (Figs 11, S3–S6). Gonimoblast filament development could not be interpreted using the available figure. Secondary medullary cells do not form around the cystocarps. Medullary cells are connected to clusters of carposporangia (Figs 12, 13). Mature carposporangia are polygonal, 12–17 µm in length and 8–11 µm in diameter. Mature cystocarps are immersed within the medulla, slightly protruding equally on both sides of the thallus, and bear a thick pericarp penetrated by multiple carpostomes (Fig. 14). Sporophytes and tetrasporoblasts were not observed.

DISCUSSION

The combined *rbc*L, *cox*1 and 28S rDNA analyses grouped four *Fredericqia* species, including *F. chiharae*, into a monophyletic natural assemblage (with strong support), in agreement with other published tree topologies (Maggs *et al*. 2013; Calderon & Boo 2016a, b, 2017; Calderon *et al*. 2016). *Fredericqia* is characterized morphologically by a cystocarp loosely immersed in the medulla and the presence of cystocarps with multiple carpostomes (Maggs *et al*. 2013; Calderon & Boo 2016a, b, 2017; Calderon *et al*. 2016). The characteristics of the *F. chiharae* cystocarp are congruent with these. Secondary medullary cells around the cystocarp, which are characteristic of *Acletoa* M.S. Calderon & S.M. Boo, *Ahnfeltiopsis* P.C. Silva & DeCew, and *Asterfilopsis* M.S. Calderon & S.M. Boo, and radial connection of the medullary cells to the carposporangia, which is characteristic of *Asterfilopsis*, were not observed in *F. chiharae* (Calderon & Boo 2016a, b, 2017; Calderon *et al*. 2016). The sporophytes of *F. decewii* and *F. deveauniensis* are crustose; *F. deveauniensis* forms cruciate tetrasporangia or bisporangia in catenate rows located in an intercalary position (Maggs *et al*. 1992, 2013). *Fredericqia chiton* produces tetrasporoblastic nemathecia (Doubt 1935; Abbott & Hollenberg 1976). No tetrasporoblasts or other sporangial structures were found on the erect thalli of *F. chiharae*. Yoshizaki (2008) reported that released carpospores of '*Gymnogongrus incurvatus*' (i.e. *F. chiharae*) developed into crusts that may form sporangia. Therefore, the life history of *F. chiharae* is presumably heteromorphic.

Molecular analyses suggested that *F. chiharae* is distinct from other *Fredericqia* species. A comparison of *F. chiharae* with three other *Fredericqia* species showed that *F. chiharae* is similar to *F. chiton* and *F. deveauniensis* (Table 1). All three species have flattened thalli, whereas *F. decewii* has a cylindrical thallus (Abbott & Hollenberg 1976; Silva 1979; Maggs *et* *al*. 1992, 2013). *Fredericqia chiharae* is distinguished from *F*. *chiton* and *F*. *deveauniensis* by an incurved thallus, the production of marginal proliferations and dioecious gametophytes (Doubt 1935; Abbott & Hollenberg 1976; Silva 1979; Maggs *et al*. 2013). *Fredericqia chiharae* is the first *Fredericqia* species reported from the northwestern Pacific region.

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LEGENDS FOR FIGURES

Fig 1. Maximum Likelihood (ML) phylogeny based on combined *rbc*L, *cox*1 and 28S rDNA sequences. Numbers below the branches indicate the bootstrap values (BP, left) and Bayesian posterior probabilities (PP, right). Only the BP ≥ 50% and PP ≥ 0.95 are shown. Asterisk (*) means BP = 100% and PP = 1.00.

Figs 2, 3. Habit of *Fredericqia chiharae sp. nov.*

Fig. 2. Habit of a plant growing on an intertidal rock. Scale bar = 1 cm.

Fig. 3. Holotype, female gametophyte (TNS-AL 209185). Arrows indicate marginal proliferations. Scale bar = 3 cm.

Figs 4–17. Vegetative and reproductive structures of *Fredericqia chiharae sp. nov*.

