A morphological and phylogenetic study of the genus *Chondria* (Rhodomelaceae, Rhodophyta)

【紅藻ヤナギノリ属（フジマツモ科）の形態学的および系統学的研究】

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June 2018

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ABSTRACT

The red algal tribe Chondrieae F. Schmitz & Falkenberg (Rhodomelaceae, Rhodophyta) currently contains seven free-living genera and two parasitic genera. The type genus is *Chondria* C. Agardh which currently includes more than 80 certain species. The tribe Chondrieae is superficially similar to the tribe Laurencieae F. Schmitz, but differs in the number of pericentral cells, origin of tetrasporangia and shape of spermatangial branches. Since the introduction of molecular tools for macroalgal systematics, some taxa of the Rhodomelaceae have been studied in attempts to clarify relationships among genera and species, especially in the tribe Laurencieae. In contrast, the Chondrieae almost has not been drawn attention, especially in terms of molecular and phylogenetic studies. Therefore, the current classification of the Chondrieae is still based almost entirely on morphological characters. This study was carried out to produce the comprehensive molecular phylogeny of *Chondria* as the type genus of the Chondrieae and to verify the current classification of *Chondria* by morphological analyses, using specimens mainly from Japan.

The molecular phylogenetic analyses were conducted based on RuBisCO large subunit (*rbc*L), small subunit of nuclear ribosomal rRNA (SSU rRNA) and mitochondrial cytochrome oxidase subunit 1 (*cox*1) gene sequences; new sequences were generated for12 species. While the Chondrieae was moderately supported and sister to the Laurencieae, the genus *Chondria* did not form a clade, being intermingled with the genera *Acanthophora* J.V. Lamouroux and *Acrocystis* Zanardini (*rbc*L and SSU rRNA trees). Morphological characters which have been adopted to identify *Chondria*

species in previous works (e.g. shape of branchlets and apices, male or female reproductive morphologies) were evaluated whether these characters reflect their molecular phylogeny or not. These taxonomic characters did not correspond to the phylogenetic trees. The phylogenetic trees were shown to be not support the subgenera *Euchondria*, *Coelochondria* and *Platychondria* in the genus *Chondria.*

Two species previously identified as a member of the genus *Chondria*, the Japanese '*Chondria capillaris*' and '*Chondria nidifica*', were segregated from *Chondria* and clustered in the same clade isolated from other species of the tribe Chondrieae in *rbc*L and SSU r RNA trees. The clade of the two species was sister to the clade of the Laurencieae and other species of the Chondrieae with moderate supports. The new genus *Neochondria* was proposed to accommodate these two species: *Neochondria ammophila* S. Sutti, M. Tani, Y. Yamagishi, T. Abe & K. Kogame *sp. nov.* (= Japanese *C. capillaris*) and *Neochondria nidifica* (Harvey) S. Sutti, T. Abe, K.A. Miller & K. Kogame *comb. nov.* (= *C. nidifica*). *Neochondria* strikingly resembles *Chondria* in gross morphology and shares several characters, but it can be distinguished from the latter by the presence of adventitious elongate cells that form densely compact layers between the pericentral cells and surround the axial strand even in ultimate branchlets.

Specimens formerly identified as *Chondria dasyphylla* (Woodward) C. Agardh in Japan were reexamined using morphological and molecular phylogenetic analyses. It was revealed that the specimens consisted of multiple species: *Chondria acuminata* sp. nov., *Chondria* cf. *curdieana* (Harvey ex J.Agardh) De Toni and an unidentified species (*Chondria* sp. 1). *Chondria acuminata* sp. nov. is characterized by its distinctively acuminate branchlets. *Chondria* cf. *curdieana*, which was firstly reported from Japan,

shares most morphological characters with the Australian *Chondria curdieana*. The last taxon, *Chondria* sp. 1 did not resemble any described *Chondria* species from Japan. Morphological and molecular data (*rbc*L, SSU and *cox*1) demonstrated the identity of these three species, suggesting that they are members of the genus *Chondria* and are distinct from the authentic *C. dasyphylla.*

ACKNOWLEDGEMENTS

Firstly, I would like to express my deep gratitude to Professor Kazuhiro Kogame from Hokkaido University, my research supervisors, for the continuous support of my Ph. D. study and for his patient guidance, enthusiastic encouragement and useful critiques of this research work. I would like to express my very great appreciation to Dr. Tsuyoshi Abe from University Museum, Hokkaido University who kindly introduced me to the herbarium specimens and gave me comments and suggestions to improve this research work. I would like to offer my special thanks to Masaya Tani, Hokkaido University, for his numerous collections and supplementary of *Chondria*. I could not have imagined having a better research work without the primary data from him. I would also like to thank Prof. Yukimasa Yamagishi, Fukuyama University, for his help with sampling. My sincere thanks go to Prof. Kathy Ann Miller from University Herbarium, University of California, who kindly sent me herbarium specimens, gave me suggestions and carefully criticized the manuscript. I would also like to extend my thanks to the staff from State Herbarium of South Australia and Herbarium of Department of Marine Biology, Punkyoung National University for sending me herbarium specimens. I am grateful to Prof. Takeo Horiguchi and Prof. Hiroshi Kajihara, Hokkaido University, for their suggestions to improve my dissertation. I wish to thank all members from Biodiversity 2, Hokkaido University, for supporting me and giving advices on laboratory works, especially Wilfred John Santianez and Masakazu Hoshino for their help in sampling. I wish also to thank my friends, family: my parents, my brother and sister for supporting me spiritually throughout my Ph. D. study.

Last but not the least, I wish to acknowledge the full scholarship from Royal Thai Government and the staff from Royal Thai Embassy, Tokyo, for their kind arrangement on the scholarship and giving me the advices about living in Japan.

GENERAL INTRODUCTION

The red algae (phylum Rhodophyta) are a distinct group of eukaryotic organisms characterized by the following combination of characteristics: the complete absence of any flagellate stages; the presence of accessory photosynthetic pigments called phycobilins (phycoerythrin and phycocyanin); the occurrence of nonaggregated photosynthetic lamellae with phycobilisomes within the chloroplast; so-called floridean starch as food reserve; the existence of oogamous sexual reproduction involving specialized female cells termed carpogonia and male gametes termed spermatia but sexuality is apparently lacking in some members (Dixon 1973; Bold & Wynne 1985; Lee 1999). Since two of those characteristics are also recognized in the cyanobacteria, some authors have suggested that red algae are primitive and related to the cyanobacteria (Bold & Wynne 1985). However, later molecular phylogenetic findings using nuclear gene sequences suggested the conflicting results, red algae were rather related to glaucophytes, green algae and land plants; comprising the super-group called Archaeplastida (Bhattacharya & Medlin 1995; Moreira *et al.* 2000; Nozaki *et al.* 2003; Adl *et al.* 2005). Red algae are a marine and freshwater assemblage (mainly marine) predominate in extensive areas of the continental shelves in tropical, temperate, and cold-water regions (Dixon 1973; Bold & Wynne 1985; Freshwater & Rueness 1994; Lee 1999).

Traditionally, Rhodophyta wasconsidered to include two classes, Bangiophyceae and Florideophyceae (Dixon 1973; Bold & Wynne 1985; Lee 1999). However, after the upheaval introduced by molecular phylogenetic studies, this outdated classification has

been revised several times (Adl *et al.* 2005, 2007, 2012). At present, Rhodophyta includes 7 accepted classes comprising Bangiophyceae, Compsopogonophyceae, Cyanidiophyceae, Florideophyceae, Porphyridiophyceae, Rhodellophyceae and Stylonematophyceae (Adl *et al.* 2012; Guiry & Guiry 2018).

Florideophyceae is the most complex and elaborate class in Rhodophyta (Freshwater 2000; Guiry & Guiry 2018). Members of the Florideophyceae have pit connections, apical growth, and sexual reproduction with a triphasic life cycle (Dixon 1973; Lee 1999). Some species are used directly by humans for food, while cell wall polysaccharides are extracted from others for use as gels, and additives in food and cosmetic products (Freshwater 2000). Currently, Florideophyceae includes certain 31 orders: Acrochaetiales, Acrosymphytales, Ahnfeltiales, Atractophorales, Balbianiales, Balliales, Batrachospermales, Bonnemaisoniales, Catenellopsidales, Ceramiales, Colaconematales, Corallinales, Entwisleiales, Gelidiales, Gigartinales, Gracilariales, Halymeniales, Hapalidiales, Hildenbrandiales, Nemaliales, Nemastomatales, Palmariales, Peyssonneliales, Pihiellales, Plocamiales, Rhodachlyales, Rhodogorgonales, Rhodymeniales, Sebdeniales, Sporolithales and Thoreales (Guiry & Guiry 2018).

The Rhodomelaceae (Ceramiales, Rhodophyta) is the largest family of the Florideophyceae, currently includes 20 tribes (Alsidieae, Amansieae, Bostrychieae, Chondrieae, Cladureae, Dipterosiphonieae, Herposiphonieae, Heterocladieae, Laurencieae, Lophosiphonieae, Lophothalieae, Neotenophyceae, Ophidocladeae, Polysiphonieae, Polyzonieae, Pterosiphonieae, Rhodomeleae, Sonderelleae, Streblocladieae and Thaumatelleae) with more than a thousand species and approximately 150 genera recognized (Diaz-Tapia *et al.* 2017; Guiry & Guiry 2018).

The present number of species and genera is probably underestimated since new genera and new species within the family have been continuously proposed based on both morphological and molecular data, e.g. *Lampisiphonia* H.-G. Choi, Díaz Tapia & Barbara (Barbara *et al.* 2013), *Coronaphycus* Metti (Metti *et al.* 2015), *Ohelopapa* F. Rousseau, Martin-Lescanne, Payri & L.Le Gall (Rousseau *et al.* 2017), *Wilsonosiphonia* D. Bustamante, Won & T.O. Cho (Bustamante *et al.* 2017). The great number of rhodomelean species reflects their high morphological diversity, especially the vegetative structures. Thallus form widely varies in the family, from simple structure types (without medullary nor cortical layers) to more complex pseudoparenchymatous types. The Rhodomelaceae is distinguished from the other families of the order Ceramiales by having a polysiphonous structure (axial cell surrounded by several pericentral cells) with monopodial growth; 4–24 pericentral cells which are cut off in alternating sequence; colorless and deciduous vegetative trichoblasts, monosiphonous holoblastic branches which develop from the axial cells and are usually present on subapical cell; tetrahedrally divided tetrasporangia borne on pericentral cells, or on cortical cells in some genera; spermatangial organs on modified trichoblasts, which are terete branches or flat plates; a (3-) 4 celled carpogonial branch, with a lateral sterile group; auxiliary cell that is cut off from the supporting cell after fertilization (Hommersand 1963; Womersley 2003). Fundamental of the current taxonomy of the Rhodomelaceae was established by Falkenberg (1901). His excellent monograph provided the massive and integrative detail of the family. Kylin (1956) mentioned that the Rhodomealceae is the most advanced group within the order Ceramiales. Later, Hommersand (1963) provided more details on the classification of the Rhodomelaceae.

However, Rhodomelaceae still includes the groups of taxa that are incomplete in systematics; taxonomic revisions have been conducted using molecular phylogenetic analyses as well as morphological analyses.

The tribe Chondrieae F. Schmitz & Falkenberg is one of the tribes in the Rhodomelaceae and currently contains seven free-living genera (*Acanthophora* J.V. Lamouroux, *Acrocystis* Zanardini, *Chondria* C. Agardh, *Cladymenia* Harvey, *Coeloclonium* J. Agardh, and *Husseya* J. Agardh) and two parasitic genera (*Benzaitenia* Yendo, and *Ululania* K.E. Apt & K.E. Schlech) (Kurihara *et al.* 2010; Diaz-Tapia *et al.* 2017; Guiry & Guiry 2018). The type genus of the tribe is *Chondria* (Schmitz & Falkenberg 1897). Most genera within the tribe have terete, or flattened branched thalli with a clear axial and five pericentral cells surrounded by a broad, compact, cellular cortex (except in *Coeloclonium*) (Womersley 2003). The tribe Chondrieae is characterized by the following features; the five pericentral cells are elongate and parallel to the axial cells; pericentral and inner cortical cells in many species are distinctive with wall thickenings. Gametophytes are dioecious; spermatangial organs are developed from trichoblasts, forming discs with sterile-cell margins; procarps are borne on lower cells of trichoblasts, with the supporting cell bearing a 4-celled carpogonial branch and 2 sterile groups; cystocarps are lateral, ovoid to slightly urceolate; tetrasporangia are formed in branchlets, occasionally in axillary clusters, cut off from pericentral cells, subspherial and tetrahedrally divided (Hommersand 1963; Stegenga *et al.* 1997).

The tribe Chondrieae is superficially similar to the tribe Laurencieae F. Schmitz and molecular phylogenetic studies have supported the close relationship of these tribes

(Kurihara *et al.* 2010; Diaz-Tapia *et al.* 2017). However, the Chondrieae differs in the number of pericentral cells (5 in Chondrieae, 2 or 4 in Laurencieae); tetrasporangia borne on pericentral cells while in the Laurencieae, they borne inside the cortical cells; and male gametophytes bear spermatangial plates which develope from trichoblasts in the Chondrieae while in the Laurencieae, spermatangia are borne on modified trichoblasts or filaments immersed in apical depressions (Hommersand 1963; Womersley 2003; Diaz-Tapia *et al.* 2017). Since the introduction of molecular tools for macroalgal systematics, some taxa of the Rhodomelaceae have been studied in attempts to clarify relationships among genera, especially in the Laurencieae (Nam *et al.* 2000; Nam 2006; Abe *et al.* 2006; Martin-Lescanne *et al.* 2010; Cassano *et al.* 2012; Metti *et al.* 2015; Francis *et al.* 2017). In contrast, the Chondrieae has been almost neglected, especially in terms of molecular and phylogenetic studies (Diaz-Tapia *et al.* 2017). Therefore, the current classification of the Chondrieae is still based almost entirely on morphological characters.

I focused on genus *Chondria* because althouh it has been recognized as the largest genus of the tribe Chondrieae (Guiry & Guiry 2018), an intensive study on this genus was missing especially molecular phylogenetic study. Molecular phylogenetic analyses as well as morphological analyses in order to verify its current classification were conducted using specimens mainly from Japan. The present thesis consists of three chapters. In Chapter 1, the molecular phylogeny of the genus *Chondria* was investigated using RuBisCO large subunit (*rbc*L), small subunit of nuclear ribosomal rRNA (SSU rRNA) and mitochondrial cytochrome oxidase subunit 1 (*cox*1) gene sequences.

Morphological characters of each *Chondria* species were evaluated whether the characters reflect their molecular phylogeny or not.

In Chapter 2, the Japanese '*Chondria capillaris* (Hudson) M.J. Wynne (= *Chondria tenuissima* (Withering) C. Agardh)', was reexamined to revise its classification. Molecular data based on *rbc*L, SSU rRNA and *cox*1 indicated that the Japanese '*Chondria capillaris*' not only differs from the typical *C. capillaris* but also is not closely related to any *Chondria* species and is even distinct from the tribe Chondrieae. Furthermore, some morphological characters strengthened the dissimilarlity between the Japanese '*C. capillaris*' and the typical *C. capillaris*, and also the separation of the Japanese '*C. capillaris*' from the genus *Chondria*. Based on both molecular and morphological results, the new genus *Neochondria* was established and *Neochondria ammophila* sp. nov. was proposed to accommodate the Japanese '*C. capillaris*'. Morphology of pericentral cells and the presence of the adventitious cells between 5 pericentral cells were evaluated as the taxonomic characters to distinguish *Neochondria* from *Chondria*.

In Chapter 3, three uncertain species regarded as '*Chondria dasyphylla* (Woodward) C. Agardh' in Japan were investigated. *Chondria dasyphylla* (Japanese name 'Yanagi nori') has been reported from Japan since 1896. Okamura (1936) gave a description of '*C. dasyphylla*' in Japan, which agrees well with other descriptions of the typical *C. dasyphylla*. However, Okamura's description did not provide much details of this taxon. My survey of specimens from the Herbarium SAP (Faculty of Science, Hokkaido University) and my samples from Japan using molecular analyses suggested three unidentified species in the specimens identified as *C. dasyphylla*. Further

investigations including comparison of these species with similar species were carried out, and they were attributed to *Chondria acuminata* sp. nov., *Chondria* cf. *curdieana* (Harvey ex J. Agardh) De Toni and *Chondria* sp. 1.

CHAPTER 1. Morphology and molecular phylogeny of the genus *Chondria* based on Japanese specimens

INTRODUCTION

The genus *Chondria* C. Agardh nom. cons. was circumscribed by C. Agardh (1817) based on *Chondria tenuissima* (Withering) C. Agardh (basionym *Fucus tenuissimus* Goodenough & Woodward) (Wynne 1991), originally comprising 29 species. The genus currently contains 80 species (Guiry & Guiry 2018) that can be found in various habitats, attached to other algae, shells or rocks (Hollenberg 1945; Abbott & Hollenberg 1976; Gordon-Mills 1987; Lee & Yoon 1996; Tani & Masuda 2003; Tani *et al.* 2003). *Chondria* species were characterized by having young branches (branchlets) basally constricted, apices of branches rounded or tapering, the subapical cells cutting off five pericentral cells in alternating order and branched trichoblasts (Harvey 1853; Womersley 2003). Five pericentral cells remain clear throughout the thallus. Pericentral cells (and often the inner cortical cells) of most species develop various forms of wall thickenings which can be the characteristics of the species (Gordon-Mills 1987).

Gametophytes of *Chondria* species are dioecious. In female gametophytes, procarps are produced on a lower cell of trichoblasts, with the fifth-formed pericentral (supporting) cell bearing a 4-celled carpogonial branch and lateral and basal sterile cell groups (Womersley 2003). Carposporophytes have a basal fusion cell and branched gominoblasts with clavate terminal carposporangia. Cystocarps are ovoid to slightly urceolate cystocarps, in some species with a basal spur developed from trichoblast cells

above the supporting cell. Male gametophytes form spermatangial plates which are flat, discoid or slightly lobed, developed from the whole or a basal branch of trichoblast, with a sterile margin of 1–3 cell broad. Tetrasporophytes produce tetrasporangia which are developed on pericentral cells of lesser branchlets (Hommersand 1963; Gordon-Mills 1987; Lee & Yoon 1996; Tani & Masuda 2003; Womersley 2003).

Most significant characters for classification of *Chondria* species are vegetative forms. Falkenberg (1901) divided *Chondria* into three subgenera based on their thallus forms. The subgenus *Euchondria* Falkenberg was characterized by a cylindrical thallus with acute apices such as the ones found in *Chondria capillaris* (Hudson) M.J. Wynne (as *Chondria tenuissima* (Withering) C. Agardh) while the subgenus *Coelochondria* Falkenberg was defined by a cylindrical thallus but with obtuse apices as observed in *Chondria dasyphylla* (Woodward) C. Agardh (Weber-van Bosse 1923), and the last subgenus, *Platychondria* Falkenberg, was established for the species which have a flattened thallus such as *Chondria viticulosa* A.J.K. Millar & M.J. Wynne (Millar & Wynne 1992). However, this system was not accepted by many researchers and was not adopted in recent works (Gordon-Mills 1987; Lee & Yoon 1996; Tani *el al.* 2003).

The other vegetative characters that have been adopted for classification of *Chondria* species are habit, size and color of thalli when alive, branching pattern, the shape of superficial epidermal cells and the presence (or absence) and shape of lenticular cell wall thickenings (Setchell & Gardner 1924; Yamada 1935; Hollenberg 1945; Dawson 1963; Gordon-Mills 1987; Lee & Yoon 1996; Tani *el al.* 2003; Bacci 2005). Some gametophytic characters, such as the number and shape of sterile cell rows surrounding a spermatangial plate and the presence or absence of a cystocarpic spur,

have been used for species identification (Dawson & Tözün 1964; Gordon-Mills 1987; Millar & Wynne 1992; Maggs & Hommersand 1993; Lee & Yoon 1996; Tani & Masuda 2003; Womersley 2003). However, species identification in the genus *Chondria* is sometimes difficult due to overlapping characters and considerable intraspecific variation (Bacci 2005).

Currently, 14 species of *Chondria* have been reported from Japan, which are *Chondria armata* (Kützing) Okamura , *Chondria capillaris* (as *Chondria tenuissima*), *Chondria crassicaulis* Harvey, *Chondria dasyphylla*, *Chondria econstricta* Tani & Masuda, *Chondria expansa* Okamura, *Chondria intertexta* P.C. Silva, *Chondria lancifolia* Okamura, *Chondria mageshimensis* Tanaka & K. Nozawa, *Chondria polyrhiza* Collins & Hervey, *Chondria repens* Børgesen, *Chondria ryukyuensis* Yamada, *Chondria stolonifera* Okamura and *Chondria xishaensis* J.-F.Zhang & B.-M.Xia (Yoshida *et al.* 1990; Yoshida 1998; Yoshida *et al.* 2015). Most records of *Chondria* species in Japan have been reported in Japanese literatures. Further, molecular analyses have not been applied to the Japanese *Chondria* in their taxonomic studies.

This Chapter aims to evaluate morphological and ecological characters used for classification and to infer the phylogeny of the *Chondria* based on molecular data in order to validate the current classification system of the genus.

MATERIALS AND METHODS

Sampling and DNA extraction

Field collections of *Chondria* were mounted on herbarium paper; some were fixed in 10% formalin in seawater (v/v) for morphological observations and some were dried in silica gel for molecular analyses. A sample of *Acrocystis nana* Zanardini was added in the analyses as the representative of other genera within the tribe Chondrieae. Voucher specimens were deposited in the Herbarium of the Faculty of Science, Hokkaido University, Sapporo (SAP). Previous formalin-preserved specimens and dried specimens deposited in SAP and specimens loaned from University Herbarium, University of California (UC) were added for molecular and morphological investigations. Total DNA was extracted from silica-gel-preserved specimens or pressed herbarium specimens (Table 1). A QuickExtract™ FFPE DNA Extraction Kit (Epicentre, Madison, USA) or a DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) were used for DNA extraction.

PCR amplification and sequencing

PCR amplifications were performed for *rbc*L, SSU and *cox*1 genes, using TaKaRa Ex Taq DNA polymerase (Takara Bio Inc, Otsu, Japan). Published (Freshwater & Rueness 1994; Shimada 2000; Saunders 2005; Abe *et al.* 2006) and original primers were used for PCR and sequencing (Table 2). PCR conditions were as follows: 94°C for 20 s, 40–50 cycles of 94°C for 20 s, 50°C (*cox*1 and *rbc*L) or 55°C (SSU) for 20 s and 72°C for 45 s, and 72°C for 5 min. PCR products were purified by PEG (polyethylene glycol) precipitation and were sequenced using a BigDye Terminator v1.1 Cycle Sequencing Kit

(Applied Biosystems, Austin, TX, USA) and a ABI Prism 310 or 3730 Genetic Analyzer (Applied Biosystems).

Sequence analyses

Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed using MrBayes v. 3.2.1 (Ronquist *et al.* 2012) and RAxML-HPC v.8 (Stamatakis 2014) on the CIPRES portal, respectively. BI analyses were run with GTR + I + G model selected by AIC in MrModeltest 2.3 (Posada & Crandall 2001). Nodal support was assessed by calculating posterior probability (PP) values at each node. The ML analyses were conducted with a GTRGAMMA model with ML estimates of base frequencies. The best-scoring ML tree and 1000 bootstrap trees were obtained using the rapid bootstrap analysis (-f a). Pairwise Distances were computed using MEGA 6.06 (Tamura *et al.* 2013).