Fig. 4. Transverse section of thallus (TNS-AL 213791) stained with cotton blue. Scale bar = 100 µm.

Fig. 5. Close-up of a section (TNS-AL 213791) stained with cotton blue showing outer compact cortex composed of anticlinal filaments, subcortical cells (arrows) and hyaline medullar cells. Scale bar = 20 µm.

Fig. 6. Transverse section of male gametophyte (TNS-AL 213914) stained with cotton blue. Spermatangia (arrows) are produced terminally on the spermatangial parental cells (arrowheads). Scale bar = 5 µm.

Fig. 7. Hand drawing of the transverse section of male gametophyte (TNS-AL 213914). One or two spermatangia (arrows) are produced terminally on the spermatangial parental cells (arrowheads). Scale bar = 5 µm.

Fig. 8. Close up of a female gametophyte (TNS-AL 213791). Cystocarps are scattered over the middle to upper portions of the thallus surface, except in the terminal portion. Scale bar = 5 mm.

Fig. 9. A procarp (TNS-AL 213791) stained with cotton blue showing a carpogonium (cp), hypogenous cell (hy), basal cell (bc), and a sterile lateral (st) from the basal cell borne on the supporting cell (sc). Scale bar = 5 µm.

Fig. 10. Hand drawing of a procarp (TNS-AL 213791) showing a carpogonium (cp), hypogenous cell (hy), basal cell (bc), and a sterile lateral (st) from the basal cell borne on the supporting cell (sc). Scale bar = 10 µm.

Fig. 11. A post-fertilization stage (TNS-AL 213791) stained with cotton blue. The auxiliary cell (a) forms gonimoblast initials (arrowheads) radially. The medullary cells (m) close to the auxiliary cell produce cells that grow into filaments similar to gonimoblast filaments (arrows). Scale bar = 20 µm.

Fig. 12 Medullary cell surrounded by clusters of carposporagia (TNS-AL 213791) stained with cotton blue. Scale bar = 30 µm.

Fig. 13. Medullary cell connected to carposporangia (arrows, TNS-AL 213791) stained with cotton blue. Scale bar = 20 µm.

Fig. 14. Transverse section of mature cystocarp (TNS-AL 213791) stained with cotton blue. Arrowheads indicate carpostomes. Scale bar = 100 µm.

Figs S1, S2. Hand drawings of vegetative structures of *Fredericqia chiharae sp. nov.*

Fig. S1. Transverse section of thallus (TNS-AL 213791) showing cortex and medulla. Arrows indicate two to three layers of subcortical cells. Scale bar = 50 µm.

Fig. S2. Longitudinal section of thallus (TNS-AL 213791) showing cortex and medulla. Arrows indicate two to three layers of subcortical cells. Scale bar = 50 µm.

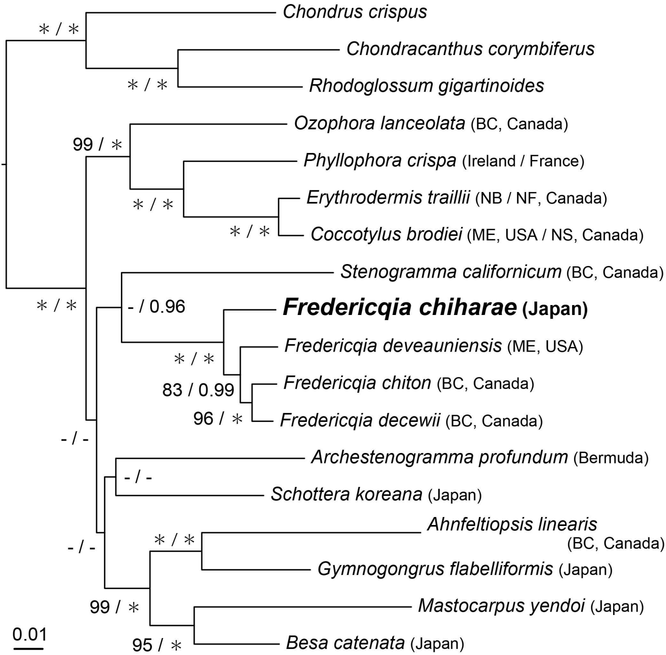
Figs S3–S6. Post-fertilization stages of *Fredericqia chiharae sp. nov.* (TNS-AL 213791) stained with cotton blue.