Published sequences of the Chondrieae and representative rhodomelacean sequences from GenBank were included in the analyses (Table 3). *Ceramium virgatum* Roth (KT250272, KP828754) (Ceramiaceae) was selected as an outgroup for *rbc*L and SSU trees. *Polysiphonia pacifica* Hollenberg (KM254964) was used as an outgroup for the *cox*1 tree (Table 3).

Morphological observations

Fresh specimens, liquid-preserved specimens and dried herbarium specimens were used for morphological observations. In case of dried herbarium specimens, specimens were

softened by soaking in filtered sea water before investigation. Sections for light microscopy were made by hand using a razor blade. Sections and fragments of thalli were stained with cotton blue in a lactic acid-phenol-glycerol water [1:1:1:1 (v/v)] solution and were mounted on microscope slides in 50% glycerol-seawater or 30% Karo corn syrup.

RESULTS AND DISCUSSION

Examined specimens

Acrocystis nana Zanardini 1872: 145

Okamura 1907; Mntangi & Farrar 1978; Norris 1988.

Type locality: "Tangion Datu" (Cape Datu), boundary between Sarawak, Malaysia and West Kalimantan, Indonesia (Silva *et al.* 1996).

Examined specimen: SAP115399 (Okinawa, Japan; 2 March 1997; with DNA; Fig. 1).

Description: Thalli 1–2 cm in height, consisting of prostrate and upright branches; upright branches terminating in a spherical or ellipsoid vesicle; axial cells baring five pericentral cells which are radially elongate.

Remarks: *Acrocystis* is a monotypic genus. *Acrocystis nana* has a unique form with vesicles. Cell wall thickenings as demonstrated in Gordon-Mills (1987) for certain species of *Chondria* were not present. Japanese specimens were well corresponded with reports from outside of Japan (Mntangi & Farrar 1978; Norris 1988).

Chondria armata (Kützing) Okamura 1907: 69

Type locality: Wagap, New Caledonia (Silva *et al.* 1996).

Examined specimens: SAP115358 (Talak Berakit, Malaysia; 26 May 1999; with DNA) and SAP115359 (Kagoshima, Japan; 2 August 1997; with DNA; Fig. 2).

Description: Pinkish red in color, thallus dendric, erect with a short, thick, firm, subcylindrical axis with 2–3 mm diam. and 5–6 cm high; lower axes are very thick while upper branches are slender and covered with short ramuli; attached to substrate by means of root-like branches; branches with acute apices and slightly constricted to unconstricted at the base; five remarkable pericentral cells.

Remarks: *Chondria armata* has been characterized by its branching pattern and shape of branches (Okamura 1907; Guiry & Guiry 2018). The present examined *C. armata* was corresponded to its original description.

Chondria capillaris (Hudson) M.J. Wynne 1991: 317

Harvey 1853; Kylin 1956; Dickinson 1963; Gordon-Mills 1987.

Type locality: England (Gordon-Mills 1987).

Examined specimens: SAP106295 (Finavarra, Ireland; 17 August 2004; with DNA) and SAP115387 (Finavarra, Ireland; 17 August 2004; Fig. 3).

Description: Thallus erect, 8–25 cm high, with discoid holdfast, irregularly radial branching, attenuate apices with axial cells being cut off from a dome-shaped apical cell, subdichotomous trichoblasts, five pericentral cells, tetrahedral tetrasporangia, disc-shaped spermatangial plates, ovoid mature cystocarps often with a marked spur at the base, and cell wall thickenings in pericentral cells and subcortical cells.

Remarks: *Chondria capillaris*, generitype of the genus *Chondria*, is the current valid name for *Chondria tenuissima*. It has been reported from Japan since 1896 as '*C. tenuissima*'. However, the Japanese '*C. tenuissima*' possesses some significant dissimilarlities from the British '*C. tenuissima*', near its type locality. An examined specimen from Ireland [SAP115387, tetrasporophyte] was well corresponded with *C. capillaris* descripted by Gordon-Mills (1987).

***Chondria crassicaulis* Harvey 1860: 330**

Harvey 1860; Okamura 1909; Lee & Yoon 1996.

Type locality: Shimoda, Shizuoka Pref., Japan (Harvey 1860).

Examined specimens: SAP115360 (Innoshima, Hiroshima, Japan; 20 April 2015; with DNA), SAP115361 (Koinoura, Fukuoka, Japan; 23 March 2015; with DNA) and SAP115362 (Oshoro, Hokkaido, Japan; 24 June 2015; with DNA; Fig. 4).

Description: Purplish-red, 1–20 cm high, tufted; holdfasts massive or discoid; main axes cartilaginous issuing branches solitarily or in groups at nodes; branchlets clavate, with constricted at the base; apices obtuse and depressed; issuing five indistinct pericentral cells; spermatangial plates discoid and undulate; ovoid cystocarps without spurs at the base.

Remarks: *Chondria crassicaulis* is distinct from the other *Chondri*a species by possessing the multicellular stalk of a pair of spermatangial plates with 2–5 rows of sterile marginal cells. Lee & Yoon (1996) suspected that based on morphology, *C. crassicaulis* may be separated from the members of the genus *Chondria*. However, the present examined specimens are corresponded to the original description of *C. crassicaulis* and recent molecular data confirmed that *C. crassicaulis* is a member of *Chondria.*

***Chondria decipiens* Kylin 1941: 41**

Abbott & Hollenberg 1976.

Type locality: California, USA (Abbott & Hollenberg 1976).

Examined specimens: UC2025838 (San Nicolas Island, California, USA; 14 November 2012; with DNA; Fig. 5A–E) and UC1844102 (Mussel Point, Pacific Grove California, USA; 26 September 1969; Fig. 5F–G).

Description: Erect thallus, 8–16 cm high, medium to deep brown; much-branched axes arising from bases of compact, discoid attachments and stolons; fusiform branchlets,

seldom exceeding 4 mm in length, terminally wrinkled or corrugated owing to small branch-initial depressions; spermatangial plate disc-shaped; ovoid cystocarps with marked cystocarpic spurs.

Remarks: *Chondria decipiens* possesses several taxonomic characters that resemble those of other *Chondria* species (Guiry & Guiry 2018), therefore, identification of this species should be done with caution. The present examined *C. decipiens* specimens loaned from UC are corresponded with its original description. *Chondria decipiens* has never been reported from Japan.

Chondria expansa Okamura 1927: 163

Lee & Yoon 1996.

Type locality: Kashiwajima, Kochi Pref., Japan (Okamura 1927).

Examined specimens: SAP115365 (Kushimoto, Wakayama, Japan; 31 March 2003; with DNA; Fig. 6) and SAP115366 (Tateyama, Chiba, Japan; 21 March 2016; with DNA).

Description: Thalli branched, intricate, reddish brown; main axes indistinct, terete or compressed, flexuous or decumbent, attaching together with haptera; fusiform branchlets with acute apices, constricted at the base; cell wall thickenings found in pericentral and subpericentral cells.

Remarks: The present examined *Chondria expansa* were drifted thalli. They were corresponded to the original description of this species (Okamura 1927).

***Chondria intertexta* P.C. Silva 1972: 204**

Synonym: *Chondria intricata* Okamura 1912: 180. nom. illeg.

Okamura 1912; Lee & Yoon 1996.

Type locality: Aburatsubo and Enoshima (Prov. Sagami), Japan (Okamura 1912).

Examined specimen: SAP115364 (Hachijo Island, Tokyo, Japan; 21 July 2005; with DNA; Fig. 7).

Description: Epiphytic, more or less intricate, pale brownish red; branches terete, indistinct and not constricted at base, bearing short ramuli; obtuse and depressed apices; haptera issuing on every erect filaments at various intervals, causing the attachment of erect filaments together in places; pericentral cells cylindrical including radiating spine-like structures.

Remarks: This species was first described as the name *Chondria intricata* by Okamura (1912). But, this name was a later homonym of *Chondria intricata* (Lamouroux) C. Agardh (1817), thus a replaced name *C. intertexta* was proposed (Silva 1972). The examined specimens were corresponded to the original description (Okamura 1912). However, radiating spine-like structures in pericentral cells that mentioned by Lee & Yoon (1996) were not found.

Chondria mageshimensis **Tanaka & K. Nozawa in Tanaka 1965**

Type locality: Mageshima, Kagoshima Pref., Japan (Tanaka 1965).

Examined specimen: SAP115367 (Innoshima, Hiroshima, Japan; 19 August 2005; with DNA; Fig. 8).

Description: Somewhat fleshy and membranaceous thallus, purplish red, 10–14 cm high; usually complanate throughout except the base and the attenuate tip, 8–12 times dichotomously branched; branchlets having depressed apices; axis with five or six pericentral cells surrounded by a loose subcortex and cortex of branching cell series; tetraspores usually ovate; male and female organs unknown.

Remarks: *Chondria mageshimensis* is a rather distinct one among the Japanese species by its thalli being slender, flattened, or complanated throughout except at the base and the attenuate apex of the thalli. The examined specimens of *C. mageshimensis* were corresponded with its original description (Tanaka 1965).

Chondria ryukyuensis **Yamada 1935: 27**

Type locality: Naha, Okinawa Pref., Japan (Yamada 1935).

Examined specimen: SAP115368 (Kagoshima, Japan; 27 August 2003; with DNA; Fig. 9).

Description: Thalli about 15 cm high, at the base nearly cylindrical and often loosely entangled in an irregular manner, becoming flattened upwards, branched repeatly and

densely in an alternate-pinnate manner; branches distichous 2–2.5 mm broad usually widened near the top; pericentral cells very large.

Remarks: *Chondria ryukyuensis* is a distinctive Japanese species by its thallus form with alternate-pinnate branching. The examined specimens of *C. ryukyuensis* were corresponded with its original description (Yamada 1935).

Neochondria ammophila* S. Sutti, M. Tani, Y. Yamagishi, T. Abe & K. Kogame *sp. nov.

(= Japanese '*Chondria capillaris*')

Type locality: Momonai, Otaru, Hokkaido, Japan (This study).

Examined specimens: SAP115347 (Momonai, Otaru, Hokkaido, Japan; 23 September 1996; with DNA), SAP115354 (Muroran, Hokkaido, Japan; 23 May 2016; Figs 10–12), SAP115369 (Innoshima, Hiroshima, Japan; 20 April 2015; with DNA), SAP115370 (Momonai, Hokkaido, Japan; 29 June 2016; with DNA; Fig. 13), SAP115349 (Muroran, Hokkaido, Japan; 28 July 1999; Fig. 14) and SAP115371 (Muroran, Hokkaido, Japan; 26 July 2016; with DNA; Fig. 15).

Description and remarks are included in Chapter 2.

Neochondria nidifica* (Harvey) S. Sutti, T. Abe, K.A. Miller & K. Kogame *comb. nov.

(= *Chondria nidifica*)

Type locality: Unknown, presumed to be in the vicinity of San Diego, California (Dawson & Tözün 1964).

Examined specimens: UC2026095 (Dana Point, California, USA; 12 December 2012; with DNA; Fig. 16A), UC2036061 (Santa Rosa Island, California, USA; 20 February 1989; Fig. 16B) and UC1022164 (Santa Rosa Island, California, USA; 27 January 1949; Fig. 16C).

Description and remarks are included in Chapter 2.

***Chondria acuminata* sp. nov. (= part of Japanese '*C. dasyphylla*')**

Examined specimens: SAP115391 (Shishi-iwa, Hokkaido, Japan; 21 August 2017; with DNA; Fig. 17), SAP115363 (Utoro, Hokkaido, Japan; 30 July 1999; with DNA; Fig. 18), SAP115401 (Utoro, Hokkaido, Japan; 9 August 1998; Fig. 19), SAP115389 (Chashikotsu, Shiretoko, Hokkaido, Japan; 11 November 2008; with DNA) and SAP115390 (Shishi-iwa, Hokkaido, Japan; 21 August 2017; with DNA).

Description: The description of this species is provided in the Chapter 3.

Remarks: This species resembles *Chondria dasyphylla* and probably has been misidentified as the species in Japan. See the Chapter 3 for details.

***Chondria* cf. *curdieana* (Harvey ex J. Agardh) De Toni, 1903**

(= part of Japanese '*C. dasyphylla*')

Examined specimens: SAP115392 (Muroran, Hokkaido, Japan; 21 August 2016; with DNA), SAP115394 (Muroran, Hokkaido, Japan; 21 August 2016; Figs 20–21),

SAP115395 (Muroran, Hokkaido, Japan; 22 August 2017; with DNA) and SAP115396 (Muroran, Hokkaido, Japan; 22 August 2017; with DNA).

Description: The description of this species is provided in the Chapter 3.

Remarks: This species resembles *Chondria dasyphylla* and probably has been misidentified as the species in Japan. See the Chapter 3 for details.

***Chondria* sp. 1**

Examined specimen: SAP115397 (Shishi-iwa, Shiretoko, Hokkaido, Japan; 21 August 2017; with DNA; Fig. 22).

Description: The description of this species is provided in the Chapter 3.

Remarks: This species does not resemble to any *Chondria* species in Japan. See the Chapter 3 for details.

Taxonomic characters

Habitat

Most examined specimens (*Acrocystis nana*, *Chondria armata*, *C. capillaris*, *C. crassicaulis*, *C. decipiens*, *C. expansa*, *C.intertexta*, *C. magashimensi*s, *C. ryukyuensis*, *C. acuminata*, *C.* sp. 1, *Neochondria ammophila*, *N. nidifica*) were epilithic species

while only *Chondria* cf. *curdieana* was epiphytic species attached on a seagrass leaf (*Phyllospadix iwatensis* Makino). Moreover, the characteristic habitat of most species was either shallow for such as *C. capillaris*, *C. crassicaulis* and *C. decipiens* or deep water for such as C. *ryukyuensis* that was found on coral reef about 2 fathoms depth (Yamada 1935; Abbott & Hollenberg 1976; Gordon-Mills 1987; Guiry & Guiry 2018).

Color, size and thallus form and attachment

Most *Chondria* species were pinkish brown or purplish red in fresh collected samples and they were usually intensified on drying. Color can be helpful for identification, but it is important that the material is fresh when collected and has been kept in darkness.

The overall size of the collected *Chondria* varied from small, intricate thalli of 2–5 cm in height [e.g. *C. intertexta* (Fig. 7A)] to erect and robust species with maximum length up to 40 cm [e. g. *N. nidifica* (Fig. 16A–C)]. Thallus forms were classified into several patterns including flattened thalli [i.e. *C. expansa* (Fig. 6A) and *C. ryukyuensis* (Fig. 9A)], prostrate and entangle thalli [i.e. *C. intertexta* (Fig. 7A) and *Chondria* sp. 1 (Fig. 22A)], moderate size and bush-like thalli [i.e. *C. mageshimensis* (Fig. 8A)], moderate size and an erect axis with branches showing a pyramidal form [i.e. *C. acuminata* (Fig. 17A)], erect and solitary without stolons [i.e. *C.* cf. *curdieana* (Fig. 20A)], erect and robust thalli with many stolons [i.e. *C. decipiens* (Fig. 5A), *N. ammophila* (Fig. 10A, B) and *N. nidifica* (Fig. 16A–C)], fleshy thalli with distinct main axes and bulbose branchlets [i.e. *C. crassicaulis* (Fig. 4A)] and fleshy thalli with distinct

main axes and bearing many pinnate branchlets but not constricted at the base [i.e. *C. armata* (Fig. 2A)].

Basically, the primary attachment of the collected *Chondria* species is by means of the discoid holdfast and rhizoidal haptera. However, it depends on the characteristic habitats of the species. For example, in species that their characteristic habitats are on rocks [e.g. *C. decipiens* (Fig. 5A) and *C. acuminata* (Fig. 17A)], several short creeping branchlets produced from the lowermost parts of erect axes were found and they became attached to the substrate. For species (i.e. *C.* cf. *curdieana*) that their habitats are on the other substrate like seagrass leaves, only a discoid holdfast was found (Fig. 20A).

Branching patterns, branchlets and apices

The most common branching pattern in *Chondria* is irregularly radial, in which branchlets (laterals) mainly are produced on both sides of the axis. In some species the branching was prolific and spreading [e.g. *C. capillaris* (Fig. 3A, B)] while in the others it was sparser [e.g. *Chondria* sp.1 (Fig. 22B)]. Degree of branching considerably varied with age and between populations within particular taxon, therefore, it was not a good taxonomic character. Clusters of small adventitious branches (axillary branching) can be found in the axils of main branches. This character was more frequent in some species (e.g. *N. ammophila*, *N. nidifica*) than in others, however, it was not constant.

Although 'branches constricted at the base' is usually listed as a generic character of the genus *Chondria* (Harvey 1853; Womersley 2003), in some species [e. g. *C. armata* (Fig. 2B), *C. econstricta*, *C. intertexta* (Fig. 7B) and *C. mageshimensis* (Fig. 8B)]

(Okamura 1907; Tanaka 1965; Womersley & Bailey 1970; Tani & Masuda 2003), the lesser branches (branchlets) are not or only slightly basally constricted. Moreover, this character related to the ages of the branches. For the younger branch at the top of the thallus, constriction was conspicuous while the older branch at the bottom or near main axis, it was ambiguous due to the thickening.

Shape of branchlets and apices is the useful features for identification of *Chondria* species (Harvey 1853; Falkenberg 1901; Kylin 1956; Taylor 1960). However, identification of *Chondria* using shape of branchlets and apices should be done with caution. For example, in *Chondria acuminata* which was previously identified as *Chondria dasyphylla* in Japan due to its obtuse and sunken apex, if considering under higher magnification, its apex was not sunken. The apical cell of *C. acuminata* was protruded from its blunt apex, making the branchlet like acuminate shape instead of obtuse shape (Fig. 17C). Besides, molecular data supported that *C. acuminata* and *C. dasyphylla* are not conspecific. Details will be given in Chapter 3.

Epidermal cell

Several intensive studies on the morphology of the genus *Chondria* provided a lot of details on the epidermal cells such as epidermal cell arrangement, shape of epidermal cells and pit connection between the cells (Gordon-Mills 1987; Lee & Yoon 1996; Tani & Masuda 2003; Tani *et al.* 2003). However, some important details were not given, for example, which part of the specimens was examined. Based on this study, shape of the epidermal cells considerably varied with age. For the most examined specimens, shape

of the epidermal cells of the younger parts was rounded in common while in the older parts, it was more or less geometric form or irregular shape (e.g. Fig. 12C, D; Fig. 21D). Therefore, shape of the epidermal cells should be used as taxonomic characters with caution.

Pericentral cells and cell wall thickening

An axial cell issuing five pericentral cells is a distinct character of the genus *Chondria* and also the other genera within the tribe Chondrieae (Harvey 1853; Womersley 2003). For most of examined *Chondria* specimens, five pericentral cells were recognized. Each pericentral cell was identified by pit connection between its cell and an axial cell, and was distinctive and larger than an axial cell in cross section and remained its identity throughout the thallus. These characters were used to distinguish the genus *Chondria* and the newly segregated genus *Neochondria*, details in Chapter 2. However, major problem regarding the use of the characters of pericentral cells as a taxonomic character is that image of cross-sections may be different depending on thallus portions. In some studies, the cross-sections were cut at the young branchlets while the main axes were cut in others (Gordon-Mills 1987; Lee & Yoon 1996; Stegenga *et al.* 1997; Tani & Masuda 2003; Tani *et al.* 2003; Womersley 2003).

Importance of cell wall thickenings in the pericentral cells and subcortical cells for classification was firstly suggested by Gordon-Mills & Womersley (1984) (Gordon-Mills 1987). The presence or absence, and form of cell wall thickenings may be species-specific (Gordon-Mills 1987; Lee & Yoon 1996). For these examined *Chondria*, cell wall

thickenings in the pericentral cells and subcortical cells were constantly found in most species including *C. capillaris* (Fig. 3D), *C. crassicaulis* (Fig. 4E), *C. expansa* (Fig. 6C), *C. intertexta* (Fig. 7D), *C. acuminata* (Fig. 17F), *C.* cf. *curdieana* (Fig. 21A) and *Chondria* sp. 1 (Fig. 22F) corresponding to the description of these species (Gordon-Mills 1987; Lee & Yoon 1996; Womersley 2003; this study). The number or the abundance of cell wall thickenings within a species varied due to their ages. Cell wall thickenings were more abundant in the elder plants. In other species including *C. armata* (Fig. 2E), *C. decipiens* (Fig. 5D), *C. mageshimensis* (Fig. 8D) and *C. ryukyuensis* (Fig. 9C), cell wall thickenings were not found or unconstant. Moreover, within the new genus *Neochondria*, cell wall thickening was not found (Figs 11B, 16D). Until now, function of the cell wall thickenings is unknown. However, based on personal observations, thalli of the species with the cell wall thickenings are firmer than the others with no cell wall thickening and are easier to cut a section.

Tetrasporophyte, male and female gametophyte

Compared to the vegetative structures, use of reproductive structures for species identification involves fertile plants, which are sometimes unavailable. Tetrasporophytes of the examined specimens shared the typical character of the *Chondria*, that is a tetrahedrally divided tetrasporangium borne on a pericentral cell. The tetrasporophytic plants of *N. nidifica* were distinctive in having tufts of tetrasporangial branchlets (Fig. 16A–C, details in Chapter 2). Patterns and arrangements of tetrasporangia of *Chondria* species were studied by Tani & Masuda (2003).

Taxonomic characters of male gametophytes used for identification include the origin and shape of a spermatangial plate and the number of sterile cells surrounding a spermatangial plate (Gordon-Mills 1987; Lee & Yoon 1996; Womersley 2003). However, generally male plants are rarely found, and in this present study male gametophytes were only found in *C.* cf. *curdieana* and *N. ammophila*. Male gametophytes of these two taxa were found in August, summer of Hokkaido Prefecture. Features of their spermatangial plates were similar: originating from the trichoblast, discoid shape with a single row of sterile cells (Fig. 14, Fig. 20C–E). In case of female gametophytes, shape of cystocarps and the presence or absence of a cystocarpic spur were regarded as taxonomic characters for identification of *Chondria* species (Gordon-Mills 1987; Lee & Yoon 1996; Womersley 2003). Cystocarps of *N. ammophila* (Fig. 15D–F), *N. nidifica* (Fig. 16G) and *C.* cf. *curdieana* (Fig. 20F) were globose and had no markedly cystocarpic spur. Cystocarps of *C. decipiens* (Fig. 5H) were globose with a cystocarpic spur, supporting the study of Abbott and Hollenberg (1976). Cystocarpic spurs are a good character to distinguish *Chondria* species.

Molecular and phylogenetic results

Lists of newly generated sequences were shown in Table 1. The *rbc*L sequences were generated from 26 specimens from 13 certain species and 1 unidentified (1119 bp except 687 bp from *A. nana* [MG843864], 715 bp from *C. decipiens* [MG255056], 720 bp from *N. nidifica* [MG255067]). ML tree represents 49 rhodomelacean sequences with a *Ceramium virgatum* as an out-group (Fig. 23). The tribes Chondrieae (excluding *Neochondria*) and

Laurencieae were supported in the tree with moderate and high supports (ML 77%/ BPP 1.00 and ML 97%/ BPP 1.00, respectively). The sequence of *C. capillaris* MG255052 from Finavarra, Ireland was identical with that of *C. capillaris* MF094050 from England. Some Chondrieae *rbc*L sequences (*A. nana*, *C. armata*, *C. crassicaulis*, *C. decipiens*, *C. expansa*, *C. intertexta*, *C. mageshimensis*, *C. ryukyuensis*, *C. acuminata*, *C.* cf. *curdieana* and *Chondria* sp. 1) were firstly sequenced in this study. All *N. ammophila* (the Japanese *C. capillaris*) sequences were almost identical. Not only differed from the typical *C. capillaris* from Ireland, *N. ammophila* were segregated from the tribe Chondrieae. Moreover, *N. ammophila* were grouped with *N. nidifica* (as *C. nidifica*). More details about the newly segregated genus *Neochondria* (Rhodomelaceae, Rhodophyta) are given in Chapter 2. *Acanthophora spicifera*, *Acrocystis nana* and *Cladhymenia lyallii* were mixed with *Chondria* species in the Chondrieae clade.

Fifteen SSU rRNA sequences were newly generated (1667–1729 bp). ML tree based on 47 rhodomelaceans and *Ceramium virgatum* KP828754 as an outgroup (Fig. 24) showed a similar topology to that of the ML tree of *rbc*L. Most *Chondria* species were clustered within the tribe Chondrieae but with low support (ML 51%/ BPP 0.92). *N. ammophila* and *N. nidifica* were grouped in the same clade with high support (ML 96%/ BPP 1.00) and isolated from the Chondrieae. *Acanthophora spicifera*, *A. pacifica* and *Acrocystis nana* were mixed with *Chondria* species. *Acanthophora spicifera* and *A. pacifica* did not cluster, *Ululania stellata* was closely related to *Chondria expansa*, and *Benzaitenia yenoshimensis* was closely related to *Chondria classicaulis.*

*Cox*1 genes (556 bp) from 21 specimens representing 11 species were amplified and sequenced in this study. ML tree was constructed from 43 Chondrieae-Laurencieae

sequences and *Polysiphonia pacifica* KM254964 as an outgroup (Fig. 25). However, *cox*1 tree did not support the tribe Chondrieae. *Acanthophora spicifera* and *A. pacifica* did not cluster. *Benzaitenia yenoshimensis* was closely related to *Chondria crassicaulis* again.

Discussion of results of molecular analyses

While species in the Laurencieae have been intensively studied in attempts to clarify relationships among genera using molecular tools (Abe *et al.* 2006; Martin-Lescanne *et al.* 2010; Cassano *et al.* 2012; Metti *et al.* 2015), species in the Chondrieae have not (Diaz-Tapia *et al.* 2017). In the present molecular analyses, the Chondrieae was supported with moderate supports and was sister to the Laurencieae. *Cladurus elatus* [MF094051], which had been included in the tribe Chondrieae in earlier classifications (Falkenberg 1901; Hommersand 1963; Kurihara *et al.* 2010) was excluded from the present analyses (Fig. 23), according to the recent study by Diaz-Tapia *et al.* (2017) in which the species was transferred to the tribe Cladueae Diaz-Tapia & Maggs.

The present trees did not highly support monophyly of the tribe Chondrieae but showed that the tribe may be paraphyly: in both *rbc*L and SSU trees, *Neochondria* was sister to the clade of Laurencieae and other species of Chondrieae with moderate supports, suggesting paraphyly of the Chondrieae. However, a new tribe to accommodate *Neochondria* is not proposed in this study because more analyses including the other genera within the Chondrieae are needed to resolve the taxonomic problem. The number of pericentral cells and shape of spermatangial branches are used

to distinguish the tribes Chondrieae (five pericentral cells and discoid spermatangial branches) and Laurencieae (2–4 pericentral cells and branched form of spermatangial branches). If this topology is true, the characters of five pericentral cells and discoid spermatagial branches, by which Chondrieae is characterized, are plesiomorphy rather than apomorphy.

The genus *Chondria* was also not supported in the present molecular trees (*rbc*L and SSU). *Benzaitenia* and *Ululania* are parasitic genera, and they were known to be closely related to their host species (Kurihara *et al*. 2010). However, *Acrocystis* and *Acanthophora* were positioned among *Chondria* species. These results have been previously reported (Kurihara *et al.* 2010), demonstrating that taxonomic revisions of genera are needed for Chondrieae based on molecular trees with higher resolution and more species and genera.

In the *rbc*L and SSU trees, two species earlier identified as a member of the genus *Chondria*, the Japanese '*C. capillaris*' (= *Neochondria ammophila*) and '*C. nidifica*' (= *Neochondria nidifica*), were segregated from *Chondria* and clustered in the same clade isolated from other species of the tribe Chondrieae. New genus *Neochondria* was proposed to accommodate them in the present study (see Chapter 2). Moreover, three *Chondria* species which did not match with any descriptions of *Chondria* species in Japan were found (see Chapter 3).

The phylogenetic trees did not support the three subgenera *Euchondria*, *Coelochondria* and *Platychondria*. Although these subgenera are characterized by shape of branches and apices (Falkenberg 1901), these characters did not show monophyly in the trees. However, the most possible clade based on shape of branchlets and apices is

the clade of *Chondria acrorhizophora* Setchell & N.L.Gardner [as *Chondria californica* (Collins) Kylin], *Chondria baileyana* (Montagne) Harvey, *Chondria dasyphylla*, *Chondria acuminata* and *Chondria* cf. *curdieana* in the *rbc*L tree with high supports (ML 94%/BPP 1.00). These species, except *C. acrorhizophora*, share the characters of the subgenera *Coelochondria* having cylindrical thalli and branchlets with depressed apices (Agardh 1817; Harvey 1853; Stechell & Gardner 1924; this study). However, in the SSU tree this clade was not supported. Considered with paraphyly of the genus *Chondria*, this system of subgenera should not be adopted.

The *cox*1 tree had lower resolution than *rbc*L and SSU trees probably due to the high evolutionary rate of the *cox*1 gene. However, each clade of species was well supported, showing usefulness for recognition of species. Similar results have been reported in Robba *et al.* (2006), Yang & Kim (2015) and Kogame *et al.* (2017).

I tried to find morphological characters that reflect the molecular phylogeny but cannot find such useful characters for revising the classification of the Chondrieae. As mentioned above, *Chondria* currently includes about 80 species, thus many species have not been investigated in molecular analyses. Further, two genera (*Coeloclonium* and *Husseya*) of the Chondrieae have not been included in molecular phylogenetic studies. Considering the results of the present molelecular analyses and existence of many species, revision of the classification of the Chondrieae including *Chondria* would be very challenging like the case of the Laurencieae (Abe *et al.* 2006; Nam 2006; Martin-Lescanne *et al.* 2010; Rousseau *et al.* 2017)

CHAPTER 2. *Neochondria* gen. nov., a segregate of *Chondria* including *N. ammophila* sp. nov. and *N. nidifica* comb. nov.

INTRODUCTION

Chondria tenuissima (Withering) C. Agardh (1817), the lectotype of the genus, was based on *Fucus tenuissimus* Withering (1796) from Portland, England. Wynne (1991) pointed out that *C. tenuissima* is a taxonomic synonym of the earlier *Ulva capillaris* Hudson (1778) (syntype localities Christchurch, Hampshire and Margate, Kent, England) and made the combination *Chondria capillaris* (Hudson) M. J. Wynne. This species has been characterized by a discoid holdfast, irregularly radial branching, attenuate apices with axial cells being cut off from a dome-shaped apical cell, subdichotomous trichoblasts, five pericentral cells, tetrahedral tetrasporangia, disc-shaped spermatangial plates, ovoid mature cystocarps with a marked spur at the base, and cell wall thickenings in pericentral cells and subcortical cells (Harvey 1853; Kylin 1956; Dickinson 1963; Gordon-Mills 1987). *Chondria capillaris* is summer annual, commonly occurring on intertidal rocks, stones and shells in Britain (Dickinson 1963, as *C. tenuissima*). This species has been reported widely from Europe, Atlantic islands, North America, South America, Caribbean Islands and Asia (Guiry & Guiry 2018).

Chondria capillaris was first recorded in Japan by Holmes (1896). Okamura (1936) provided a description of Japanese specimens (as *C. tenuissima*): thalli with tufts of several axes from a stoloniferous base; pyramidal outline, 10–25 cm in height; terete branches 1 mm in diameter, pinkish red, mostly irregularly branched with radially

arranged, alternate, fusiform branchlets with acute apices. Although there are few published records of '*C. capillaris*' from Japan (Yoshida *et al.* 1990; Yoshida 1998; Yoshida *et al.* 2015), specimens are available in the herbarium of Hokkaido University (SAP).

Since some morphological characters of Japanese specimens of *C. capillaris* differ from its original description and other publications on this taxon, the Japanese *C. capillaris* should be reexamined. The object of this Chapter is to reexamine and clarify the classification of the Japanese '*C. capillaris*'. The Japanese *C. capillaris* resembled *Chondria nidifica* Harvey, thus herbarium specimens of the latter were also investigated.

MATERIALS AND METHODS

Specimens of '*Chondria capillaris*' were collected at low tide from Hokkaido and Hiroshima Prefectures, Japan (Table 4). Field collections were mounted on herbarium paper; some were fixed in 10% formalin in seawater (v/v) for morphological observations and some were dried in silica gel for molecular analyses. Voucher specimens were deposited in the Herbarium of the Faculty of Science, Hokkaido University, Sapporo (SAP). Other specimens (Table 5) in SAP were also examined to determine patterns of distribution and phenology. Specimens of *Chondria nidifica* Harvey housed in the University Herbarium, University of California (UC) were examined (Table 5). For anatomical observations, sections of thalli were made by hand using a razor blade. Sections and fragments of thalli were stained with cotton blue in a

lactic acid-phenol-glycerol water [1:1:1:1 (v/v)] solution and were mounted on microscope slides in 50% glycerol-seawater or 30% Karo corn syrup.

Methods for molecular investigations, including DNA extraction, PCR amplification and sequence analysis, are mentioned in Chapter 1

RESULTS

Neochondria S. Sutti, M. Tani, Y. Yamagishi, T. Abe & K. Kogame *gen. nov.*

DIAGNOSIS: With characters of the genus *Chondria*; cartilaginous, branched thallus with polysiphonous branches constricted at the base; distinct axial cells, each with 5 pericentral cells; female plants bearing ovoid, globose cystocarps; male plants bearing fan-shaped spermatangial branches; tetrasporangial plants bearing tetrahedrally divided tetrasporangia borne on pericentral cells. Characters unique to the genus: adventitious elongate cells between the pericentral cells, which are evident only at the apex of young branches, forming densely compact layers surrounding the central axial cells throughout, including the ultimate branchlets; cystocarps without spur branches at the base.

GENERITYPE: *Neochondria ammophila* S. Sutti, M. Tani, Y. Yamagishi, T. Abe & K. Kogame *sp. nov.*

ETYMOLOGY: The generic name refers to the resemblance to *Chondria*; neo-, from the Greek “neos” meaning new, young, fresh, recent.

Neochondria ammophila* S. Sutti, M. Tani, Y. Yamagishi, T. Abe & K. Kogame *sp. nov.

Figs 10–15

DESCRIPTION: Thalli terete, cartilaginous, with 0.5–2.0 mm diameter discoid holdfasts and several erect axes tufted from stoloniferous bases. Size 8–20 cm in length and 0.5–1.0 mm in diameter, purplish red in color. Branching mostly irregularly and radially alternate. Lateral branches fragile, fusiform with acute tips, and strongly constricted at the base while lower branches firm and less constricted. In transverse section at tip of a lateral branch, an axial cell issuing 5 pericentral cells and adventitious cells with a single layer of pigmented, palisade-like cortical cells and 2–3 rows of subcortical cells. Five pericentral cells are generally equal as the axial cell, 30–40 µm in diameter while adventitious cells 10–20 µm in diameter. Tetrasporangial branchlets irregularly arranged, sometimes in inconspicuous tufts. Male branchlets with several rounded spermatangial plates, each with a single row of sterile cells. Female branchlets bearing cystocarps lacking spurs.

HOLOTYPE: SAP115370, Momonai (43°12'37.4"N 140°53'43.1"E), Otaru, Hokkaido Prefecture, Japan, 29 June 2016 (Herbarium SAP, Faculty of Science, Hokkaido University, Sapporo, Japan).

ISOTYPE: SAP115355

PARATYPES: Specimens listed in Tables 1, 4 and 5.

ETYMOLOGY: From the Greek ‘ámmos’ (noun), meaning ‘sand’, and ‘-philia’, meaning ‘lover’.

DISTRIBUTION: Hokkaido, Honshu and Kyushu, Japan.

DNA SEQUENCES OF THE TYPE: *rbc*L= MG255065, SSU= MG272243, *cox*1= MG272230

MISAPPLIED NAMES IN JAPAN: *Chondria tenuissima* sensu Okamura 1936: 842; *Chondria capillaris* (Hudson) M.J. Wynne 1991: 317

Vegetative features

Terete and cartilaginous to fleshy thalli, deep purplish red when fresh, dried specimens firmly attached to paper and brown or reddish brown (Fig. 10A). Densely branched near the base, some branches becoming stoloniferous (Fig. 10B). Occasional unbranched, single-celled rhizoidal haptera attach stoloniferous branches to the substrate (Fig. 10C). In middle to upper portions, branches tufted at unequal intervals. Ultimate branchlets fusiform, obviously constricted at the base and 200–250 µm in diameter and 500–1000 µm in length (Fig. 10D). Sub-dichotomously branched trichoblasts conspicuous at apices (Fig. 10E). Apices attenuated with a single dome-shaped apical cell 10–15 µm in diameter (Fig. 11A). Cross-sections of branchlet apices show single layer of pigmented, palisade-like cortical cells, and 2–3 rows of subcortical cells. Each axial cell bearing,

near its midpoint, five pericentral cells of equal size, about the same size and shape as the axial cell, 30–40 µm in diameter. Adventitious cells 10–20 µm in diameter, cut off from pericentral cells, are conspicuous among the pericentral cells (Fig. 11B, C). Axial cell and 5 pericentral cells can be distinguished only in the distal portions of branches and not in mature position (Fig. 11D). Cell wall thickenings in the pericentral cells were not observed.

Longitudinal sections of branchlet show arrangement of axial cells, pericentral cells and adventitious pericentral cells. Trichoblast basal cells originate from axial cells (Fig. 12A, B). Epidermal cells in the upper (younger) branches rounded to oval or square (10 × 15 µm in surface view); in the lower (older) branches, epidermal cells larger and square or rectangular (10–15 µm in width and 15–30 µm in length) (Fig. 12C, D).

Reproductive morphology

The uppermost branches of tetrasporophytes are lighter in colour and more highly branched than vegetative plants, and are easily recognised in the field (Fig. 13A). Tetrasporangial branchlets irregular (Fig. 13B), frequently tufted (Fig. 13C), often with truncate apices and sparse trichoblasts. Mature tetrasporangial branchlets swollen (Fig. 13D) with tetrahedrally divided tetrasporangia, 100–120 µm in diameter, borne on pericentral cells (Fig. 13E).

Male gametophytes rare, found only once in Muroran, Hokkaido (Table 4). Spermatangial plates rounded, produced from first or second order trichoblast cells in short, pedicellate fans (Fig. 14A). One to three plates arise from a single trichoblast cell (Fig. 14B). Spermatangial plates small near the apex, enlarging and maturing below.

Mature plates 100–400 µm in diameter, their size dependant upon the size of the branchlets that bear them. Each plate with a single row of flattened or irregular vegetative cells around its margin (Fig. 14C).

The branching pattern of female gametophytes is more tufted than that of sterile and tetrasporophytic plants (Fig. 15A). Procarps initiated near the apex from the suprabasal cell of a trichoblast and intermingled with sterile trichoblasts (Fig. 15B). Occasionally, a trichogyne can be seen still issuing from a young cystocarp (Fig. 15C). There is no evidence of a basal spur at any stage of cystocarpic development (Fig. 15D, E). Mature cystocarps spherical or ovoid, 500–800 µm in diameter, with a single ostiole (Fig. 15F).

Habitat and phenology

This species was usually found attached to rocks in sand in the low intertidal zone (Table 6). Male and female gametophytes were collected only in July, the summer season in Hokkaido Prefecture. Tetrasporangial plants were found from June to September (Table 4).

Neochondria nidifica* (Harvey) S. Sutti, T. Abe, K.A. Miller & K. Kogame *comb. nov.

Fig. 16

BASIONYM: *Chondria nidifica* Harvey 1858, Nereis boreali-americana. Part III. Chlorospermeae: p. 125, pl. L.B.

TYPE LOCALITY: Unknown ("NW coast?", collected by Dr. A. Schott during the Mexican Boundary Survey, according to the label on the type specimen); presumed to be in the vicinity of San Diego, California (Dawson & Tözün 1964).

EXAMINED SPECIMENS: Specimens collected from California, USA (Table 5).

Morphological observation

Pressed specimens of *Chondria nidifica* were examined and compared with the description of Dawson & Tözün (1964) and the type illustration by Harvey (1858). Morphology of all examined specimens agreed well with the description of Dawson & Tözün (1964): terete thalli with fusiform branchlets, each axial cell with 5 pericentral cells, visible only in apices of young branches; conspicuous tufted tetrasporangial branchlets; and cystocarps lacking spurs (Table 6, Fig. 16).

Molecular phylogenetic results

In *rbc*L sequences, four *Neochondria ammophila* (SAP115347, SAP115369, SAP115370 and SAP11537) were almost identical and made a clade (ML100%, BPP 1.00) in the tree (Fig. 23). They formed the clade with *N. nidifica* UC2026095, with high supports, and segregated from the tribe Chondrieae. However, *N. ammophila* differed from *N. nidifica* with 8.8% sequence divergence. Newly sequenced *Chondria capillaris*

SAP106295 from Finavarra, Ireland was identical with *C. capillaris* MF094050 from England. *Neochondria ammophila* differed from those *C. capillaris* with 16.0% sequence divergence in *rbc*L. The SSU rRNA tree represented the corresponding result: *N. ammophila* formed a clade with *N. nidifica* with high supports (ML 96%, BPP 1.00) and segregated from the tribe Chondrieae (Fig. 24). On the contrary to *rbc*L tree, *N. nidifica* sequence intermingled with *N. ammophila*, not separated. Moreover, *N. ammophila* differed from *C. capillaris* GU223767 with 3.4% sequence divergence in SSU rRNA.

In *cox*1 tree, the tribe Chondrieae was not supported, due to a lower resolution than those of the *rbc*L and SSU rRNA trees. Three *N. ammophila* (SAP115369, SAP115370, SAP115371) were almost identical (2 bp difference) and formed a clade with *N. nidifica* with high supports (ML 100%, BPP 1.00) (Fig. 25). *Neochondria ammophila* sequences differed from *C. capillaris* MF094021 with 21% divergence. Sequence divergence between *N. ammophila* and *N. nidifica* was 14%. All molecular phylogenetic trees demonstrated the corresponding results that *Neochondria ammophila* and *N. nidifica* grouped together in a clade with strong support and did not cluster with *Chondria capillaris*, the generitype of *Chondria*.

DISCUSSION

Neochondria strikingly resembles *Chondria* in gross morphology and shares several characters with the latter, e.g., five pericentral cells from each axial cell and fan-shaped,

pedicellate spermatangial branches (Table 7). However, *Neochondria* can be distinguished from *Chondria* by the presence of adventitious elongate cells that form densely compact layers between the pericentral cells and surrounding the axial strand, visible even in ultimate branchlets. In cross-sections of *Chondria* species with terete thalli, the five pericentral cells are significantly larger than the axial cell and are arranged in striking pentaradial symmetry throughout the thallus (Gordon-Mills 1987; Lee & Yoon 1996; Womersley 2003; Tani & Masuda 2003). In *Neochondria*, the pericentral cells are comparable in size to the axial cells in transverse section even at the apices. Both of which are obscure in the main axes.

Because herbarium specimens identified as *C. tenuissima* sensu Okamura in SAP had the characteristics of the new species, *N. ammophila*, we conclude that they represent the same species, and are distinct from the typical *C. capillaris* (Table 6). '*Chondria tenuissima*' (and *C. capillaris*) can therefore be removed from the Japanese seaweed flora.

Three terete species currently in the genus *Chondria* possess prostrate stolons and acute apices, resembling *N. ammophila*, but are clearly distinguished by molecular sequences (Figs 23–25) and morphology (Table 6). *Chondria capensis* (Harvey) Askenasy is endemic to South Africa (Wynne 1986; Stegenga *et al.* 1997). It shares most characters with *N. ammophila* but can be distinguished from *N. ammophila* by its blackish color in nature and five pericentral cells remaining throughout the thallus (Stegenga *et al.* 1997). *Chondria decipiens* was first described from California by Kylin (1941) and has been reported from other regions: western Atlantic (Wynne 2011), Brazil (Creed *et al.* 2010), Far East Russia (Perestkenko 1980; Titlyanov & Titlyanov 2012). It

is distinguished from *N. ammophila* by possessing cystocarps with marked spurs at the base and five pericentral cells remaining throughut the thallus. This species, as well as *Chondria dasyphylla* (Woodward) C. Agardh and *Chondria capillaris* from localities in ocean basins far from their type localities, should be re-investigated to elucidate their distributions, which may prove to be narrower, as were Japanese specimens of *C. capillaris* in the present study.

Chondria nidifica was described by Harvey (1858) and has been reported from California and Baja California, Mexico (Dawson 1963; Dawson & Tözün 1964; Abbott & Hollenberg 1976) and the Arabian Gulf (John & Al-Thani 2014). Dawson & Tözün (1964) described *C. nidifica* in detail using specimens from California and Baja California, Mexico. Its habitat is similar to that of *N. ammophila*: both species grow on sand-influenced rocks in the low intertidal or shallow subtidal zone.

Tetrasporophytes of *Chondria nidifica* have long been identified by distinctive and conspicuous tufts of tetrasporangial branchlets (Dawson & Tözün 1964; Fig. 16). In contrast, tetrasporophytes of *N. ammophila* bear only inconspicuous tufts of a few of tetrasporangial branchlets (Fig. 13). Such morphological differences can distinguish these two species. Molecular analyses (Figs 23–25) indicated the close relationship between the two species, suggesting that the two species can be in the same genus. On the other hand, the analysis based on *cox* 1 sequences strengthened the difference between these two species although they belonged to the same clade. Mitochondria *cox* 1 markers have been proposed as standard markers for cataloging red algal biodiversity and resolving differences between closely related species (Saunders 2005; Robba *et al*.

2006; Le Gall & Saunders 2010; Kucera & Saunders 2012). Therefore, the new combination *Neochondria nidifica* has been proposed.

CONCLUSION

The new genus *Neochondria*, the new species *N. ammophila* and the new combination *N. nidifica* are newly proposed (Sutti *et al.* 2018). Molecular data (*rbc*L and SSU) suggested the segregation of the new genus *Neochondria* from the genus *Chondria.* Generally, characters of *Neochondria* resemble those of *Chondria*. However, *Neochondria* can be distinguished from *Chondria* by the presence of adventitious elongate cells between the five pericentral cells which are evident only at the apex of young branches. *Neochondria* currently consists of its generitype *Neochondria ammophila* and *Neochondria nidifica*.