Fig. S3. The medullary cells (m) close to the auxiliary cell (a) produce cells that grow into filaments similar to gonimoblast filaments (arrows). Scale bar = 20 µm.

Figs S4-S6. Close up of the auxiliary cell and the medullary cell in three different focal planes.

Fig. S4. Gonimoblast initials (gi) and secondary pit connection between auxiliary cell and medullary cell (arrowhead). Scale bar = 10 µm.

Figs S5, S6. Secondary pit connection between auxiliary cell and medullary cell (arrowhead) and produced filaments (arrows). Scale bar = 10 µm.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Erythrodermis traillii (NB / NF, Canada)
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Stenogramma californicum (BC, Canada)
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Fredericqia chiharae (Japan)
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Fredericqia chiton (BC, Canada)
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96 / *
Fredericqia decewii (BC, Canada)
- / -
Archestenogramma profundum (Bermuda)
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Schottera koreana (Japan)
Ahnfeltiopsis linearis (BC, Canada)
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Mastocarpus yendoi (Japan)
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Besa catenata (Japan)

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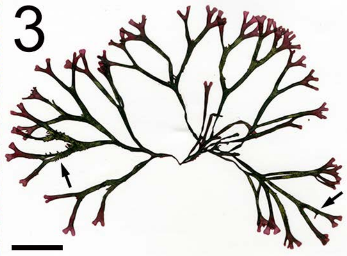

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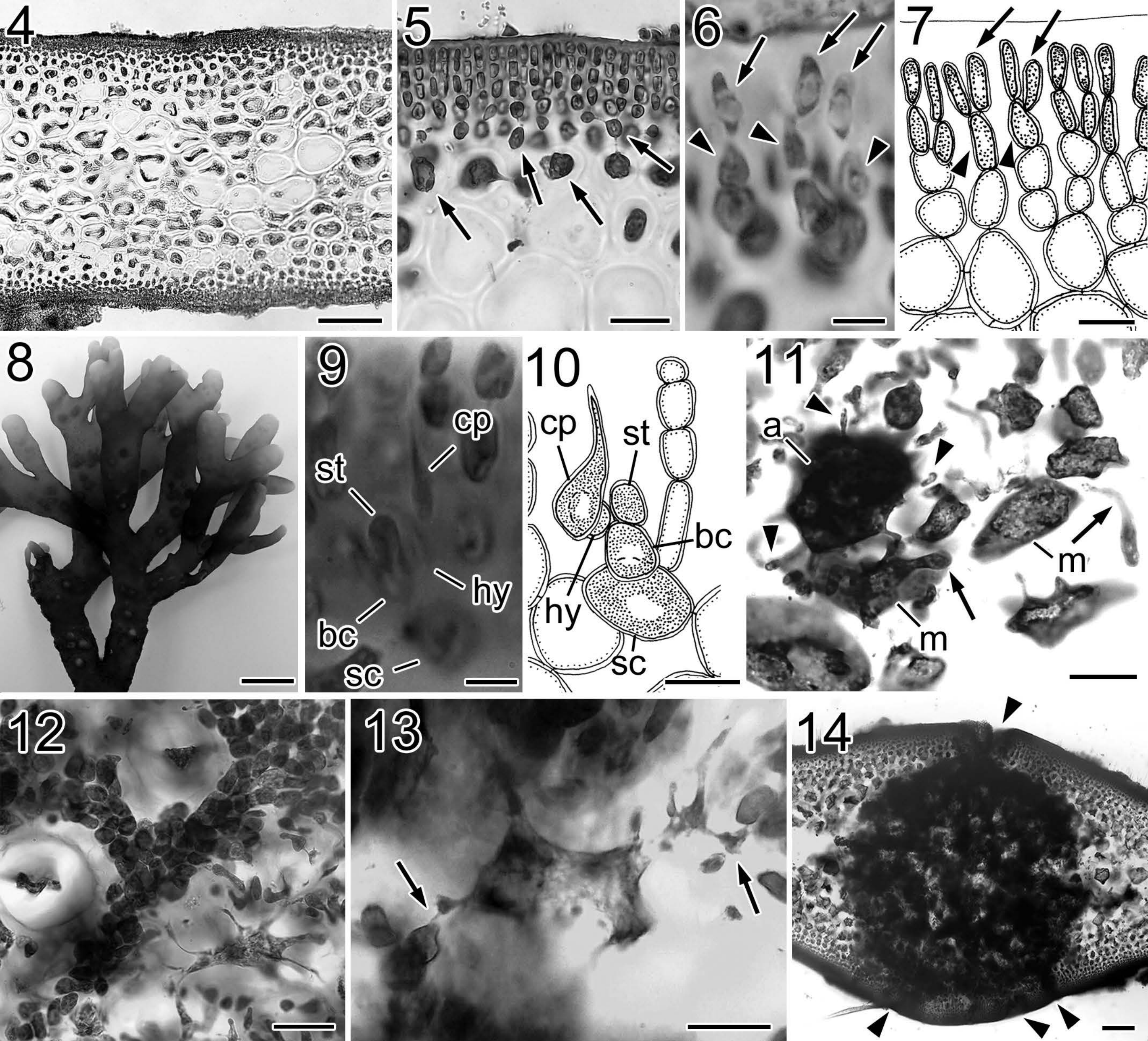