In terms of morphology, *N. ammophila* differs from *N. nidifica* by its tetrasporophyte. *N. nidifica*, as '*C. nidifica*', has long been identified by its distinctive tetrasporophyte which forms conspicuous tufts of tetrasporangial branchlets (Dawson & Tözün 1964). On the other hand, tetrasporophytes of *N. ammophila* bear only inconspicuous tufts of a few of tetrasporangial branchlets. Molecular data revealed the difference between these two taxa.

CHAPTER 3. Yanagi nori—the Japanese *Chondria dasyphylla* including a new species and a probable new record of *Chondria* from Japan

INTRODUCTION

Chondria dasyphylla (Woodward) C. Agardh (1817) was first described by Woodward (1794), as *Fucus dasyphyllus*, with characteristics of a cartilaginous but considerably gelatinous texture, 4 to 6 inches, axes divided immediately from the holdfast which is not fibrous but discoid, into very numerous branches; a ramulus (branchlet) cylindrical, terminating in a blunt point and with a constricted base. The plants might grow in the subtidal zone. Maggs and Hommersand (1993) designated a specimen of Turner's collections from Essex, Yarmouth (England), as the neotype of this species. *Chondria dasyphylla* was a representative of the subgenus *Coelochondria* described by Falkenberg (1901).

Chondria dasyphylla is a bushy plant, 10–20 cm in height, purplish to reddish brown in color. The primary erect axis originates from a discoid holdfast with subsequently further erect axes, giving a clumped appearance (Woodward 1794; Turner 1808; Gordon & Mills 1987). The branching pattern is irregularly radial, similar to that of *Chondria capillaris* (Hudson) M.J. Wynne [as *Chondria tenuissima* (Withering) C. Agardh], but all the parts of *C. dasyphylla* are slightly broader than those of *C. capillaris* (as *C. tenuissima*) (Dickinson 1963). In a transverse section of *C. dasyphylla*, an axial cell is surrounded by five large pericentral cells. Cell wall thickenings are formed in pericentral cells and subcortical cells as band-like caps (Gordon-Mills 1987).

Tetrasporangia are tetrahedral. Spermatangial plates are developed in a manner similar to *C. capillaris*; however, the sterile cells have a flattish edge rather than the curved edge as in *C. capillaris*. Mature cystocarps are urceolate and lack an obvious basal spur (Kylin 1928; Gordon-Mills 1987).

Chondria dasyphylla is a summer annual, commonly occurring on stones and shells, or piers in subtidal zone generally where the surface is covered with sand and mud (Dickinson 1963; Gordon-Mills 1987; Guiry 2012). This species is widespread in warm temperate seas, very common in shallow waters and it has been reported from Europe (Maggs & Hommersand 1993; Ludwig & Schnittler 1996; Gómez *et al.* 2001; Hardy & Guiry 2003), Atlantic islands (Neto 1994; Schneider 2003; John *et al.* 2004), North America (Abbott & Hollenberg 1976; Miller 2012), South America (Ramírez & Santelices 1991; Creed *et al.* 2010), Caribbean islands (Taylor 1960; Suárez 2005), Africa (Silva *et al.* 1996; Ateweberhan & Prud'homme van Reine 2005), Pacific islands (Tsuda & Walsh 2013) and Asia (Holmes 1896; Lewis & Norris 1987; Lee & Oh 1986; Yoshida *et al*. 1990). However, misidentification as *C. dasyphylla* has been reported in some regions. For example, the case of *Chondria curdieana* (Harvey ex J.Agardh) De Toni and *Chondria succulenta* (J.Agardh) Falkenberg, these two Australian species were previously misidentified as *C. dasyphylla*. Later detailed examinations revealed important differences among *C. curdieana*, *C. succulenta* and British *C. dasyphylla* (Gordon-Mills 1987; Womersley 2003). Another case of misidentification as *C. dasyphylla* was reported in Korea. *Chondria pellucida* Y.-P.Lee was previously identified as *C. dasyphylla* in Korea but Lee and Yoon (1996) pointed out that, based on their morphological differences, the species is not *C. dasyphylla*. Therefore, *C. dasyphylla*

in regions far from the type locality (England) should be re-examined with caution and molecular approaches.

Chondria dasyphylla (Japanese name "yanagi nori") was first recorded in Japan by Holmes (1896). Okamura (1936) provided a description of the Japanese *C. dasyphylla* as: growing on rocks; erect thalli, 10–20 cm in height, terete branches 1–1.5 mm in diameter, dark purple in color; pyramidal outline; branchlets clavate shaped with rounded to obtuse apices; in section, thallus polysiphonous with cortical layer to completely obscuring five pericentral cells of polysiphonous axis; tetrasporangia formed in a stichidia, divided tetrahedrally. Likewise those of the Japanese '*Chondria tenuissima*', only few published records of *C. dasyphylla* have been reported from Japan (Yoshida 1998; Yoshida *et al.* 1990, 2015). SAP possesses some collections identified as *C. dasyphylla*; however, they seem to include more than one species.

This Chapter 3 aims to reexamine the specimens identified as *Chondria dasyphylla* in Japan, to clarify their classification.

MATERIALS AND METHODS

Plants similar to *Chondria dasyphylla* were collected from Muroran and Shiretoko, Hokkaido Prefecture, Japan (Table 8). Field collections were mounted on herbarium paper; some were fixed in 10% formalin in seawater (v/v) for morphological observations and some were dried in silica gel for molecular analyses. Voucher specimens were deposited in the Herbarium of the Faculty of Science, Hokkaido University, Sapporo (SAP). Other specimens identified as '*Chondria dasyphylla*' housed

in SAP were selected and used for morphological observation. Specimens of *Chondria curdieana* from State herbarium of South Australia (AD) and *Chondria pellucida* from Herbarium of Department of Marine Biology, Pukyoung National University, Korea were examined (Table 9). Methods of the morphological observation and molecular investigation are mentioned earlier in Chapter 1.

RESULTS

Chondria acuminata* S. Sutti, M. Tani, T. Abe & K. Kogame *sp. nov.

Figs 17-19.

DESCRIPTION: Epilithic, tufted, reddish-brown, 10–20 cm high; holdfast subdiscoid, sprouting several erect axes; distinct and fleshy main axes; branching mostly irregularly radial. Lateral branches (branchlets) clavate with markedly acuminate tips and basally constricted at the base. In transverse section at a distal end of young branch, an axial cell issuing distinct 5 pericentral cells. Cell wall thickening formed as band-like caps or lobed in pericentral and subcortical cells. Tetrasporangial branchlets longlish clavate, arranged in irregularly radial manner. Female branchlets bearing cystocarps lacking marked spurs at the base. Male gametophyte unknown.

HOLOTYPE: SAP115363, Utoro (44°04'36"N 144°59'38"E), Shiretoko, Hokkaido Prefecture, Japan, 30 July 1999 (Herbarium SAP, Faculty of Science, Hokkaido University, Sapporo, Japan).

ISOTYPE: SAP 115402

ETYMOLOGY: From the Italian ‘acuminata’ (adjective), meaning acuminate: narrowing to a slender point.

DISTRIBUTION: Hokkaido, Honshu, Kyushu, Japan.

DNA SEQUENCES OF THE TYPE: *rbc*L= MG255062, SSU= MG272240, *cox*1= MG272237

MISAPPLIED NAMES: *Chondria dasyphylla* sensu Okamura 1936: 843.

Vegetative features

Terete, tufted and fleshy thalli, deep red to reddish brown when fresh, dried specimens firmly attached to paper and deep brown. Thalli are 10–20 cm in diameter and possess more or less massive basal disc sprouting several erect axes. Stolons are very small or absent. Main axes are terete and distinct (Fig. 17A). Branches are issued radially on erect axes, appearing alternately and shortening acropetally (Fig. 17B). Ultimate branchlets strongly constricted at the base, clavate with markedly acuminate apices. An apical filament with dome shaped apical cell protrudes from an apex of a branchlet. Branchlets are 100–200 µm in diameter and 200–500 µm in length (Fig. 17C). Epidermal cells in the upper (younger) branches are rounded or polygonal (10 × 15 µm

in surface view) (Fig. 17D); in the lower (older) branches, epidermal cells are larger and rectangular (8–10 µm in width and 25–40 µm in length) with pit connections between upper and lower next cells (Fig. 17E). In a cross section of a branchlet, a single row of square to rectangle cortical cells and 2–3 rows of subcortical cells are found. An axial cell is distinct in the centre and issues five pericentral cells. Five pericentral cells are slightly larger than an axial cell. Cell wall thinckenings are formed as band-like caps or ring shaped in pericentral cells (Fig. 17F). In some cross-sections, cell wall thickenings are present in subcortical cells. An axial cell issuing five pericentral cells with cell wall thickenings, remains its identity even in main axes (Fig. 17G).

Reproductive morphology

Tetrasporangial branchlets are formed radially on the upper part of a thalli, clavate, swollen and constricted at the base (Fig. 18A, B). Tetrasporagia are formed on pericentral cells and tetrahedrally divided (Fig. 18C).

Female gametophytes are rare, slightly smaller than vegetative plants and tetrasporophytes, 8–10 cm in height (Fig. 19A). A basal disc is massive and sprouting several axes (Fig. 19B). Mature cystocarps ovoid to urceolate, 600–800 µm in diameter, irregularly branched. Markedly cystocarpic spur was not found (Fig. 19C).

Remarks

Chondria acuminata was previously identified as *Chondria dasyphylla* in Japan. In general, the plants in hand share most characters with the original description of *C. dasyphylla* given by Woodward (1794) and '*C. dasyphylla*' in Japan given by Okamura (1936) such as epilithic; terete and gelatinous thallus; branchlets cylindrical with obtuse to rounded apices, constricted at the base. Moreover, *C. acuminata* resembles the British *C. dasyphylla* in having cell wall thickenings formed as band-like caps or lobed in pericentral and subcortical cells; urceolate cystocarps without a spur. However, *C. acuminata* differs from those *C. dasyphylla* descriptions by possessing branchlets with apices issuing a protruding apical filament or an acuminate apex instead of obtuse to rounded apices.

Chondria acuminata is more or less related to *Chondria pellucida* and *Chondria succulenta*. It differs from the latter two species in terms of the shape of the branchlets, the presence or absence of cell wall thinckenings and cystocarps with or without cystocarpic spurs (Lee & Yoon 1996; Womersley 2003). *Chondria acuminata* shares some morphological characters with *Chondria chejuensis* such as basal appearance and cystocarps without a spur. Nevertheless, these two species differ from each other in terms of thallus size and shape and apex of a branchlet (Lee & Yoon 1996). Details of comparison among the above-mentioned *Chondria* species are given in Table 10.

This proposal of *C. acuminata* sp. nov. in this thesis is not formal according to ICN (International Code of Nomenclature for algae, fungi, and plants) and should be formally proposed in a scientific journal. Moreover, additional samplings of this taxon are needed, especially the gametophytes.

Chondria* cf. *curdieana (Harvey ex J. Agardh) De Toni, 1903: 884

Figs 20, 21

DESCRIPTION: With the characters of *Chondria curdieana* (Harvey ex J. Agardh) De Toni 1903: terete thallus, 5–12 cm in high, irregularly radial branching; epilithic or epiphytic; holdfast discoid; ultimate branchlets 100–300 µm in diameter, cylindrical, basally constricted; apices rounded or usually slightly depressed with an apical filament cutting off 5 pericentral cells and conspicuous trichoblasts; cell wall thickenings usually present in pericentral and inner cortical cells; gametophyte dioecious; cystocarps ovoid, subsessile, without a spur; spermatangial plates discoid, margin smooth and 2–3 cells broad.

MISAPPLIED NAMES: *Chondria dasyphylla* sensu Okamura 1936: 843

TYPE LOCALITY: "S. Australia" (Curdie), probably SE S. Australia (Womersley 2003)

EXAMINED SPECIMENS: Specimens noted as *Chondria* cf. *curdieana* from Muroran, Hokkaido, Japan (Table 8); specimens noted as '*Chondria dasyphylla*' from Akkeshi, Hokkaido, Japan (Table 9).

Remarks

Chondria cf. *curdieana* is another taxon formerly identified as *Chondria dasyphylla* in Japan. *Chondria* cf. *curdieana* differs from the original description of *C. dasyphylla* given by Woodward (1794) and '*C. dasyphylla*' in Japan given by Okamura (1936) in having smaller thallus, 5–12 cm high; no epilithic specimen was found; thallus solitary, a single axis sprouting from a discoid holdfast. The recent *C.* cf. *curdieana* specimens from Japan share most characters with *Chondria curdieana*. However, the recent Japanese *C.* cf. *curdieana* specimens slightly differ from the Australian *C. curdieana* described by Womersley (2003) and herbarium specimens from AD (Table 9) in terms of spermatangial plates. Most spermatangial plates of the Japanese *C.* cf. *curdieana* possess a single row of sterile marginal cells although some of them possess 2 rows of sterile marginal cells (Fig. 20E) while the Australian *C. curdieana* possesses spermatangial plates with distinct 2–3 rows of sterile marginal cells.

Unidentified *Chondria* sp. 1

Fig. 22

DESCRIPTION: Thallus entangled and creeping, appearing in a tuft of loosely intricate bush; epilithic; 5–15 cm high, reddish brown to pale green; main axis indistinct; basal disc and stolon absent or very small; branching pattern sparsely radial to irregularly radial; ultimate branchlets 100–300 µm in diameter, basally constricted; apices rounded to truncate with an apical filament cutting off 5 pericentral cells and conspicuous trichoblasts; distinct ring-shaped cell wall thickenings present in pericentral and inner cortical cells, remaining constantly through branches and axes.

EXAMINED SPECIMNES: Specimens noted as *Chondria* sp. 1, known only from Shiretoko, Hokkaido, Japan (Table 1)

Remarks

Chondria sp. 1 differs from those two described species, *Chondria acuminata* and *Chondria* cf. *curdieana*, and the other selected *Chondria* species listed in Table 10. According to the lists of *Chondria* species that had been reported from Japan mentioned in Chapter 1: *Chondria armata* (Kützing) Okamura, *Chondria capillaris* (Hudson) M.J.Wynne (as *Chondria tenuissima*), *Chondria crassicaulis* Harvey, *Chondria dasyphylla*, *Chondria econstricta* Tani & Masuda, *Chondria expansa* Okamura, *Chondria intertexta* P.C. Silva, *Chondria lancifolia* Okamura, *Chondria mageshimensis* Tanaka & K. Nozawa, *Chondria polyrhiza* Collins & Hervey, *Chondria repens* Børgesen, *Chondria ryukyuensis* Yamada, *Chondria stolonifera* Okamura and *Chondria xishaensis* J.-F. Zhang & B.-M. Xia (Yoshida 1998; Yoshida *et al.* 1990, 2015), no any of them was related to *Chondria* sp. 1. Morphological details of some Japanese *Chondria* were given in Chapter 1. In addition, *C. econstricta* differs from *Chondria* sp. 1 by having unconstricted branchlets and absence of cell wall thickenings (Tani & Masuda 2003). *C.lanciofolia*. *C. polyrhiza* and *C. xishaensis* are the species possessing branchlets with acute apices (Collins & Hervey 1917; Okamura 1935; Tani & Masuda 2003), hence, they are not related to *Chondria* sp. 1. *Chondria repens* can be distinguished from *Chondria* sp. 1 by having minute thallus, only 1–2 cm in dimension (Børgesen 1924). The last taxon, *C. stolonifera* can be distinguished from *Chondria* sp. 1

by having compressed to flattened thallus sprouting from a discoid holdfast (Okamura 1935). Reproductive specimens of *Chondria* sp. 1 were not found.

Molecular and phylogenetic results

In *rbc*L analysis (Fig. 23), four specimens of *Chondria acuminata* [SAP115363, SAP115389, SAP115390, SAP115391] were identical and made a clade (ML 100%, BPP 1.00), being sister to a clade of *Chondria* cf. *curdieana* [SAP115392, SAP115395, SAP115396]. The clade of *C. acuminata* and the clade of *C.* cf. *curdieana* were related with low supports (ML 69%, BPP 0.72). A sequence of *Chondria* sp. 1 was located at the base of the tribe Chondrieae. *Chondria dasyphylla* U04021 from USA differed from those *C. acuminata*, *C.* cf. *curdieana* and *Chondria* sp. 1 with 9%, 12% and 17% sequence divergences, respectively. SSU rRNA tree provided a corresponding result to the *rbc*L tree; a clade of *C. acuminata* (ML 98%/BPP 0.99) was being sister to a clade of *C.* cf. *curdieana* (ML 99%/BPP 0.94) (Fig. 24). A sequence of *Chondria* sp. 1 located within the tribe Chondrieae. *C. dasyphylla* GU223771 from Ireland differed from the mingled clade of *C. acuminata*-*C.* cf. *curdieana* and a sequence of *Chondria* sp. 1 with 2% and 6% divergences, respectively.

The tribe Chondrieae was not supported in *cox* 1 tree (Fig. 25). The clade of *C. acuminata* sequences differed from the clade *C.* cf. *curdieana* with 14% divergence and these two clades were related with moderate supports (ML 75%/BPP 0.99). Moreover, the clade of *C.* cf. *curdieana* specimens clustered with *Chondria arcuata* Hollenberg (HQ423044) from USA, *Chondria* sp. ARS-2010 (GU223883) from France and

Chondria sp. ARS-2011 (HQ422895) from USA with high supports (ML 100%, BPP 1.00). A sequence of *Chondria* sp. 1 did not relate to any clade. *Chondria dasyphylla* sequence was not added in the analysis.

DISCUSSION

A taxon formerly known as *Chondria dasyphylla* in Japan is recognized as a new species, *Chondria acuminata,* mainly based on morphological comparisons (Table 10). This species mainly occurs in Hokkaido and can be found in Honshu and Kyushu, Japan (Tables 8, 9). *Chondria acuminata* is characterized by its cylindrical, basally constricted ultimate branchlet with markedly acuminate apices; thalli having a more or less massive basal disc sprouting several erect axes; 5 distinct pericentral cells with cell wall thickenings; cystocarps without a markedly cystocarpic spur. These characters have been accepted as taxonomic characters to identify the species within the genus *Chondria* (Falkenberg 1901; Setchell & Gardner 1924; Yamada 1935; Hollenberg 1945; Dawson 1963; Dawson & Tözün 1964; Gordon-Mills 1987; Millar & Wynne 1992; Maggs & Hommersand 1993; Lee & Yoon 1996; Tani & Masuda 2003; Tani *el al.* 2003; Womersley 2003; Bacci 2005). Molecular data strengthened the identity of *C. acuminata* in the tribe Chondrieae, showing that this species is different from any examined *Chondria* species including *C. dasyphylla.*

Another taxon formerly identified as *Chondria dasyphylla* in Japan is *Chondria* cf. *curdieana*. *Chondria curdieana* is an Australian species that has a limited distribution in South to Western Australia (Womersley 2003; Guiry & Guiry 2018). Japanese *C.* cf.

curdieana shares most morphological characters with those of Australian *C. curdieana* except the number of sterile cell rows surrounding a spermatangial plate. The number and shape of sterile cell rows surrounding a spermatangial plate have been used as a taxonomic character to identify a species within the genus *Chondria* (Lee & Yoon 1996; Tani & Masuda 2003; Tani *el al.* 2003; Womersley 2003; Bacci 2005). For example, Lee & Yoon (1996) used this character to distinguish *C. chejuensis* from *C. curdieana*: a single row of sterile marginal cells surrounding a spermatangial plate in *C. chejuensis* and 2–3 rows of sterile marginal cells surrounding a spermatangial plate in *C. curdieana*. However, not only by this mentioned character, *C. chejuensis* and *C. curdieana* were also distinguished by thallus size and the size of tetrasporangium (Lee & Yoon 1996). For the Japanese *C.* cf. *curdieana*, even most spermatangial plates possess a single row of sterile marginal cells, some of them possess 2 overlapping rows of sterile marginal cells (Fig. 20E) that resemble a figure of *C. curdieana* given by Womersley (2003). Molecular data (*rbc*L, SSU, *cox* 1) demonstrated that *C.* cf. *curdieana* are not identical to any other *Chondria* sequences. Because of the absence of molecular data from the typical *C. curdieana* near its type locality, the Japanese specimens are assigned as *Chondria* cf. *curdieana* in this study. This is the first record of *C. curdieana*, as *C.* cf. *curdieana*, in Japan. Based on newly collected specimens (Table 8) and the former specimens from SAP (Table 9), the Japanese *C.* cf. *curdieana* shows a limited distribution within Akkeshi and Muroran, Hokkaido, Japan.

Chondria sp. 1 was found only from Shiretoko, Hokkaido, Japan. It possesses common characters of the genus *Chondria* in having young branches (branchlets) basally constricted, apices of branches rounded, an axial cell issuing five pericentral

cells and branched trichoblasts (Harvey 1853; Womersley 2003). *Chondria* sp. 1 is distinct by its markedly ring-shaped cell wall thickenings in all five pericentral cells. Morphological characters such as thallus structure, shape of branchlets and morphology of cell wall thickenings indicate that *Chondria* sp. 1 does not resemble any *Chondria* reported from Japan to date. In addition, molecular data supported the position of this species in the tribe Chondrieae and distinction of this species from other *Chondria* used in the molecular analyses. However, *Chondria* sp. 1 is not assigned as a certain species due to lack of information of necessary taxonomic characters to identify a species, especially reproductive organs, due to the limited number of specimens available. Therefore, more samples are needed to obtain better understanding on this unidentified species.

CONCLUSION

Based on specimens formerly identified as *Chondria dasyphylla* in Japan, a new species, a new record of an accepted species and an unidentified species are described in Chapter 3. They are *Chondria acuminata* sp. nov., *Chondria* cf. *curdieana* and *Chondria* sp. 1, respectively. Morphological and molecular data demonstrated the identity of these mentioned taxa, suggesting that they are members of the genus *Chondria* and are distinct from the authentic *C. dasyphylla*. *Chondria acuminata* and *C.* cf. *curdieana* should be added in the Japanese seaweed flora. More samples of *Chondria* sp. 1 are needed to clarify its classification.

Differring from the case of misidentified *Chondria capillaris* in Japan leading to

the proposal of the new genus *Neochondria* mentioned in Chapter 2, it is premature to conclude whether the authentic *C. dasyphylla* exists in Japan or not. The identification of *C. dasyphylla* in Japan is problematic due to the presence of more than a species. Further, other specimens identified as *C. dasyphylla* in Japan should be reexamined using molecular approaches as well as morphological analyses.

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Table 1. Specimens from which DNA sequences were newly determined in the present study. GenBank accession numbers of sequences are also shown.

Species	Locality (date)	Condition	Voucher specimen	*rbc*L	SSU	*cox*1
Acrocystis nana	Okinawa, Japan (2 March 1997)	Silica gel	SAP115399	MG843864	--	MG843856
Chondria armata	Malaysia (26 May 1999)	Silica gel	SAP115358	MG255050	--	MG255068
Chondria armata	Kagoshima, Japan (02 August1997)	Silica gel	SAP115359	MG255051	--	--
Chondria capillaris	Finavarra, Ireland (17 August 2004)	Silica gel	SAP106295	MG255052	--	--
Chondria crassicaulis	Innoshima, Hiroshima, Japan (20 April 2015)	Silica gel	SAP115360	MG255053	--	MG255069
Chondria crassicaulis	Koinoura, Fukuoka, Japan (23 March 2015)	Silica gel	SAP115361	MG255054	--	MG255070
Chondria crassicaulis	Oshoro, Hokkaido, Japan (24 June 2015)	Silica gel	SAP115362	MG255055	MG272238	MG255071

Chondria decipiens	San Nicolas Island, California, USA (14 November 2012)	Pressed	UC2025838	MG255056	--	MG272232
Chondria expansa	Kushimoto, Wakayama, Japan (31 March 2003)	Silica gel	SAP115365	MG255057	--	MG272233
Chondria expansa	Tateyama, Chiba, Japan (21 March 2016)	Silica gel	SAP115366	MG255058	MG272239	MG272234
Chondria intertexta	Hachijo, Tokyo, Japan (21 July 2005)	Pressed	SAP115364	MG255059	--	--
Chondria mageshimensis	Innoshima, Hiroshima, Japan (19 August 2005)	Silica gel	SAP115367	MG255060	--	--
Chondria ryukyuensis	Kagoshima, Japan (27 August 2003)	Silica gel	SAP115368	MG255061	--	MG272236
Chondria acuminata (Japanese '*C. dasyphylla*')	Utoro, Shiretoko, Hokkaido, Japan (30 July 1999)	Silica gel	SAP115363	MG255062	MG272240	MG272237
Chondria acuminata (Japanese '*C. dasyphylla*')	Shishi-iwa, Shiretoko, Hokkaido, Japan (11 November 2008)	Silica gel	SAP115389	MG843865	MG831939	MG843857

Chondria acuminata (Japanese '*C. dasyphylla*')	Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017)	Silica gel	SAP115390	MG843866	MG831940	MG843858
Chondria acuminata (Japanese '*C. dasyphylla*')	Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017)	Silica gel	SAP115391	MG843867	MG831941	MG843859
Chondria cf. *curdieana* (Japanese '*C. dasyphylla*')	Muroran, Hokkaido, Japan (21 August 2016)	Silica gel	SAP115392	MG843868	MG831942	MG843860
Chondria cf. *curdieana* (Japanese '*C. dasyphylla*')	Muroran, Hokkaido, Japan (22 August 2017)	Silica gel	SAP115395	MG843869	MG831943	MG843861
Chondria cf. *curdieana* (Japanese '*C. dasyphylla*')	Muroran, Hokkaido, Japan (22 August 2017)	Silica gel	SAP115396	MG843870	MG831944	MG843862
Chondria sp. 1	Shiretoko, Hokkaido, Japan (21 August 2017)	Silica gel	SAP115397	MG843871	MG831945	MG843863
Neochondria ammophila (Japanese '*C. capillaris*')	Momonai, Otaru, Hokkaido (23 September1996)	Silica gel	SAP115347	MG255063	MG272241	--
Neochondria ammophila (Japanese '*C. capillaris*')	Innoshima, Hiroshima, Japan (20 April 2015)	Silica gel	SAP115369	MG255064	MG272242	MG272229

Neochondria ammophila (Japanese '*C. capillaris*')	Momonai, Hokkaido, Japan (29 June 2016)	Silica gel	SAP115370	MG255065	MG272243	MG272230
Neochondria ammophila (Japanese '*C. capillaris*')	Muroran, Hokkaido, Japan (26 July 2016)	Silica gel	SAP115371	MG255066	MG272244	MG272231
Neochondria nidifica (= *Chondria nidifica*)	Dana Point, California, USA (12 December 2012)	Pressed	UC2026095	MG255067	MG272245	MG272235

Table 2. Primers used for amplification and sequencing.

Gene	Sequence (5´ to 3´)			References
*rbc*L	F8	Forward	GGTGAATTCCATACGCTAAAATG	Abe *et al* (2006)
	R753	Reverse	GCTCTTTCATACATATCTTCC	Freshwater & Rueness (1994)
	F605	Forward	CCATTTCATGCGTTGGAAAGAAAGAT	Shimada (2000)
	RH5	Reverse	TAGAAACTCCAACAGCTTACGTTTAA	Abe *et al* (2006)
*cox*1	GazF1	Forward	TCAACAAATCATAAAGATATTGG	Saunders (2005)
	GazR1	Reverse	ACTTCTGGATGTCCAAAAAAYCA	Saunders (2005)
SSU	SRrh1	Forward	GCTTGTCTCAAAGACTAAGCC	This study
	SRrh5	Reverse	GCCAAAATCCGACTACGAGC	This study
	SRrh4	Forward	ACCAGCAGAGGGCAAGTCTG	This study
	SRrh9	Reverse	CCTATTTAGCACGCCCAGGT	This study
	SRrh8	Forward	GGAAAACTTACCAGGTCCAG	This study
	SRrh12	Reverse	CCTTCTGCAGGTTCACCTAC	This study

Table 3. GenBank accession numbers of the published sequences included in the phylogenetic analyses.

Species	Location; Collection date	GenBank accession number		
		*rbc*L	SSU	*cox*1
Acanthophora pacifica	USA, Hawaii; 24-Jan-2008		GU223750	
Acanthophora pacifica	USA, Hawaii; --			HQ422947
Acanthophora spicifera	USA, Oahu; 11-Mar-2008		GU223753	
Acanthophora spicifera	Japan, Okinawa; 03-Feb-2002		GU223763	
Acanthophora spicifera	USA, HI, Kihei, Maui; 05-Apr-2006	GQ252538		
Acanthophora spicifera	USA, Hawaii; --			HQ422873
Acrocystis nana	Japan, Nagasaki; 01-Jun-1999		GU223764	
Alsidium cymatophilum	USA, Oahu; 19-Apr-2008		GU223765	
Benzaitenia yenoshimensis	Japan, Chiba; 21-Apr-2008		GU223735	
Benzaitenia yenoshimensis	Japan, Kashiwazaki; 03-May-2008			GU223853
Benzaitenia yenoshimensis	Japan, Kashiwazaki; 03-May-2008			GU223854
Bostrychia moritziana	Australia, Western Port Bay; 25-Oct-1986		AF203893	
Ceramium virgatum	Spitsbergen, Kapp Thordsen; 11-Aug-2010		KP828754	

Ceramium virgatum	USA, Massachusetts; 13-Apr-2010	KT250272		
Chondria arcuata	USA, Hawaii; --			HQ423044
Chondria armata	Japan, Kagoshima; 28-May-1998		GU223766	
Chondria baileyana	Canada, Prince Edward Island; 29-Jul-2008	KU564500		
Chondria baileyana	Canada, Nova Scotia; 16-Aug-2012			KU564345
Chondria californica	USA, California; 01-Jul-1996	AY172578		
Chondria capillaris	Ireland, Finavarra; 17-Aug-2004		GU223767	
Chondria capensis	South Africa; 02-Mar-2011	KY927799		
Chondria coerulescens	Spain, Playa del Sarello; 28-Jul-2010			KF671147
Chondria collinsiana	Brazil, Rio de Janeiro; 2005	GU330225		
Chondria crassicaulis	Japan, Chiba; 21-Apr-2008		GU223754	
Chondria crassicaulis	Japan, Chiba; 21-Apr-2008			GU223870
Chondria crassicaulis	China; 12-Oct-2011			KC795910
Chondria dangeardii	USA, Molokai, Hawaii; 21-Mar-2008		GU223770	
Chondria dangeardii	USA: Molokai, Hawaii; 21-Mar-2008			GU223879
Chondria dasyphylla	Ireland, Finavarra; 03-Jun-2004		GU223771	

Chondria dasyphylla	USA, NC; --	U04021		
Chondria expansa	Japan, Kochi; 04-May-2000		GU223772	
Chondria ryukyuensis	Japan, Kagoshima; 27-Aug-2003		GU223773	
Chondria scintillans	France, Brittany; 05-Dec-2002	KF492775		
Chondria scintillans	France, Brittany; 05-Dec-2002			KF492717
Chondria tenuissima	England, Swanage; 7-Jun-2015	MF094050		
Chondria tenuissima	England, Swanage; 7-Jun-2015			MF094021
Chondria sp. ARS-2010	France, Brittany; 23-Mar-2012			GU223882
Chondria sp. ARS-2010	France, Brittany; 23-Mar-2013			GU223883
Chondria sp. ARS-2011	USA, Hawaii; --			HQ422895
Chondria sp. ARS-2011	USA, Hawaii; --			HQ422964
Chondria sp. ARS-2011	USA, Hawaii; --			HQ423059
Chondrophycus papillosus	USA: Florida; --	AF465807		
Chondrophycus succisus	USA, Molokai, Hawaii; 11-Feb-2007		GU223778	
Cladhymenia lyallii	New Zealand, Lyall Bay; 21-Apr-1994	AF259496		
Cladurus elatus	Australia, Victoria; 11-Jan-2015	MF094051		

Halopithys incurve	UK; --	AF281882		
Herposiphonia parca	South Korea, Gyeongbuk; --		JX828166	
Herposiphonia tenella	USA, North Carolina; 26-Oct-2003	KT825867		
Janczewskia hawaiiana	USA, Oahu, Hawaii; 08-Apr-2007		GU223742	
Laurencia complanata	South Africa: Port Edward; --	AF465813		
Laurencia dendroidea	Spain, Canary Islands; 15-Jan-2013			KF492728
Laurencia flexuosa	South Africa: Palm Beach; --	AF465815		
Laurencia intricate	Mexico, Campeche; --	AF465809		
Laurencia majuscula	USA, Molokai, Hawaii; 10-Feb-2007		GU223784	
Laurencia majuscula	USA, Hawaii; --			HQ423051
Laurencia nipponica	Russia, Sakhalin; 23-Jun-2003		GU223758	
Laurencia obtuse	Ireland; --	AF281881		
Laurencia pyramidalis	Spain, Canary Islands; 10-Sep-2012			KF492756
Laurencia viridis	Portugal, Madeira; 06-Jul-2011			KF492761
Lembergia allanii	New Zealand, Three Kings Islands; 24-Nov-1998--		AF373215	

Lenormandia spectabilis	Australia, Cockburn Sound; 25-Aug-1999		AF339896	
Melanamansia mamillaris	Australia, Port Denison; 9-Nov-1995		AF203889	
Micropeuce strobiliferum	Australia, Port Macdonnell; 11-Jul-1997		AF203896	
Murrayella periclados	Philippines, Mindanao; 20 May-1998		AF203887	
Ohelopapa flexilis	Philippines; --	AF489860		
Osmundea pinnatifida	Ireland, Spiddal; 06-Apr-2004		GU223795	
Osmundea pinnatifida	Ireland; --	AF281876		
Osmundea splendens	Mexico, Baja California; 02-Jul-1996	AY172576		
Palisada flagellifera	Spain, Canary Islands; 14-Jul-2006	EF685998		
Palisada parvipapillata	USA, Oahu; 18-Sep-2007		GU223796	
Polysiphonia harveyi	Ireland, Maghery; --	AF342897		
Polysiphonia howei	USA, Hawaii; 23-Jan-2008		GU223798	
Polysiphonia pacifica	USA, California; 21-May-2010			KM254964
Polysiphonia stricta	UK, Flambourough; 16-July-1998		AF427535	
Rhodomela confervoides	France, Brittany; --		AY617145	
Rhodomela confervoides	Germany, Kiel Bight; --	AF083381		

Sonderella linearis	Australia, Warrnambool; 12-Apr-1997	AF203888	
Tolypiocladia glomerulata	USA, Hawaii; 13-Apr-2008	GU223799	
Ululania stellata	USA, Oahu, Hawaii; 11-Mar-2008	GU223744	
Ululania stellata	USA, Maui, Hawaii; 11-Dec-2007		GU223865

Table 4. Samples of Japanese '*Chondria capillaris*' (=*Neochondria ammophila*) used in this study.

Locality (Date)	Voucher specimen	Condition	Note
Momonai, Otaru, Hokkaido (26.v.1996)	SAP115346	Pressed	Vegetative plant
Momonai, Otaru, Hokkaido (23.ix.1996)	SAP115347	Pressed	Tetrasporophyte; with molecular data
Momonai, Otaru, Hokkaido (10.iii.1997)	SAP115348	Pressed	Vegetative plant
Muroran, Hokkaido (28.vii.1999)	SAP114349	Transection	Male gametophyte
Muroran, Hokkaido (28.vii.1999)	SAP115350	Pressed	Tetrasporophyte
Muroran, Hokkaido (18.vii.2001)	SAP115351	Pressed	Female gametophyte
Utsumi-Cho, Hiroshima (19.iv.2004)	SAP115352	Pressed	Vegetative plant

Innoshima, Hiroshima (20.iv.2015)	SAP115369	Pressed	Vegetative plant; with molecular data
Momonai, Otaru, Hokkaido, (29.vii.2015)	SAP115353	Pressed	Tetrasporophyte
Muroran, Hokkaido, (23.v.2016)	SAP115354	Pressed	Vegetative plant
Momonai, Otaru, Hokkaido, (29.vi.2016)	SAP115355	Pressed	Tetrasporophyte
Momonai, Otaru, Hokkaido, (29.vi.2016)	SAP115370	Pressed	Tetrasporophyte; with molecular data
Muroran, Hokkaido, (26.vii.2016)	SAP115371	Pressed	Female gametophyte; with molecular data

Table 5. List of additional herbarium specimens (from UC and SAP) used for morphological observations.

Species (identified as)	Date	Locality	Code
Chondria decipiens	26 September 1969	Pacific grove, California, USA	UC1844102
Chondria decipiens	14 November 2012	San Nicolas Island, California, USA	UC2025838
Chondria nidifica	27 January 1949	Santa Rosa Island, California, USA	UC1022164
Chondria nidifica	20 February 1989	Santa Rosa Island, California, USA	UC2036061
Chondria nidifica	12 December 2012	Dana Point, California, USA	UC2026095
Chondria nidifica	6 January 2013	San Nicolas Island, California, USA	UC2025809
Chondria capillaris	17 August 2004	Frinavarra, Ireland	SAP115387
'Chondria tenuissima'	3 March 1932	Himi, Toyama, Japan	SAP108861
'Chondria tenuissima'	18 May 1956	Fukuoka, Japan	SAP094404
'Chondria tenuissima'	8 May 1962	Noto, Ishikawa, Japan	SAP105508
'Chondria tenuissima'	21 July 1970	Muroran, Hokkaido, Japan	SAP063400
'Chondria tenuissima'	20 July 1992	Hokkaido, Japan	SAP097647
'Chondria tenuissima'	11 April 1993	Hiroshima, Japan	SAP091695

'Chondria tenuissima'	21 May 2001	Iyo, Ehime, Japan	SAP094422
'Chondria tenuissima'	14 July 2002	Hakodate, Hokkaido, Japan	SAP110738
'Chondria tenuissima'	23 November 2002	Hakodate, Hokkaido, Japan	SAP110739

Table 6. Comparative morphology of the Japanese '*Chondria capillaris*' (*Neochondria ammophila*), *C. capillaris* and three terete *Chondria* species with acute apices and stolons.

Characters	**Japanese '*C. capillaris*' (*Neochondria ammophila*)**	***C. capillaris*** [3, 4]	***C. capensis*** [5]	***C. decipiens*** [1, 6]	***C. nidifica*** [1, 2, 6] **(*Neochondria nidifica*)**
Distribution	Japan	Europe, Atlantic Islands, North America, Caribbean Islands, South America, Africa, Asia	Africa, Indian Ocean Islands	North America, South America, Western Atlantic, Asia (Far East Russia)	North America, South-west Asia
Habitat	on rocks, lightly covered with sand	on rocks and stones	sublittoral fringe of expose localities	on rocks, sheltered, intertidal	on rocks, in sand between tide marks
Branchlet	fusiform	fusiform	fusiform	fusiform	fusiform
Holdfast	discoid	discoid	unknown	discoid	discoid

Stolon	present	absent	present	present	present
Pericentral cells	5 with adventitious elongate cells; retain identity only in the young branch	5 remaining conspicuous throughout the thallus	5 remaining conspicuous throughout the thallus	5; remaining conspicuous throughout the thallus	5; retain identity only in the young branch
Cell wall thickening	absent	present	unknown	absent	absent
Tetrasporangial branchlets arrangement	irregular	alternate to irregular	unknown	irregular	whirled and tufted
Spermatangial plate	disc-shaped	disc-shaped	disc-shaped	disc-shaped	disc-shaped
Shape of cystocarp	ovoid to urceolate	ovoid to urceolate	globose	ovoid	ovoid
Cystocarpic spur	without marked spur	marked spur	unknown	marked spur	without marked spur

[1] Abbott & Hollenberg (1976), [2] Dawson & Tözün (1964), [3] Gordon-Mills (1987), [4] Guiry & Guiry (2017), [5] Stegenga *et al.* (1997), and [6] This study

Table 7. Comparative morphology among the genus *Neochondria* and the genera (excluding the parasitic genera, *Benzaitenia* and *Ululania*) within the tribe Chondrieae.

Character	***Neochondria***[9]	**Chondrieae**					
		Chondria[4,6,8,9]	***Acanthophora***[7,8]	***Acrocystis***[2,5]	***Cladhymenia***[1,3]	***Coeloclonium***[8]	***Husseya***[8]
Branchlet	fusiform, constricted	terete or compressed, constricted	short and spine, not constricted	clavate and hollow, constricted	foliose, constricted	partly hollow, constricted	clavate, constricted
Number of pericentral cell	5	5	5	5	5	5	5
Pericentral cell identity	indistinct, present with adventitious elongate cell; retain only in the young branch	distinct; remain throughout the thallus	distinct; remain throughout the thallus	distinct; remain throughout the thallus	distinct, non-stratified	distinct; elongate at right angles to axial cells	distinct; remain throughout the thallus
Cell wall thickening	absent	present	present	unknown	present	absent	present

Tetrasporangia	spirally arrange; cut off from pericentral cell	cut off from pericental cells	cut off from pericental cells	cut off from pericental cells	embedded in cortex	cut off from pericental cells	cut off from pericental cells
Cystocarp	ovoid, short stalk	ovoid	ovoid, sessile	unknown	ovoid	ovoid, sessile	ovoid
Spermatangial organ	discoid with a sterile margin	discoid with a sterile margin	discoid with a sterile margin	unknown	discoid with a sterile margin	discoid with a sterile margin	unknown

[1] Hooker & Harvey (1845), [2] Okamura (1907), [3] Saenger *et al.* (1971), [4] Gordon-Mills (1987), [5] Norris (1988), [6] Lee & Yoon (1996), [7] De Jong *et al.* (1999), [8] Womersley (2003) and [9] This study.

Table 8. Collected samples identified as Japanese '*Chondria dasyphylla*' (Yanagi nori) used for morphological observations.

Locality (Date)	Voucher specimen	Condition	Note
Utoro, Hokkaido, Japan (30 July 1999)	SAP115363	Pressed	= *Chondria acuminata*; tetrasporophyte
Utoro, Hokkaido, Japan (9 August 1998)	SAP115401	Pressed	= *Chondria acuminata*; female gametophyte
Utoro, Hokkaido, Japan (30 July 1999)	SAP115402	Pressed	= *Chondria acuminata*; female gametophyte
Chashikotsu, Shiretoko, Hokkaido, Japan (11 November 2008)	SAP115389	Pressed	= *Chondria acuminata*; tetrasporophyte
Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017)	SAP115390	Pressed	= *Chondria acuminata*; tetrasporophyte
Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017)	SAP115391	Pressed	= *Chondria acuminata*; tetrasporophyte

Muroran, Hokkaido, Japan (21 August 2016)	SAP115392	Pressed	= *Chondria* cf. *curdieana*; male and female gametophyte
Muroran, Hokkaido, Japan (21 August 2016)	SAP115394	Pressed	= *Chondria* cf. *curdieana*; male and female gametophyte
Muroran, Hokkaido, Japan (21 August 2017)	SAP115395	Pressed	= *Chondria* cf. *curdieana*; male gametophyte
Muroran, Hokkaido, Japan (21 August 2017)	SAP115396	Pressed	= *Chondria* cf. *curdieana*; female gametophyte

Table 9. List of additional herbarium specimens: *Chondria curdieana* from AD, '*Chondria dasyphylla*' from SAP and C*hondria pellucida* from Herbarium of Department of Marine Biology, Punkyoung National University, used for morphological observations.

Species (identified as)	Date	Locality	Code
Chondria curdieana	19-Feb-58	Eyre, Wanna, South Australia	AD-A22460A
Chondria curdieana	19-Jan-65	Eyre, Pennington Bay, South Australia	AD-A28937
Chondria curdieana	17-May-65	Otway, Robe, South Australia	AD-A29274A
Chondria curdieana	12-Feb-78	Otway, Robe, South Australia	AD-A49751A
Chondria curdieana	31-Dec-81	Otway, Cape Lannes, South Australia	AD-A52065A
Chondria curdieana	9-Feb-82	Otway, Robe, South Australia	AD-A52071A
Chondria curdieana	12-Sep-83	Otway, Robe, South Australia	AD-A53718
Chondria curdieana	28-Oct-96	Otway, Inner Reef, South Australia	AD-A67160A
'*Chondria dasyphylla*' (*Chondria acuminata*)	-- August 1936	Muroran, Hokkaido, Japan	SAP113945
'*Chondria dasyphylla*' (*Chohdria acuminata*)	26-Apr-05	Innoshima, Hiroshima, Japan	SAP102343

‘*Chondria dasyphylla*’ (*Chondria acuminata*)	29-Jul-06	Aburatsubo, Kanagawa	SAP103075
‘*Chondria dasyphylla*’ (*Chondria acuminata*)	26-Aug-06	Otaru, Hokkaido, Japan	SAP111274
‘*Chondria dasyphylla*’ (*Chondria acuminata*)	25-Feb-09	Mitoma, Fukuoka, Japan	SAP107367
‘*Chondria dasyphylla*’ (*Chondria* cf. *curdieana*)	5-Sep-33	Akkeshi, Hokkaido, Japan	SAP112321
‘*Chondria dasyphylla*’ (*Chondria* cf. *curdieana*)	5-Sep-33	Akkeshi, Hokkaido, Japan	SAP112322
‘*Chondria dasyphylla*’ (*Chondria* cf. *curdieana*)	5-Sep-33	Akkeshi, Hokkaido, Japan	SAP112323
‘*Chondria dasyphylla*’ (*Chondria* cf. *curdieana*)	29-Jun-04	Akkeshi, Hokkaido, Japan	SAP098589
Chondria pellucida	1-Sep-08	Byeonggok, Korea	N080104603

Table 10 Comparative morphology of *Chondria acuminata*, *Chondria* cf. *curdieana*, *Chondria* sp. 1, *Chondria dasyphylla* and four *Chondria* species that are related to *C. dasyphylla*.