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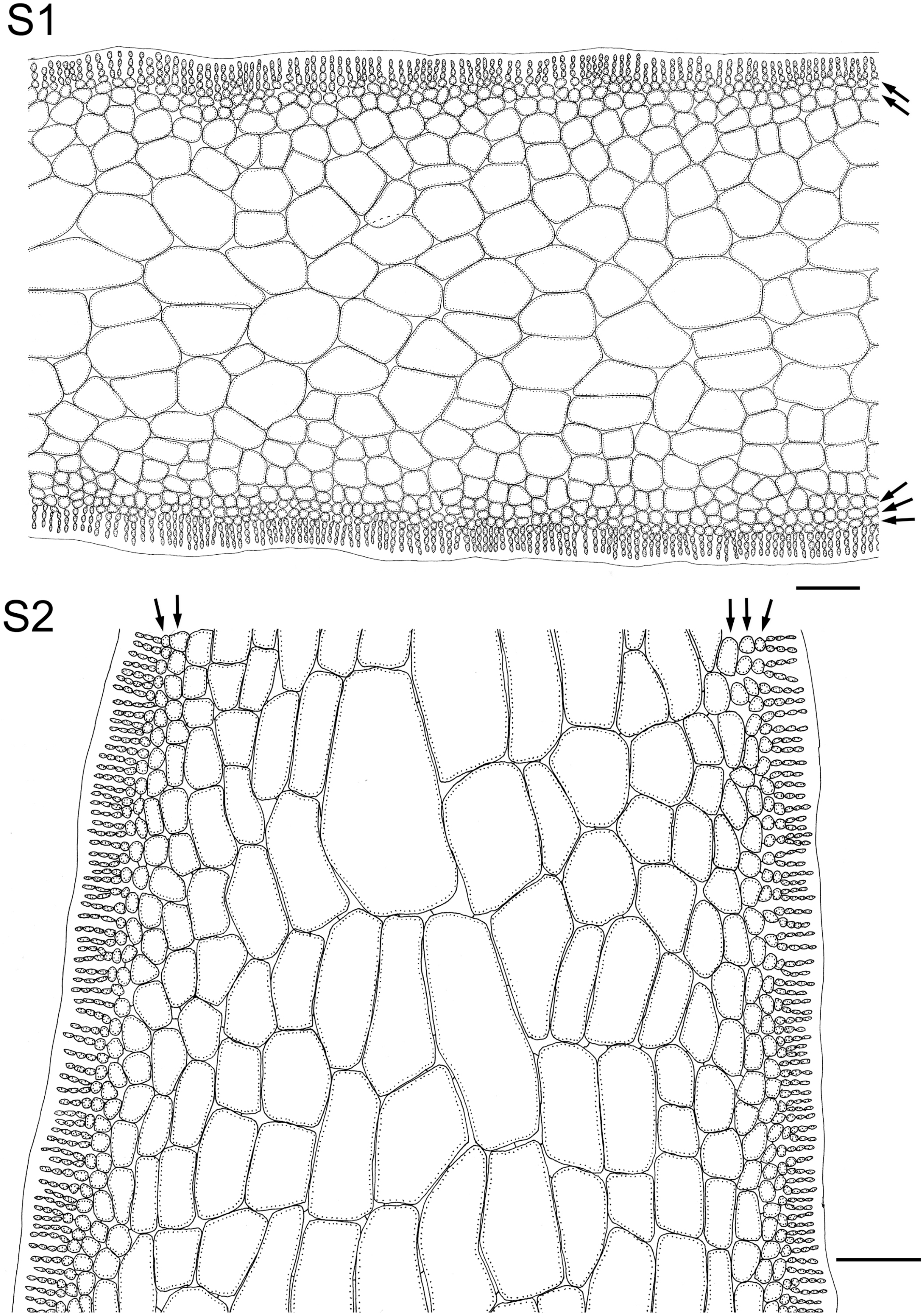
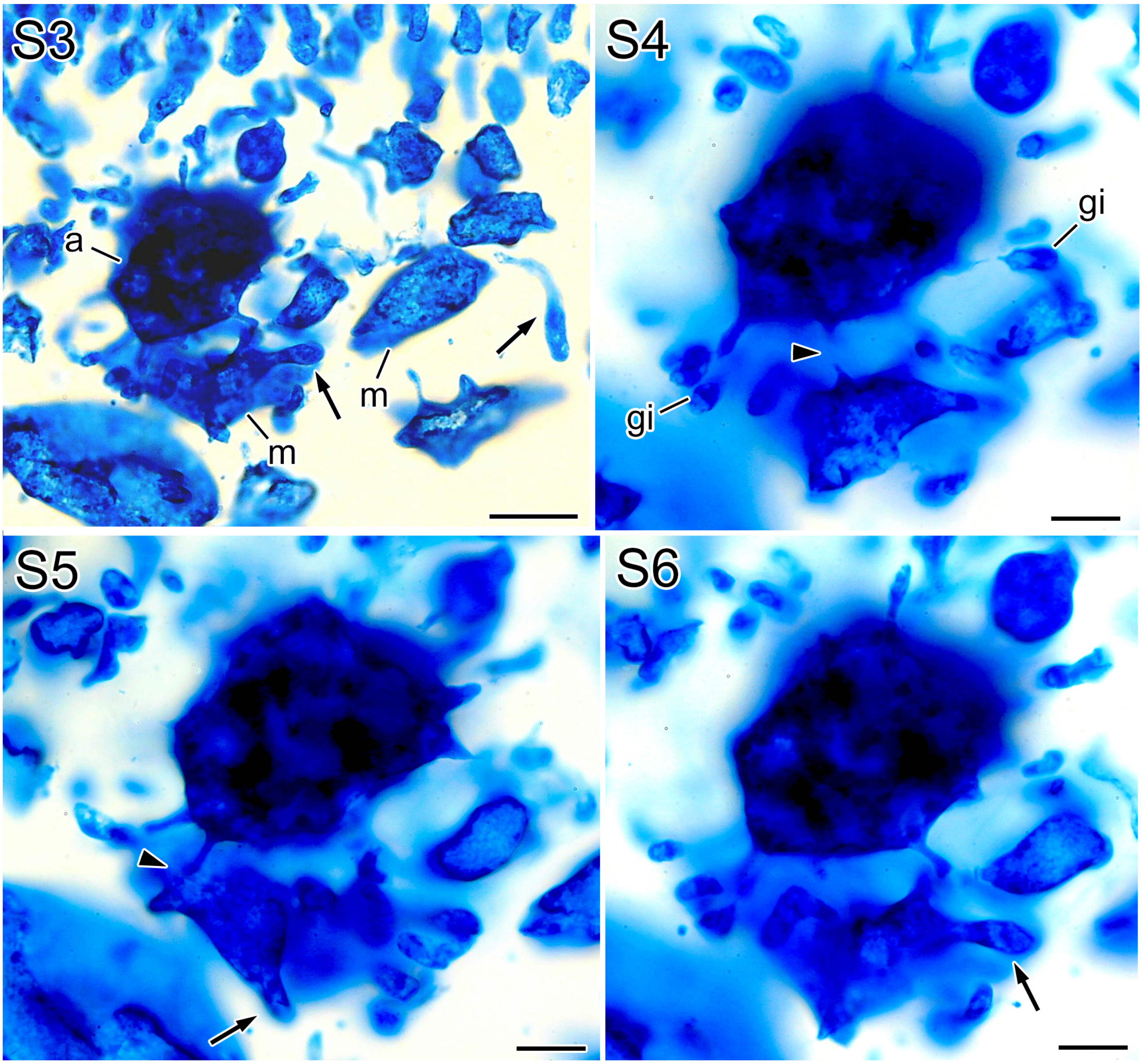


Figs S1, S2. Hand drawings of vegetative structures of *Fredericqia chiharae sp. nov*.

Fig. S1. Transverse section of thallus (TNS-AL 213791) showing cortex and medulla. Arrows indicate two to three layers of subcortical cells. Scale bar = 50 µm.

Fig. S2. Longitudinal section of thallus (TNS-AL 213791) showing cortex and medulla. Arrows indicate two to three layers of subcortical cells. Scale bar = 50 µm.



Figs S3–S6. Post-fertilization stages of *Fredericqia chiharae sp. nov*. (TNS-AL 213791) stained with cotton blue.

Fig. S3. The medullary cells (m) close to the auxiliary cell (a) produce cells that grow into filaments similar to gonimoblast filaments (arrows). Scale bar = 20 µm.

Figs S4-S6. Close up of the auxiliary cell and the medullary cell in three different focal planes.

Fig. S4. Gonimoblast initials (gi) and secondary pit connection between auxiliary cell and medullary cell (arrowhead). Scale bar = 10 µm.

Figs S5, S6. Secondary pit connection between auxiliary cell and medullary cell (arrowhead) and produced filaments (arrows). Scale bar = 10 µm.

Table S1. Collection locations and details, and INSD (DDBJ/EMBL/GenBank) accession numbers of samples used in the *rbc*L, *cox*1, and 28S rDNA sequence analyses. Accession numbers in bold were determined for this study.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
Gigartinaceae				
Chondracanthus corymbiferus (Kützing) Guiry	Indian Is., Kitsap Co., WA, USA; 10 June 1994; M.H. Hommersand; Hughey & Hommersand (2008)	DQ869094		
	Wizard Islet, Vancouver Is., BC, Canada; G.W. Saunders; GWS002830; Le Gall & Saunders (2010)		GQ398090	GQ338088
Chondrus crispus Stackhouse	Cheyne Beach, Devon, England; 3 Apr. 2010; K.M. Kim & K.M. Lee; CNU034179; Calderon & Boo (2016)	KU640262	KU640326	
	Harper & Saunders (2001)			AF419120
Rhodoglossum gigartinoides (Sonder) Edyvane & Womersley	Flinders Jetty, VIC, Australia; G.T. Kraft & G.W. Saunders; G0098; Schneider *et al*. (2011)	JN403074		GQ338091
	Robe, Cape Dombey Jetty, SA, Australia; 17 Nov. 2011; G.W. Saunders & K. Dixon; GWS029663; Kraft & Saunders (2014)		KF026468	
Phyllophoraceae				
Ahnfeltiopsis linearis (C. Agardh) P.C. Silva & DeCew	Bradys Beach, Bamfield, BC, Canada; 15 Jun. 2005; G.W. Saunders, B. Clarkston & D. McDevit; GWS003998; Le Gall & Saunders (2010)	GQ338148		
	Bradys Beach, Bamfield, BC, Canada; 8 Jun. 2005; G.W. Saunders; GWS002887; Le Gall & Saunders (2010)		GQ380028	GQ338104
Archestenogramma profundum C.W. Schneider, Chengsupanimit & G.W. Saunders	Cathedral Rock off Castle Harbour, Bermuda; 23 August 2010; C.W. Schneider, C. Lane, D. McDevit & T. Popolizio; BDA0365; Schneider *et al*. (2011)	JN403075	HQ933371	
	Cathedral Rock off Castle Harbour, Bermuda; 23 August 2010; C.W. Schneider, C. Lane, D. McDevit & T. Popolizio; BDA0368; Schneider *et al*. (2011)			JN403059
Besa catenata (Yendo) M.S. Calderon & S.M. Boo	Katada (34°15’ N, 136°50’ E), Shima Town, Shima City, Mie Pref., Japan; 14 Apr. 2017; M. Suzuki; TNS-AL 209180; this study	**LC589694**1)	**LC589698**2)	**LC589691**
Coccotylus brodiei (Turner) Kützing	Starboard, ME, USA; 25 Apr. 2006; G.W. Saunders, L. Le Gall, D. McDevit, S. Clayden & C. Lane; GWS003640; Le Gall & Saunders (2010)	GQ338135	GQ380037	

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
	Bras d'Or, Cape Breton, NS, Canada; 2 Sep. 2004; G.W. Saunders; GWS002350; Le Gall & Saunders (2010)			GQ338109
Erythrodermis traillii (Holmes ex Batters) Guiry & Garbary	Meadow Cove, NB, Canada; 3 Dec. 2006; G.W. Saunders, D. Saunders, L. Le Gall & D. McDevit; GWS005655; Le Gall & Saunders (2010)	GQ338133	GQ380105	
	English Harbour Eastern Cove, NF, Canada; 20 Jul. 2006; L. Le Gall, D. McDevit & J. Utge; GWS007553; Le Gall & Saunders (2010)			GQ338108
Fredericqia chiharae Mas. Suzuki & Kitayama *sp. nov.*	Tokawa (35°41' N, 140°51' E), Choshi City, Chiba Pref., Japan; 22 Jun. 2008; M. Suzuki; TNS-AL 207116; this study	**LC589695**	**LC589699**	**LC589692**
	Tokawa (35°41' N, 140°51' E), Choshi City, Chiba Pref., Japan; 12 May 2017; M. Suzuki; TNS-AL 209185 *Holotype; this study	**LC589696**$^{3)}$	**LC589700**$^{4)}$	
Fredericqia chiton (M. Howe) Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders	Point Holmes, Comox, BC, Canada; 15 Jun. 2007; G.W. Saunders, B. Clarkston, D. McDevit & K. Roy; GWS008640; Le Gall & Saunders (2010)	GQ338149	GQ380107	
	Scotts Bay, Bamfield, BC, Canada; 13 Sep. 2005; J. Mortimer; GWS003109; Le Gall & Saunders (2010)			GQ338107
Fredericqia decewii Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders	Pachena Beach, Bamfield, BC, Canada; 5 Jun. 2007; D. McDevit, B. Clarkston, K. Roy & H. Kucera; GWS008273; Le Gall & Saunders (2010)	GQ338150	GQ380018	
	Pachena Beach, Bamfield, BC, Canada; 4 Jun. 2005; G.W. Saunders; GWS002712; Le Gall & Saunders (2010)			GQ338105
Fredericqia deveauniensis Maggs, L.Le Gall, Mineur, Provan & G.W. Saunders	Cape Neddick, ME, USA; 3 Nov. 2006; G.W. Saunders & D. McDevit; GWS005620; Le Gall & Saunders (2010)	GQ338151	GQ380113	
	Cape Neddick, ME, USA; 14 Apr. 2005; G.W. Saunders; GWS002678; Le Gall & Saunders (2010)			GQ338106
Gymnogongrus flabelliformis Harvey	Ebisujima (34°39' N, 138°57' E), Susaki, Shimoda City, Shizuoka Pref., Japan; 14 May 2014; M. Suzuki; TNS-AL 188599; Suzuki *et al*. (2021), this study	LC473155	**LC473168**	**LC473147**
Mastocarpus yendoi Masuda & T. Yoshida	Enoshima (35°17' N 139°29' E), Fujisawa City, Kanagawa Pref., Japan; 17 Apr. 2014; M. Suzuki; TNS-AL 188596; this study	**LC589697**	**LC589701**	**LC589693**