Character	***C. acuminata* (Japanese '*C. dasyphylla*')**	***C.* cf. *curdieana* (Japanese '*C. dasyphylla*')**	***Chondria* sp. 1**	***Chondria chejuensis***	***Chondria curdieana***	***Chondria dasyphylla***	***Chondria pellucida***	***Chondria succulenta***
Distribution	Honshu, Kyushu and Hokkaido, Japan	Denshin-hama, Muroran, Hokkaido, Japan	Shishi-iwa, Shiretoko, Hokkaido, Japan	Cheju, Korea	Australia and New Zealand	common in temperate waters	Cheju, Korea	Western Australia
Habitat	epilithic on rock in lower litteral zone	epiphytic on sea grass leaf	epilithic	epilithic in the lower tidal mark	epilithic or epiphytic on *Posidonia* or larger algae	on stones and shells or piers	on bedrock in the intertidal zone; intertidal habitat	epilithic
Thallus structure	distinct and terete main axes	distinct and terete main axes	entangle and creeping; appearing in a tuft of loosely intricate bush	distinct and terete main axes with several prostrating filaments	distinct and terete main axes	distinct and terete main axes	distinct and terete main axes	distinct and terete main axes
Size	10-15(20) cm	5-10 cm	5-15 cm	2-5 cm	5-12(17) cm	10-21 cm	4-15 cm	3-18 (30) cm
Color	reddish brown	pinkish red	reddish brown to pale green	pale purplish brown	red-brown, fading to yellow-brown	reddish brown	purplish red or pale green	reddish brown
Basal disc	more or less	solitary and discoid	absence or very	discoid holdfast	solitary discoid	discoid holdfast	holdfast subdiscoid	discoid holdfast

	massive holdfast sprouting several erect axes	holdfast	small	sprouting several erect axes	holdfast	sprouting several erect axes	sprouting several erect axes	sprouting several erect axes
Stolon	absence or very small	absence	absence or very small	absence or very small	absence	absence	several short stolons near basal discs	absence or very small
Branching pattern	radial to irregularly radial	radial to irregularly radial	sparsely radial	radial to irregularly radial	radial to irregularly radial	radial to irregularly radial	radial to irregularly radial	radial to irregularly radial
Shape of branchlets and apices	clavate; acuminate apices; constricted	clavate; rounded apices; constricted	clavate; rounded apices; constricted	longish clavate; blunt to depressed apices; constricted	clavate; rounded apices; constricted	clavate; obtuse apices; constricted	clavate; obtuse to depressed apices; constricted	clavate; rounded to depressed apices with protruding apical filaments
Pericentral cells	5	5	5	5	5	5	5	5
Cell wall thickening	formed as band-like caps or lobed in pericentral and subcortical cells	formed as band-like or ring-shaped in pericentral and subcortical cells	formed as ring shape-shape in pericentral and subcortical cells; distinct, constant presence in all 5 pericentral cells	formed as band-like caps in pericentral and subpericentral cells	usually present in pericentral and inner cortical cells, on the inner walls or band-like around the cell and becoming hooked and lobed	formed as band -liked caps on the upper ends of pericentral and subcortical cells	absence	formed as hemisperial caps

Tetrasporangial branchlets	clavate shaped with depressed apices	no data	no data	longish clavate with depressed apices	clavate shaped with depressed apices; tetraspore borne in 1-2 pericentral cells per axial cell	clavate shaped with depressed apices; 1-3 fertile peri- centrals are produced per fertile axial cell	with an acute apex; smaller size of the trasporangia (than *C. dasyphylla*); pinnate laterals	tetrasporangia near ends of branchlets; on 2-3 pericentral cells per axial cells
Spermatangial organ	no data	discoid; 1-2 marginal sterile layers with flattish edge	no data	discoid with single marginal sterile layer	discoid; 2 marginal sterile layers; sterile with flattish edge	discoid to bilobed; single marginal sterile layer; sterile cells with a flattish edge	single marginal sterile layer; single cell stalk; heart shape; distinct vein of dichotomous branches	discoid to slightly lobed with a single row of sterile marginal cells
Cystocarp	ovoid to urceolate, without markedly cystocarpic spur	ovoid, without markedly cystocarpic spur	no data	urceolate, without markedly cystocarpic spur	ovoid, without markedly cystocarpic spur	urceolate, cystocarps without markedly cystocarpic spur	ovoid, cystocarp with a spur at the base	ovoid to slightly urceolate, with a distinct basal spur
Sequences	*rbc*L, SSU, *cox*1	*rbc*L, SSU, *cox*1	*rbc*L, SSU, *cox*1	no data	no data	*rbc*L, SSU	no data	no data
References	newly collected and former SAP specimens	newly collected and former SAP specimens	newly collected specimens	Lee & Yoon 1996	AD specimens; Womersley 2003	Woodward 1794; Gordon-Mills 1987	Korean specimen; Lee & Yoon 1996	Womersley 2003

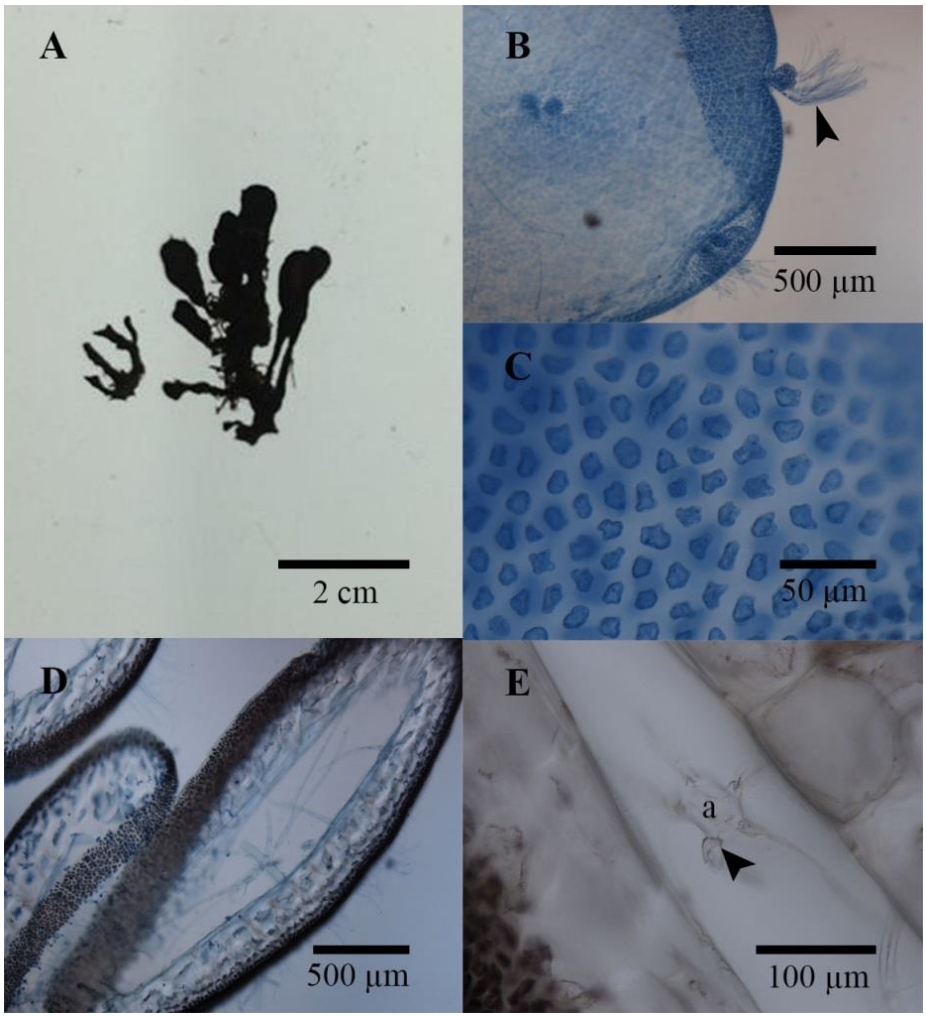

Fig. 1. Vegetative morphology of an examined *Acrocystis nana.*

A. *Acrocystis nana* (SAP115399, *rbc*L [MG843864], *cox*1 [MG843856]) collected from Okinawa Prefecture, Japan on 2 March 1997.

B. Rounded apex with dichotomously branched trichoblasts (arrowhead).

C. Epidermal cell arrangement.

D. Cross-sections of a hollow bulbous branch.

E. High magnification of a cross-section of a hollow bulbous branch, showing an axial cell (a). Arrowhead indicates pit connection.

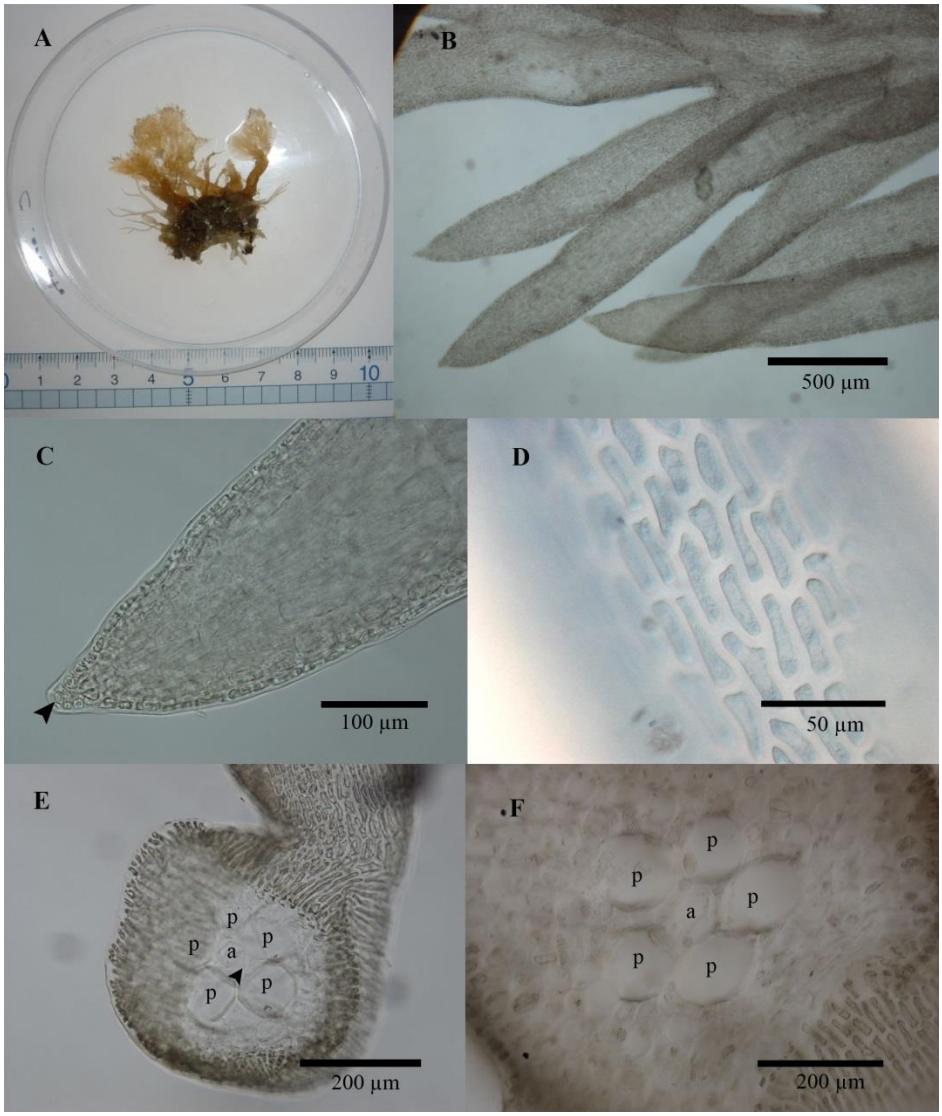

Fig. 2. Vegetative morphology of *Chondria armata.*

A. *Chondria armata* (SAP115359, *rbc*L [MG255051]) collected from Kagoshima Prefecture, Japan on 1 August 1997.

B. Unconstricted, cylindrical branches with acute apices.

C. High magnification of an acute apex.

D. Epidermal cell arrangement.

E. Cross-section of a branch (near an apex). An axial cell (a) issuing 5 pericentral cells (p). Arrowhead indicates pit connection.

F. Cross-section of a median portion of an axis showing an axial cell (a) issuing 5 pericentral cells (p).

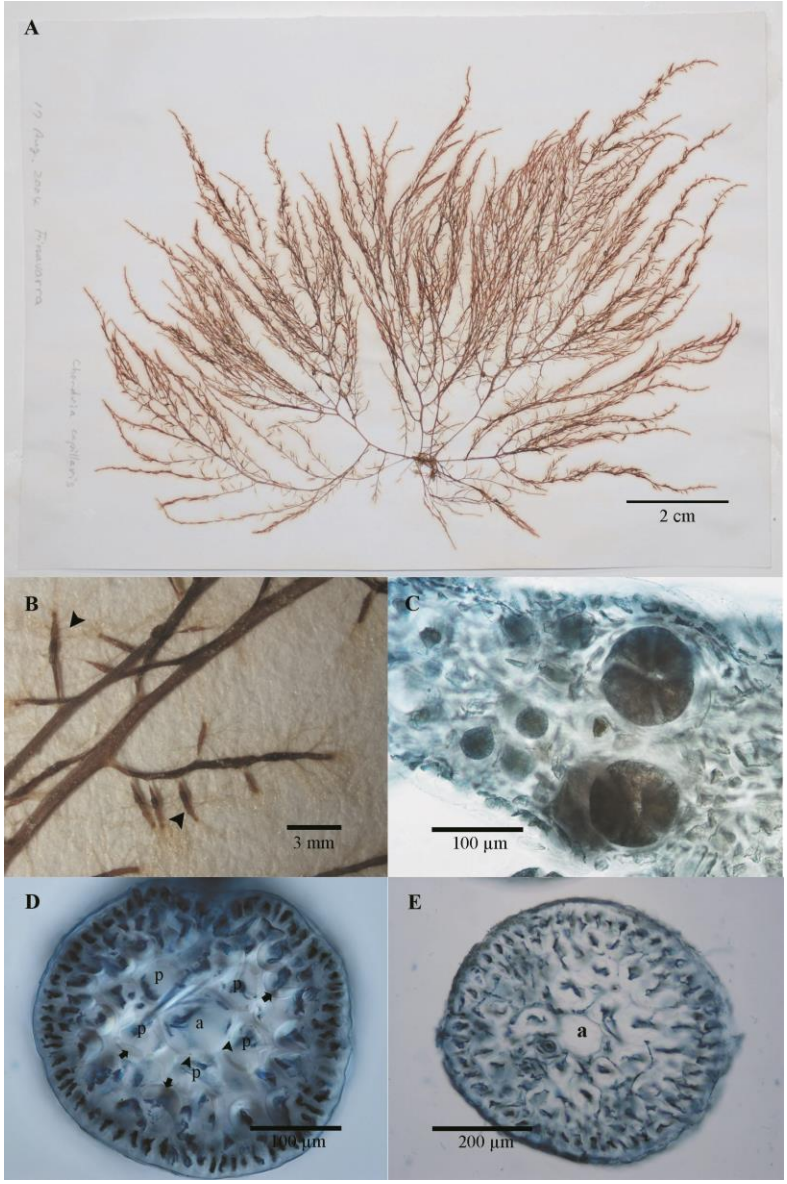

Fig. 3. Morphology of tetrasporophyte of an examined *Chondria capillaris.*

A. *Chondria capillaris* (SAP116387) collected from Finavarra, Ireland on 17 August 2004.

B. Fusiform branchlets bearing tetrasporangia with conspicuous trichoblasts (arrowheads).

C. High magnification showing tetraspores borne in pericentral cells.

D. Cross-section of a branchlet (near apex) showing an axial cell (a) issuing 5 pericentral cells (p). Arrowheads indicate pit connection. Arrows indicate cell wall thickenings in pericentral cells and sub-cortical cells.

E. Cross-section of a median portion of an axis showing an axial cell (a) issuing pericentral cells. Number of pericentral cells cannot be distinguished.

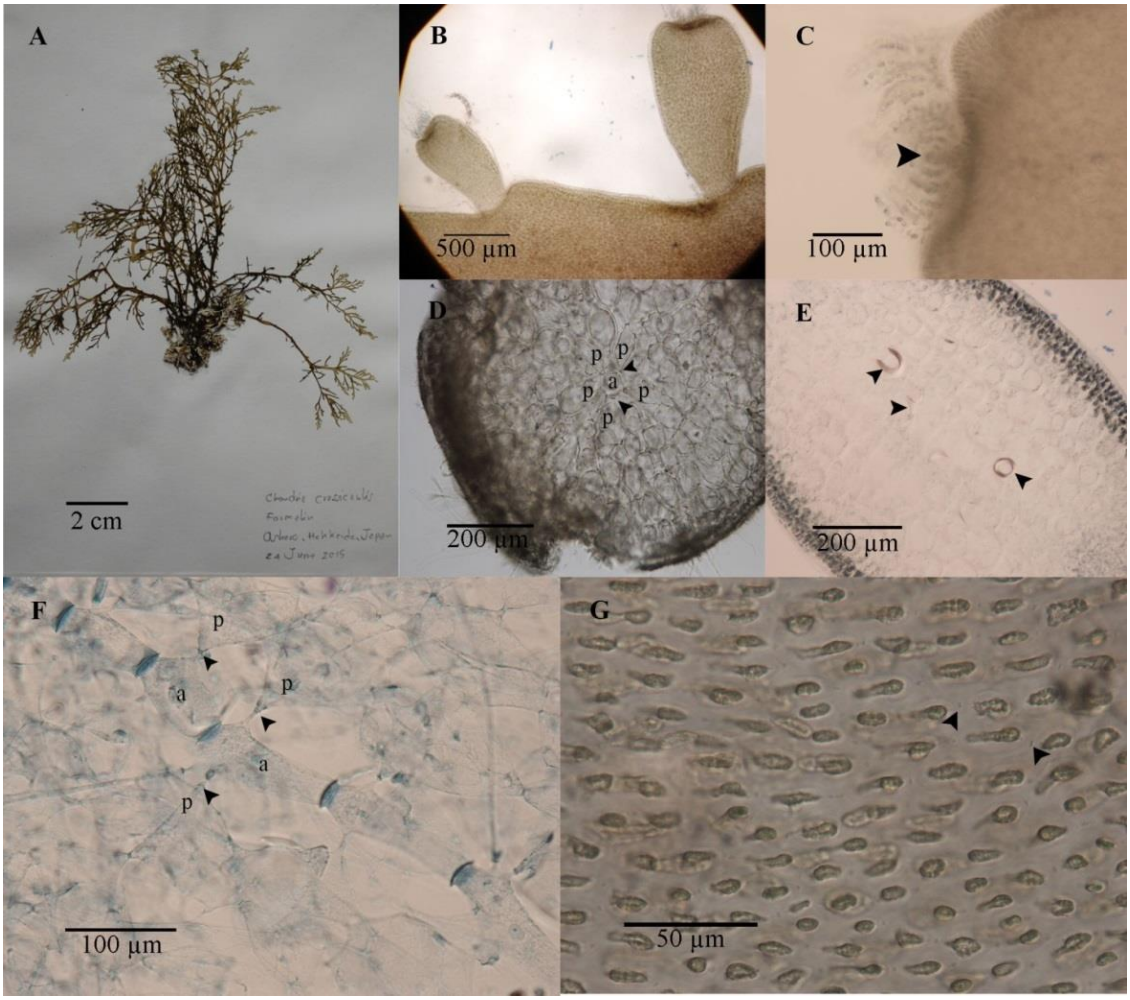

Fig. 4. Vegetative morphology of an examined *Chondria crassicaulis.*

A. *Chondria crassicaulis* (SAP115362, *rbc*L [MG255055], SSU [MG272238], *cox*1 [MG255071]) collected from Oshoro, Hokkaido, Japan on 24 June 2015.

B. Clavate-shaped branchlets with depressed apices.

C. High magnification of a depressed apex with protruding apical cell.

D. Cross-section of a branchlet showing an axial (a) issuing 5 pericentral cells (p). Arrowheads indicate pit connection.

E. Cell wall thickenings in medullary cells (arrowheads) appearing in crescent shape, horseshoe shape and round shape.

F. Longitudinal section of a branchlet showing a single row of axial cells (a) connected to pericentral cells (p) by pit connections (arrowheads).

G. Epidermal cell arrangement with pit connections (arrowheads).

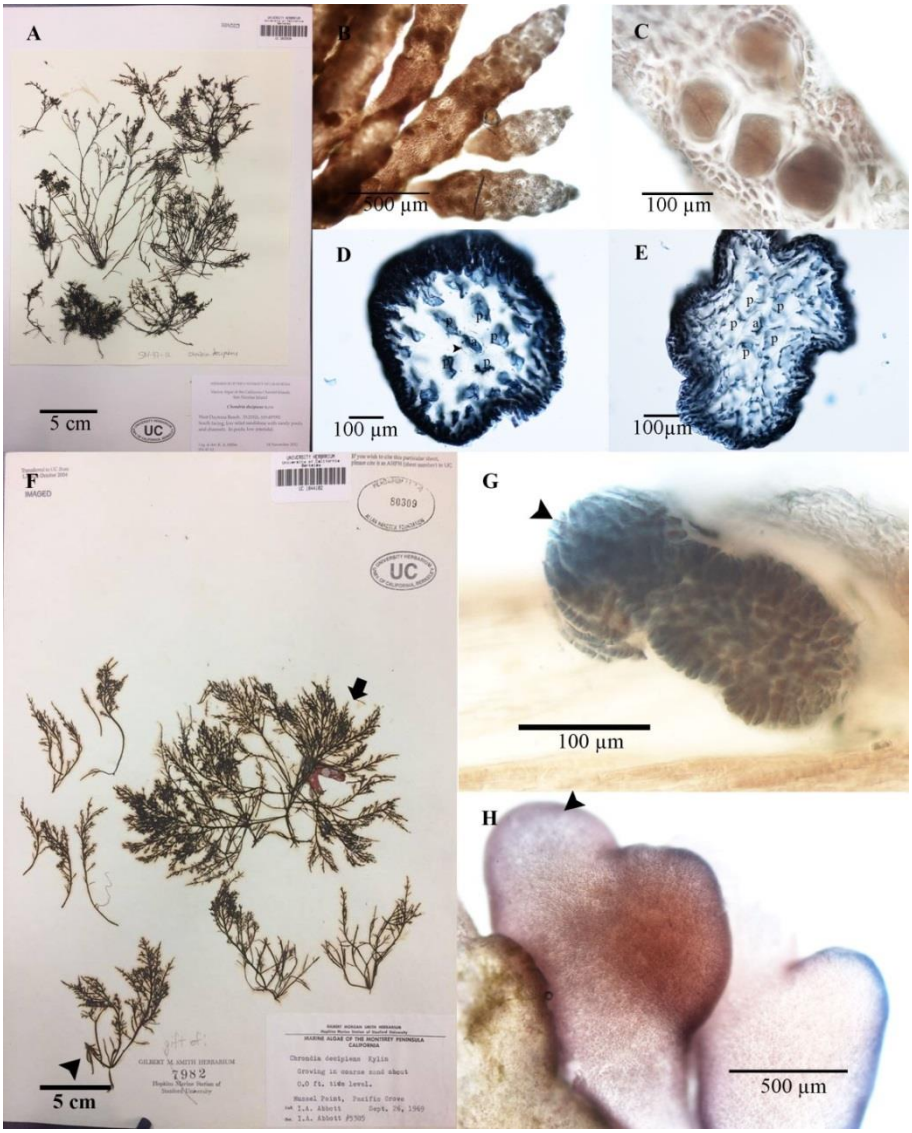

Fig. 5. Morphology of the examined *Chondria decipiens*.

A. *Chondria decipiens* (UC2025838, *rbc*L [MG255056], *cox*1 [MG272232]) collected from San Nicolas Island, California, USA on 14 November 2012.

B. Branchlets bearing tetrasporangia.

C. High magnification of a branchlet bearing tetrasporangia showing tetraspores borne in pericentral cells.

D. Cross-section of a branchlet (near an apex) showing axial cell (a) issuing 5 pericentral cells (p). Arrowhead indicates pit connection.

E. Cross-section of a median portion of an axis showing an axial cell (a) issuing 5 pericentral cells (p).

F. *Chondria decipiens* [tetraspophytes (arrow) and female gametophyte (arrowhead). UC1844102] collected from Mussel Point, Pacific Grove California, USA on 26 September 1969.

G. High magnification of a branchlet with acute apex. Arrowhead indicates apical cell.

H. High magnification of young developed cystocarps. Arrowhead indicates cystocarpic spur.

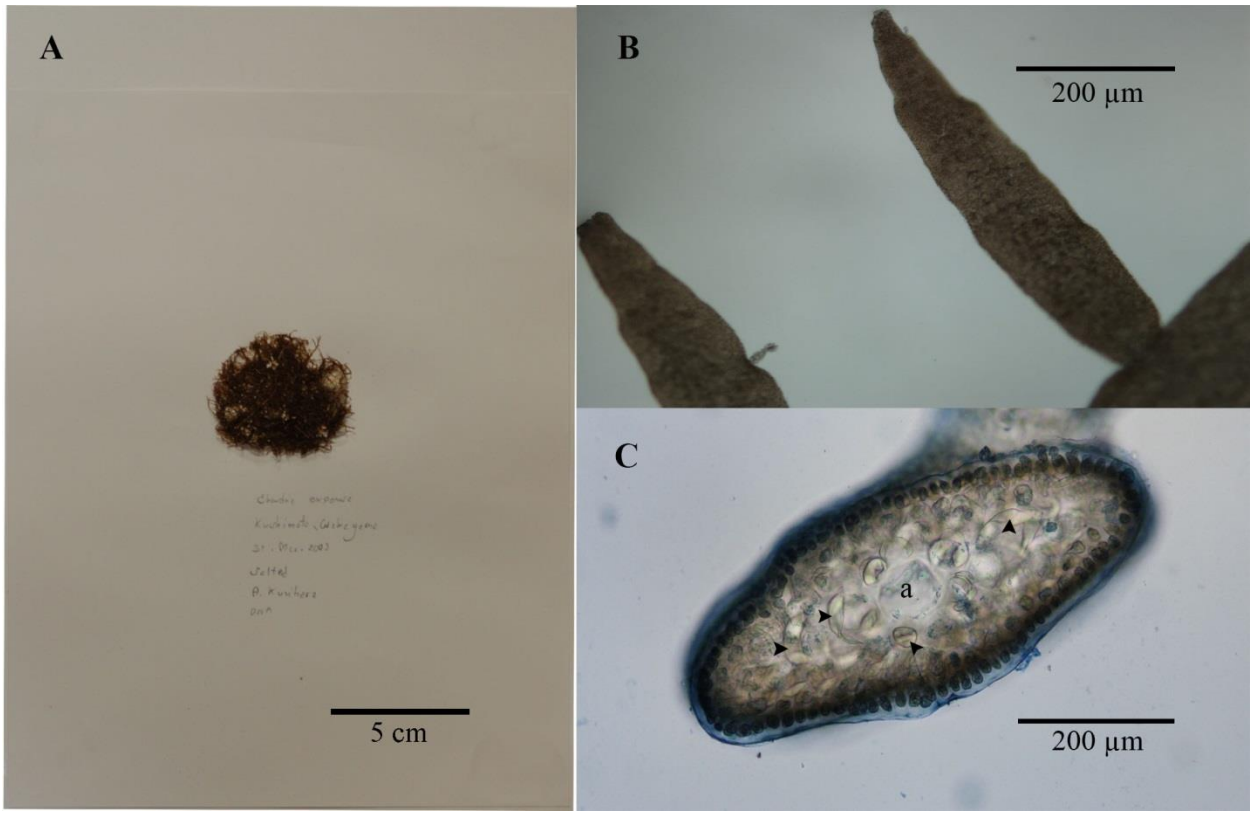

Fig. 6. Vegetative morphology of *Chondria expansa*.

A. *Chondria expansa* (SAP115365, *rbc*L [MG255057], *cox*1 [MG272233]) collected from Kushimoto, Wakayama Prefecture, Japan on 31 March 2003.

B. Flattened and fusiform branchlets.

C. Cross-section of a branchlet showing an axial cell (a). Arrowheads indicate cell wall thickenings in pericentral cells, medullary cells and sub-cortical cells.

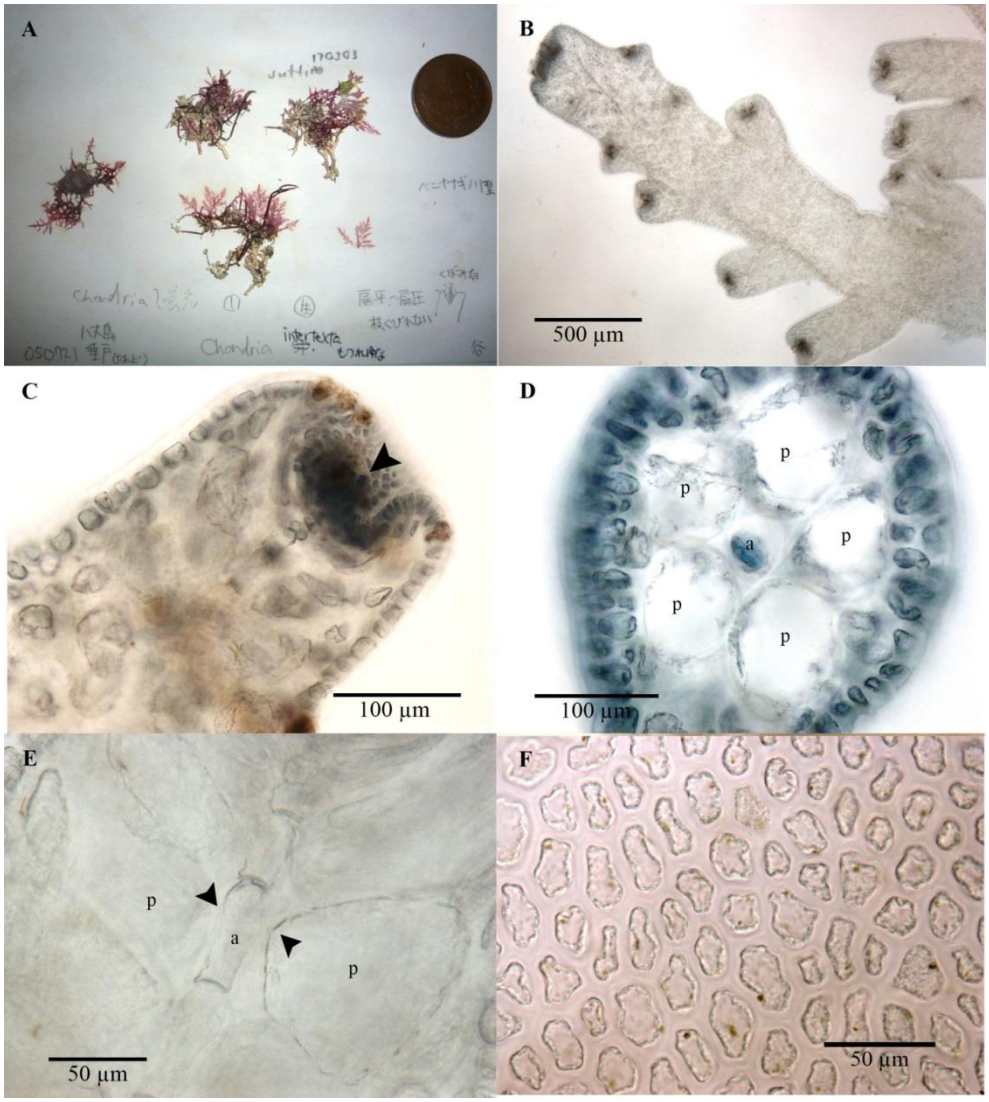

Fig. 7. Vegetative morphology of *Chondria intertexta*.

A. *Chondria intertexta* (SAP115364, *rbc*L [MG255059]) collected from Hachijo Island, Tokyo, Japan on 21 July 2005)

B. Unconstricted, cylindrical branchlets with depressed apices.

C. High magnification of a branchlet. Arrowhead indicates apical cell sunken in a depressed apex.

D. Cross-section of a branch (near an apex) showing an axial cell (a) issuing 5 pericentral cells (p).

E. Longitudinal section of a branch showing arrangement of axial cells and pericentral cells. Arrowheads indicate pit connection.

F. Epidermal cell arrangement.

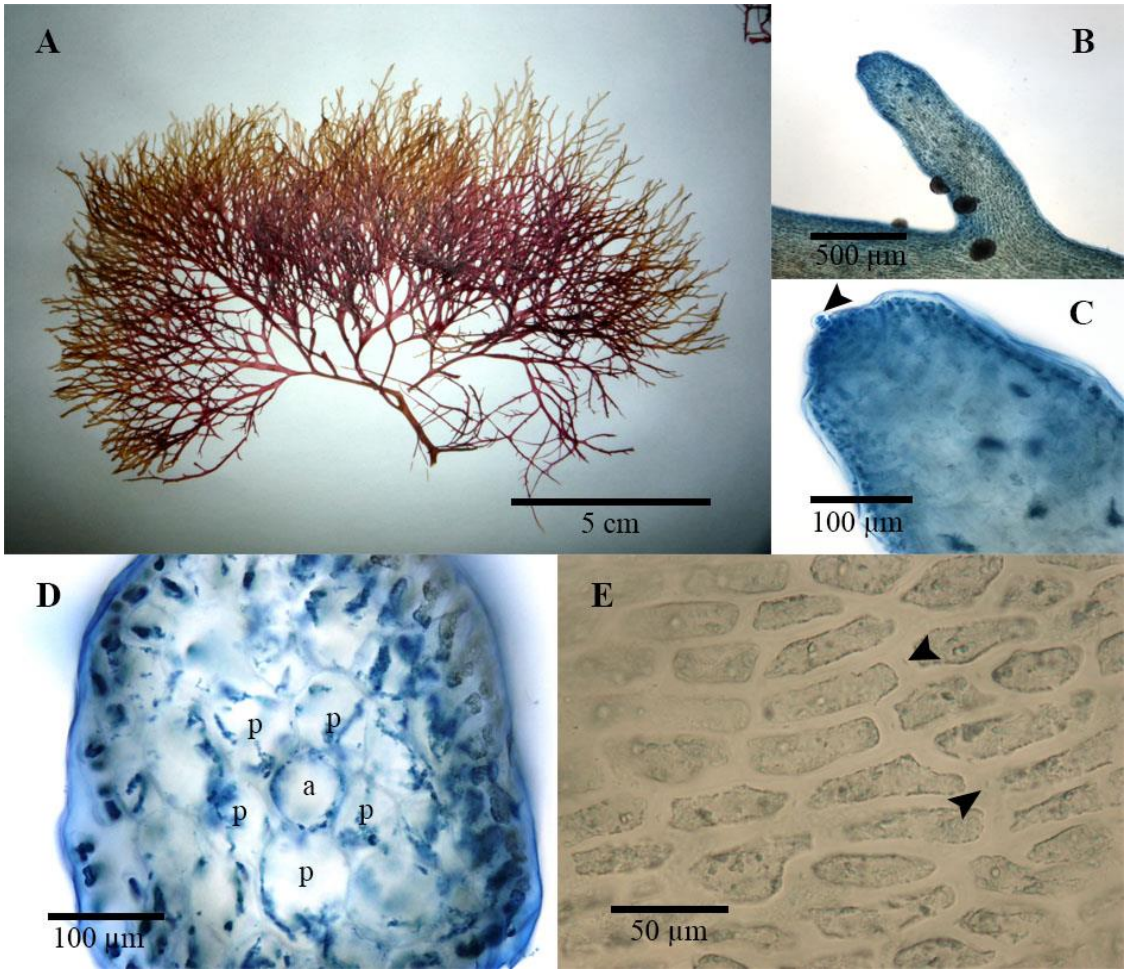

Fig. 8. Vegetative morphology of *Chondria mageshimensis*.

A. *Chondria mageshimensis* (SAP115367, *rbc*L [MG255060]) collected from Innoshima, Hiroshima Prefecture, Japan on 19 August 2005.

B. Unconstricted, complanate branchlet.

C. High magnification of a branchlet. Arrowhead indicates apical cell protruding from a rounded apex.

D. Cross-section of a young branch showing an axial cell (a) issuing 5 pericentral cells (p).

E. Epidermal cell arrangement. Arrowheads indicate pit connection.

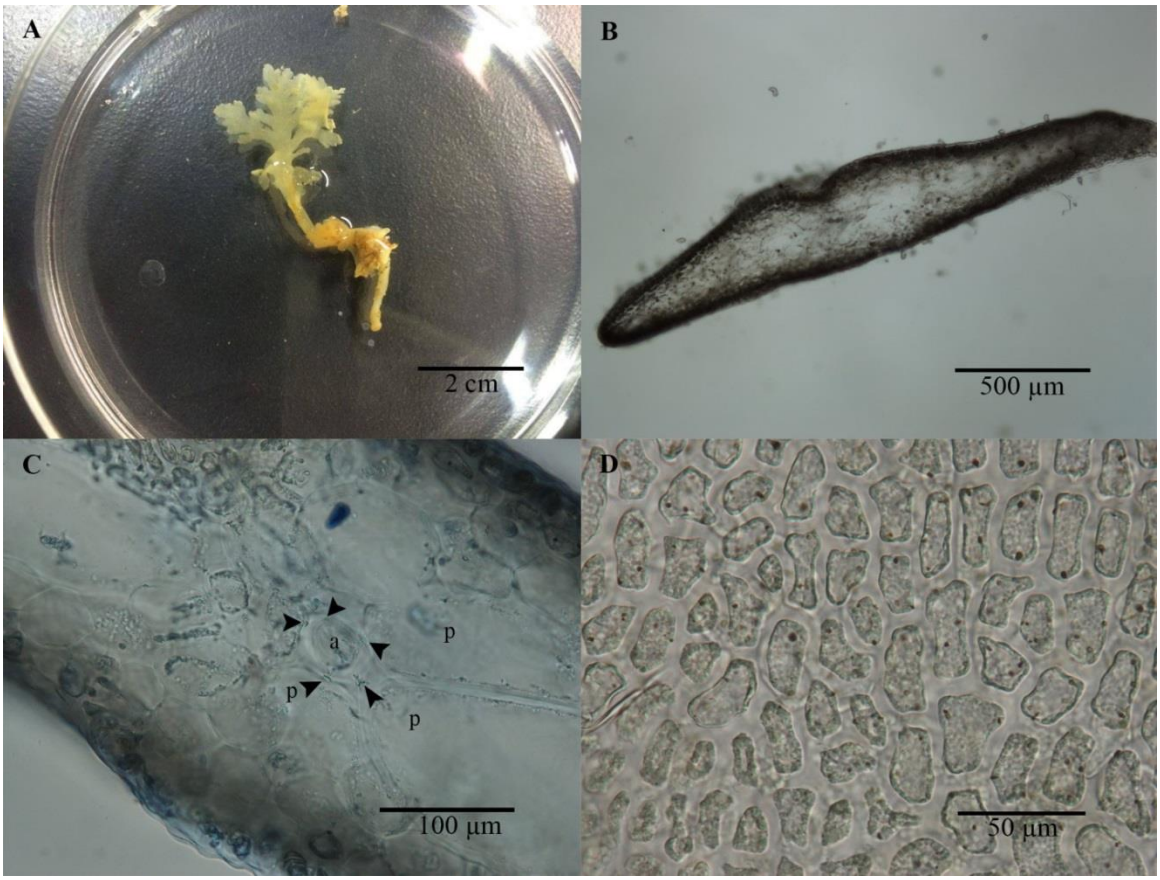

Fig. 9. Vegetative morphology of *Chondria ryukyuensis*

A. *Chondria ryukyuensis* (SAP115368, *rbc*L [MG255061], *cox*1 [MG272236]) collected from Kagoshima Prefecture, Japan on 27 August 2003.

B. Cross-section of a flattened branchlet.

C. High magnification of a cross-section of a flattened branchlet showing an axial cell (a) issuing pericentral cells (p). Arrowheads indicate pit connection.

D. Epidermal cell arrangement.

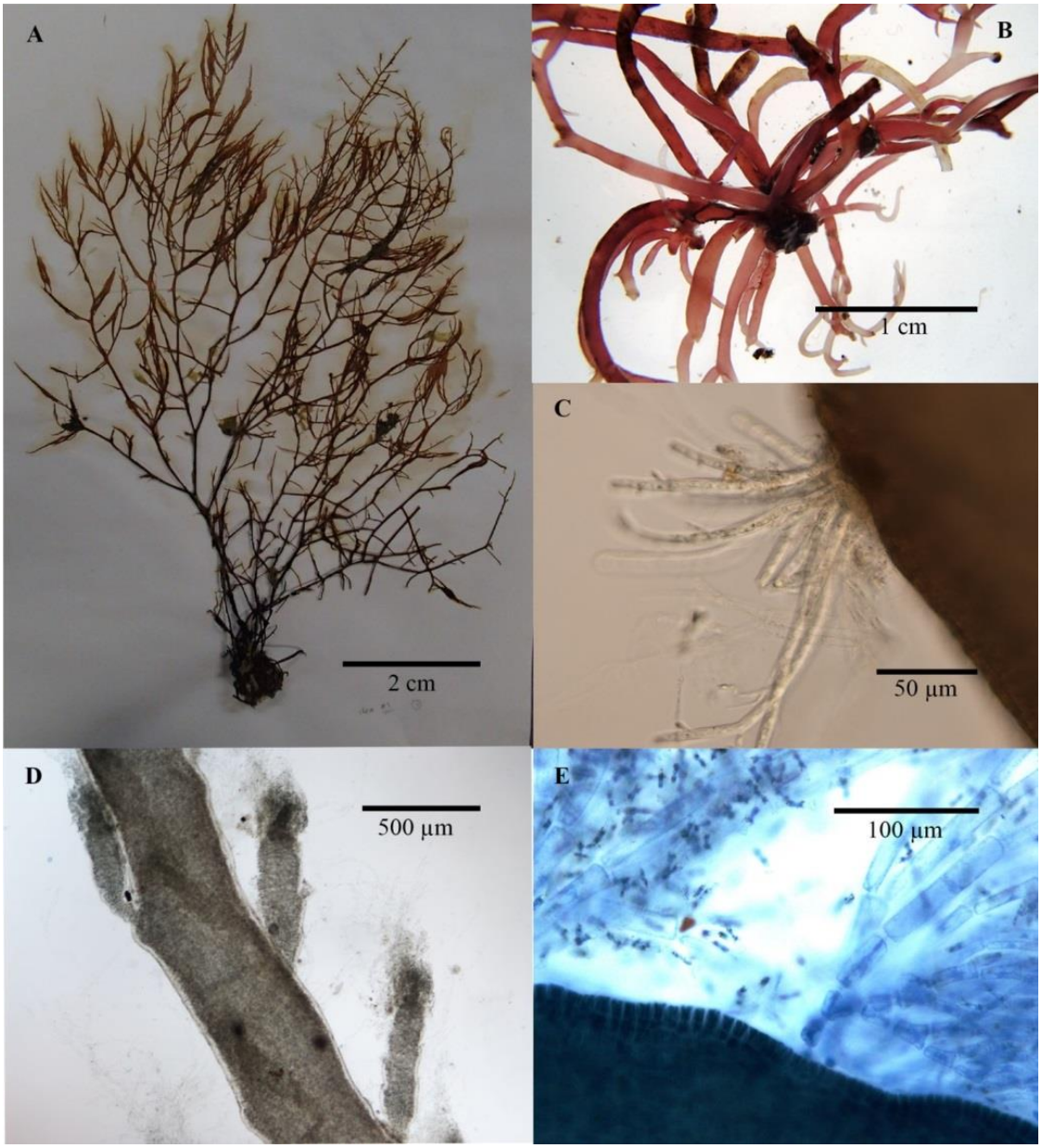

Fig. 10. Vegetative morphology of *Neochondria ammophila* sp. nov.

A. A specimen of *Neochondria ammophila* (sterile plant, SAP115354) collected at Denshin-hama, Muroran, Hokkaido, Japan on 23 May 2016.

B. Basal part with prostrate branches, discoid holdfasts (arrowheads) and stolon (arrow).

C. Unbranched, single-celled rhizoidal haptera on a prostrate branch.

D. Ultimate branchlets with basal constrictions.

E. High magnification view of trichoblasts with sub-dichotomous branching.

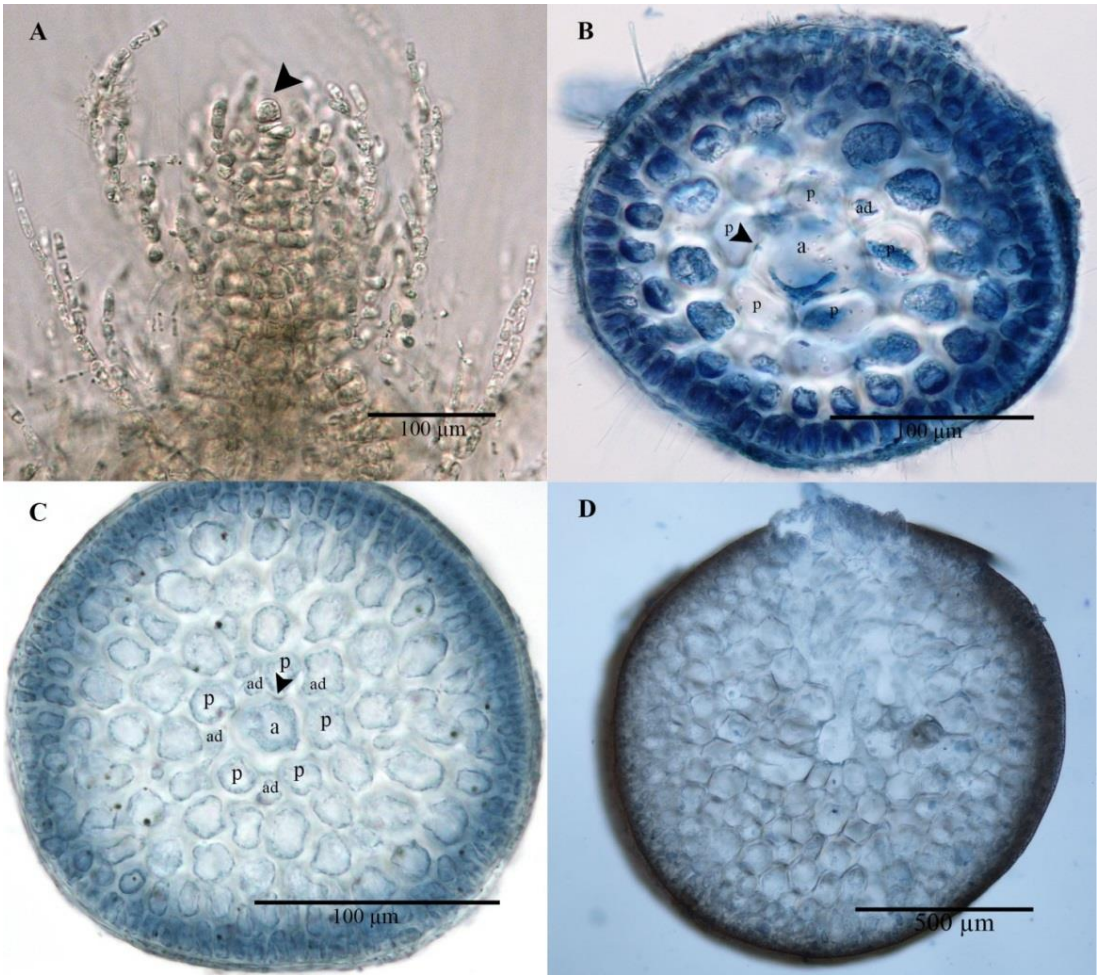

Fig. 11. Morphology of apical cell, axial cell, pericentral cell and adventitious elongate cells of *Neochondria ammophila* sp. nov.