Table S1. Continued.

Species	Collection information (locality; date; collector, voucher; Reference)	*rbc*L	*cox*1	28S rDNA
Ozophora lanceolata L.Le Gall & G.W. Saunders	Bradys Beach, Bamfield, BC, Canada; 15 Jun. 2006; G.W. Saunders, B. Clarkston & D. McDevit; GWS004011; Le Gall & Saunders (2010)	GQ338128	GQ380341	GQ338112
Phyllophora crispa (Hudson) P.S. Dixon	Mullaghmore Head, Ireland; 28 Jul. 2003; G.W. Saunders; GWS001813; Le Gall & Saunders (2010)	GQ338131		
	St Michel de Plouguerneau, Brittany, France; 15 Jul. 2007; J. Martin-Lescanne; JML017; Le Gall & Saunders (2010)		GQ380358	GQ338114
Schottera koreana M.S. Calderon, T.H. Seo & S.M. Boo	Shiraishinohana (33°54' N, 132°42' E), Takahama Town, Matsuyama City, Ehime Pref., Japan; 9 Apr. 2016; M. Suzuki; TNS-AL 207074; Suzuki *et al.* (2021), this study	LC538294	**LC538301**	**LC538303**
Stenogramma californicum Harvey	Wizard I., Bamfield, BC, Canada; 3 Jun. 2008; B. Clarkston & S. Toews; GWS010565; Le Gall & Saunders (2010)	GQ338125	GQ380375	GQ338121

1) This sequence was determined from the specimen used in Shibneva *et al*. (2021), and identical (longer 5' read) to MT188617.

2) This sequence was determined from the specimen used in Shibneva *et al*. (2021), and identical (longer 5' and 3' read) to MT188618.

3) This sequence is identical to LC589695.

4) This sequence is identical to LC589699.

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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, *cox*1, and 28S rDNA sequences.

	Combined *rbc*L, *cox*1, and 28S rDNA analyses
Number of taxa	18
Number of nucleotides (bp)	*rbc*L: 1275, *cox*1: 645, 28S rDNA: 2620
Substitution model for ML analyses[1)]	*rbc*L: 1st codons (TrN+I+G4), 2nd codons (TIM2+I+G4), 3rd codons (TIM2+I+G4) *cox*1: 1st codons (TIM1ef+I+G4), 2nd codons (TPM3uf+I), 3rd codons (TPM2uf+I+G4) 28S rDNA: TIM3+I+G4
Substitution model for BI analyses[2)]	*rbc*L: 1st codons (GTR+I+G), 2nd codons (GTR+I), 3rd codons (GTR+I+G) *cox*1: 1st codons (SYM+G), 2nd codons (HKY+I), 3rd codons (GTR+I+G) 28S rDNA: GTR+I+G

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

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

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