A. A dome-shaped apical cell cutting off axial cells (arrowhead).

B, C. Cross-sections of an ultimate branchlet (near the tip). An axial cell (a) issuing adventitious elongate cells (ad) among 5 pericentral cells (p). Arrow indicates pit connection.

D. Cross-section of a median portion of an axis. Axial cell, pericentral cells and adventitious elongate cells are indistinguishable.

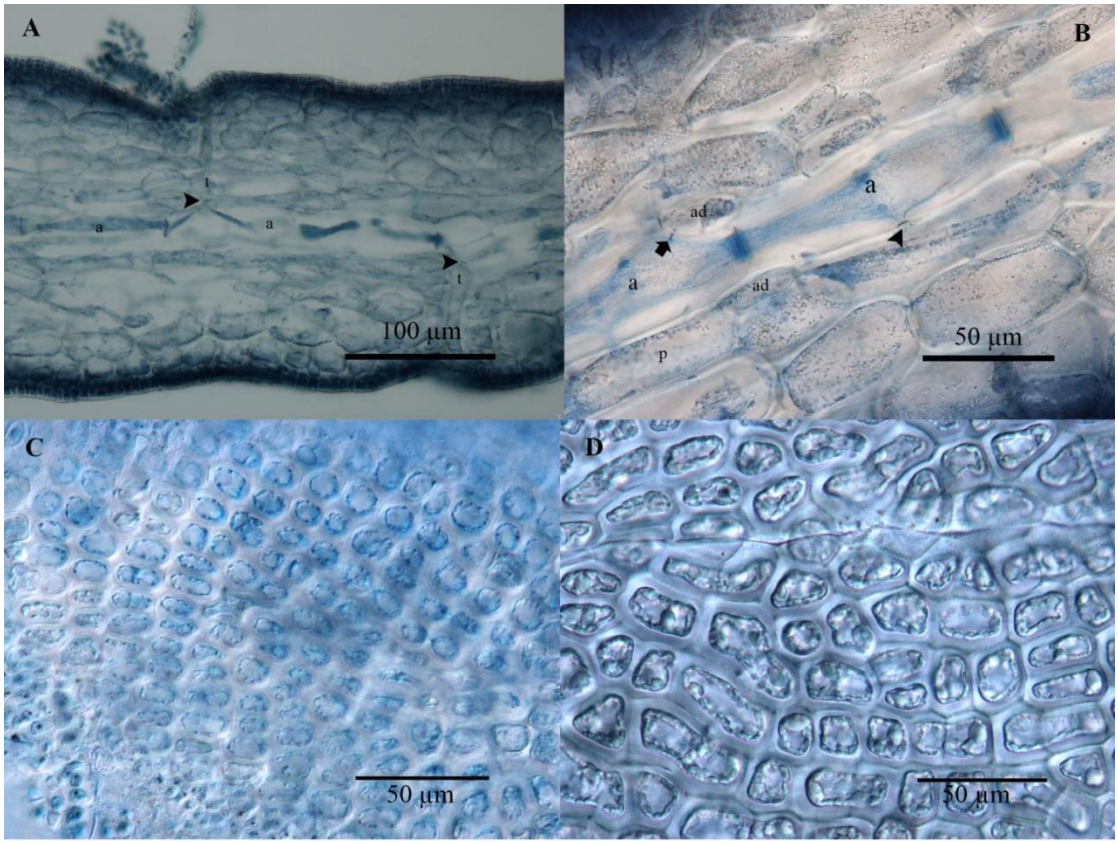

Fig. 12. Morphology of axial cells, pericentral cells, adventitious cells, trichoblast basal cells in longitudinal sections and epidermal cell arrangement of *Neochondria ammophila* sp. nov.

A. Longitudinal section of an ultimate branchlet. Arrowhead indicates pit connection between a trichoblast basal cell (t) and an axial cell (a).

B. Longitudinal section of an ultimate branchlet. Arrowhead indicates pit connection between axial cell (a) and pericentral cell (p); arrow indicates pit connection between axial cell and adventitious elongate cell (ad).

C. Rounded to oval epidermal cell arrangement in a branchlet.

D. Polygonal epidermal cell arrangement in a median portion of an axis.

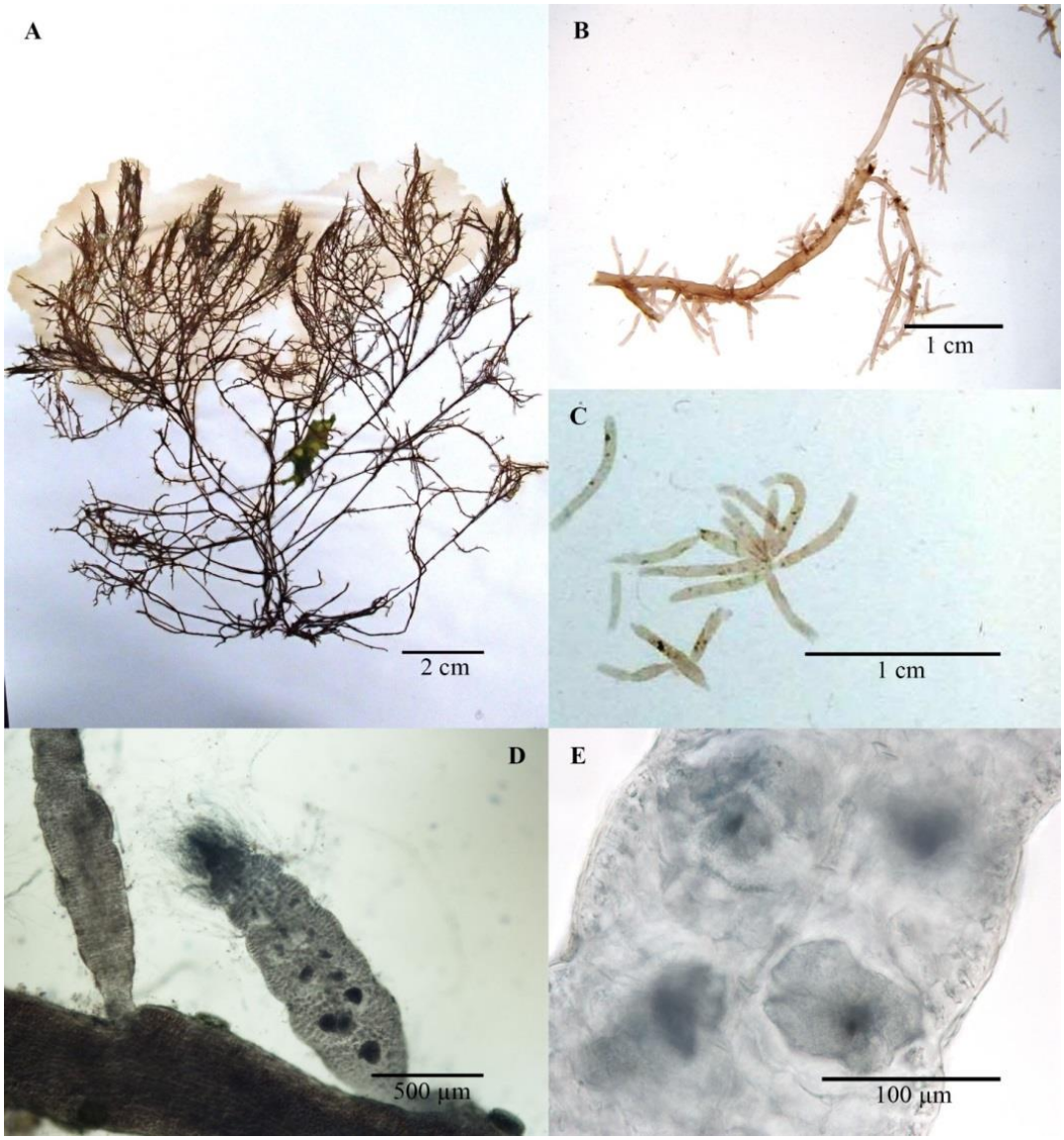

Fig. 13. Morphology of tetrasporophyte of *Neochondria ammophila* sp. nov.

A. Holotype specimen of *Neochondria ammophila* (tetrasporophyte, SAP115370, *rbc*L [MG255065], SSU [MG272243], cox 1 [MG272230]) collected from Momonai, Otaru, Hokkaido, Japan on 29 June 2016.

B. Fragments of upper part of a tetrasporophyte. Arrowhead indicates a tetrasporangial branchlet.

C. Tetrasporangial branchlets forming loose tufts.

D. Branchlet bearing tetrasporangia.

E. Tetrasporangia borne on pericentral cells.

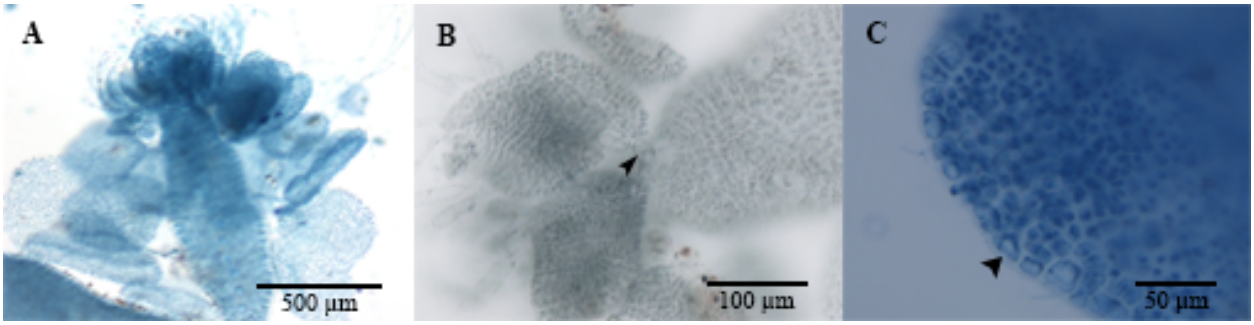

Fig. 14. Male gametophyte of *Neochondria ammophila* sp. nov. collected from Muroran, Hokkaido, Japan on 28 July 1999 (formalin preserved specimen, SAP115349).

A. Branchlet bearing spermatangial plates. The plates are basipetally formed on the branchlet.

B. Two spermatangial plates developing from a trichoblast cell (arrowhead).

C. A mature spermatangial plate with a single row of sterile cells (arrowhead).

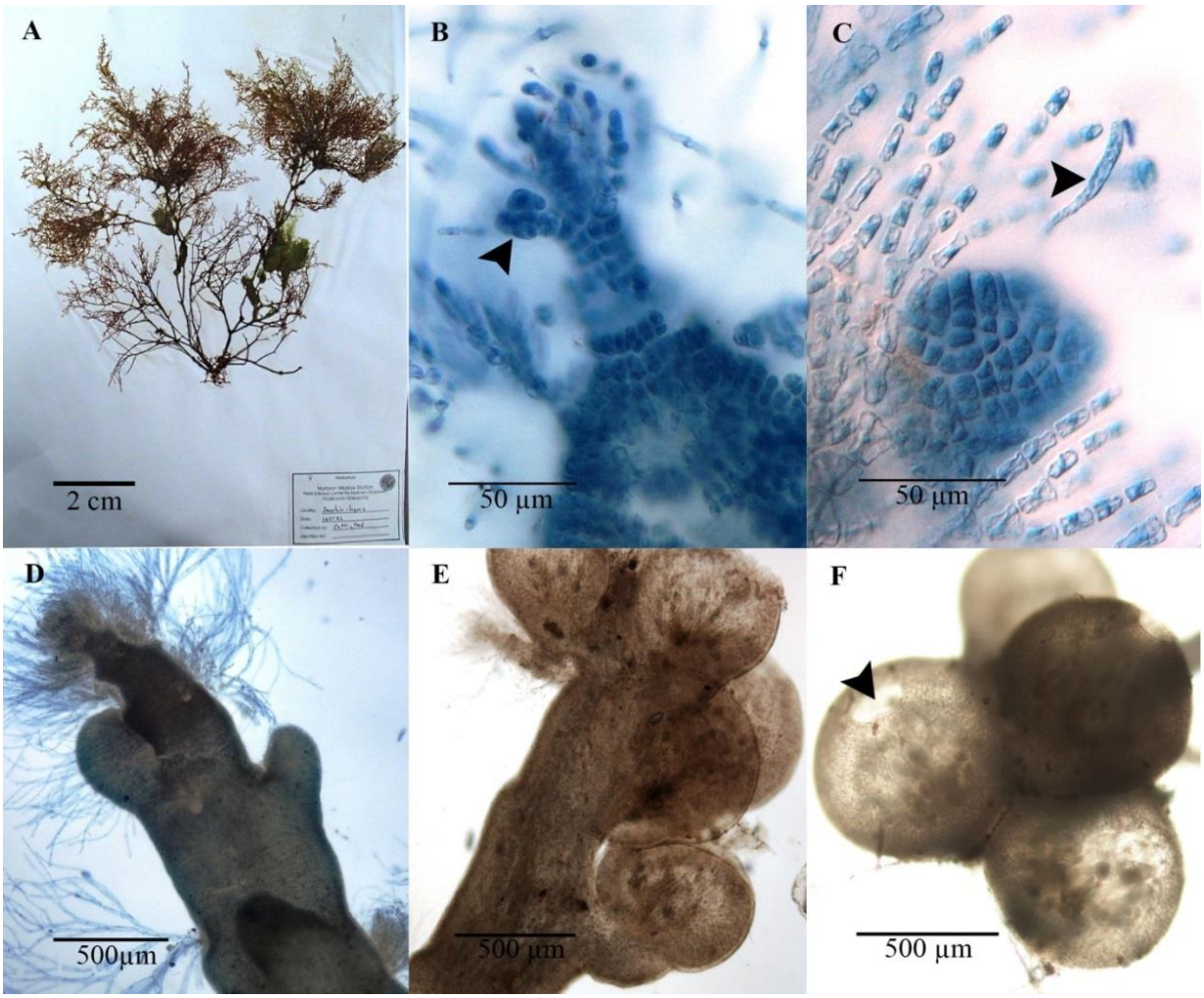

Fig. 15. Female gametophyte of *Neochondria ammophila* sp. nov.

A. Specimen of *Neochondria ammophila* (female gametophyte, SAP115371, *rbc*L [MG255066], SSU [MG272244], cox 1 [MG272231]) collected from Denshin-hama, Muroran, Hokkaido, Japan on 26 July 2016.

B. Early stage gonimoblast (arrowhead) showing 3 or 4 cells initiated post-fertilization.

C. Young cystocarp (out of focus) bearing a trichogyne (arrowhead).

D. Branchlet with developing cystocarps.

E. Branchlet with mature cystocarps.

F. High magnification view of mature cystocarps, showing an ostiole (arrowhead) opposite the base of the cystocarp.

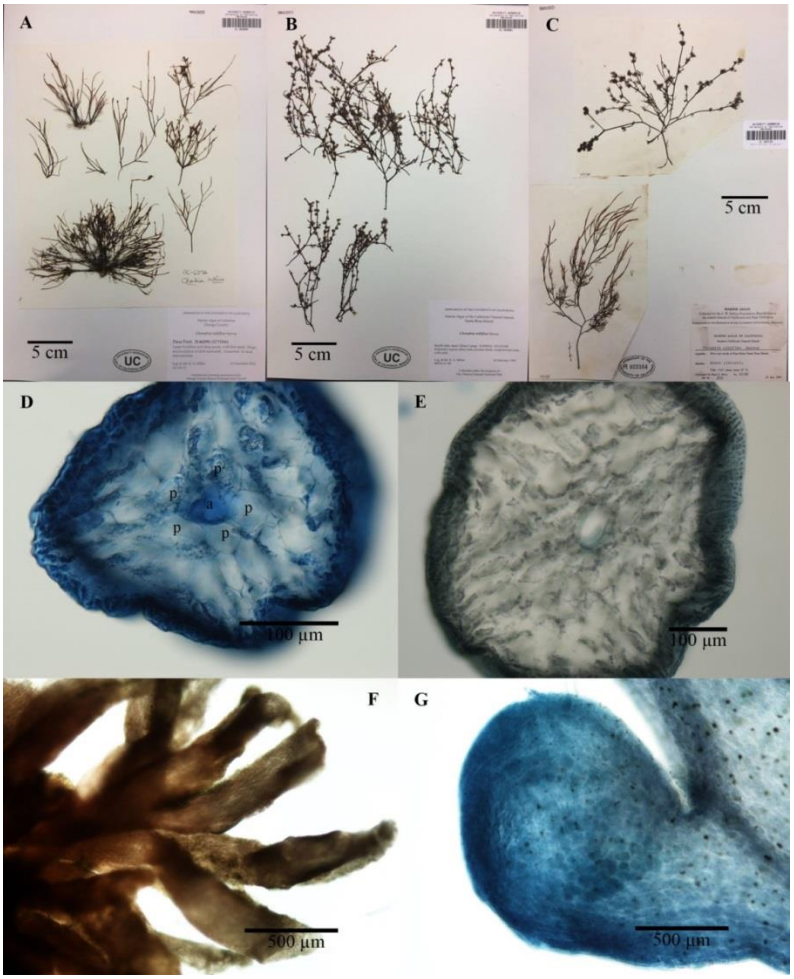

Fig. 16. Morphology of *Neochondria nidifica* comb. nov. (= *Chondria nidifica*)

A. *Chondria nidifica* (UC2026095, tetrasporophyte, *rbc*L [MG255067], SSU [MG272245], *cox*1 [MG272235]) collected from Dana Point, California, USA on 12 December 2012.

B. *Chondria nidifica* (UC2036061, tetrasporophyte) collected from Santa Rosa Island, California, USA on 20 February 1989.

C. *Chondria nidifica* (UC1022164, tetrasporophyte and female gametophyte) collected from Santa Rosa Island, California, USA on 27 February 1949.

D. Cross-section of an ultimate branchlet near the tip showing an axial cell (a) and pericentral cells (P).

E. Cross-section of an axis.

F. Tufted tetrasporangial branchlets.

G. Cystocarp without cystocarpic spur.

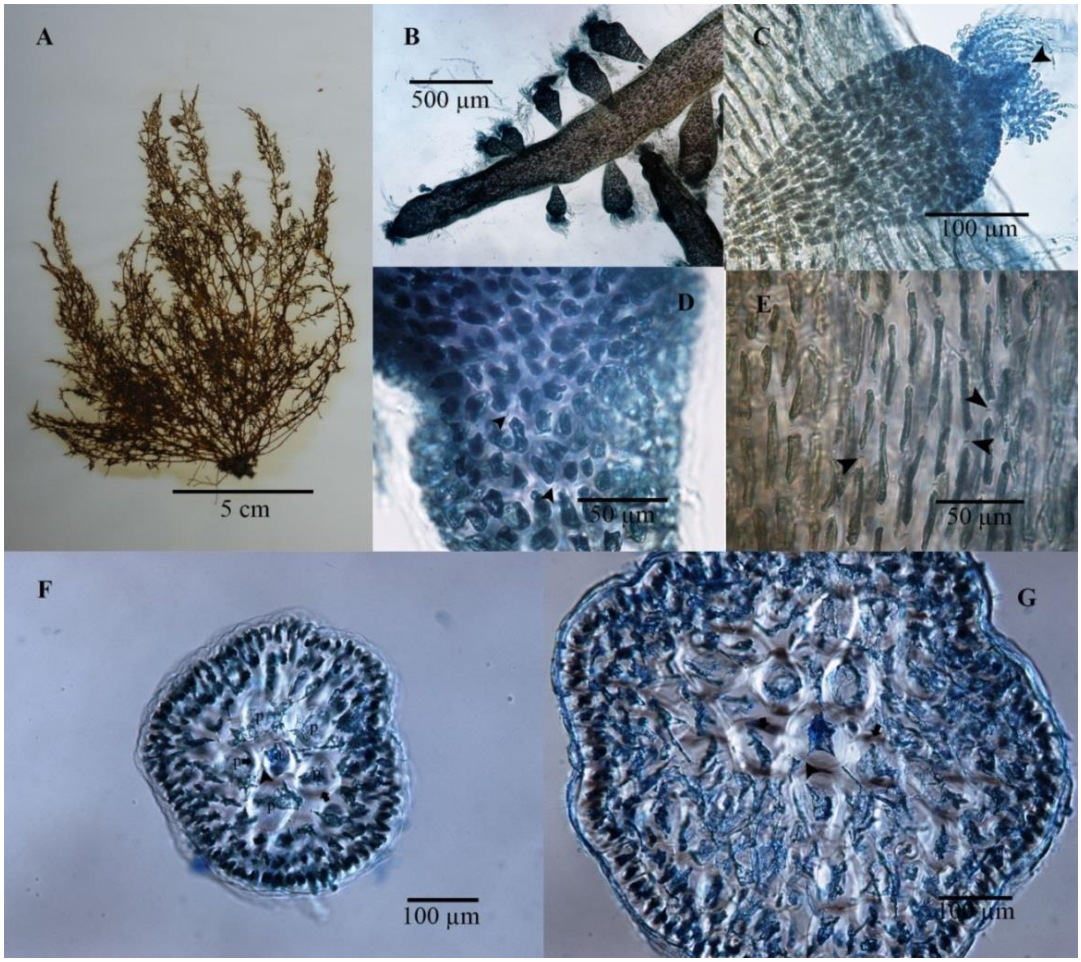

Fig. 17. Vegetative morphology of *Chondria acuminata* sp. nov. (= Japanese '*Chondria dasyphylla*')

A. *Chondria acuminata* (SAP115391, *rbc*L [MG843867], SSU [MG831941], *cox*1 [MG843859]) collected from Shiretoko, Hokkaido, Japan on 21 August 2017.

B. Young branch bearing branchlets in acropetal outline.

C. High magnification of a clavate branchlet with acuminate apex (arrowhead).

D. Rounded to polygonal, epidermal cell arrangement in a branchlet. Arrowheads indicate pit connection.

E. Rectangular, epidermal cell arrangement in a median portion of an axis. Arrowheads indicate pit connection.

F. Cross-section of a young branch showing an axial cell (a) issuing 5 pericentral cells (p). Arrowhead indicates pit connection, arrows indicate cell wall thickenings in pericentral cells.

G Cross-section of a median portion of an axis showing the remaining of an axial cell issuing 5 pericentral cells. Arrowhead indicates pit connection, arrow indicate cell wall thickening.

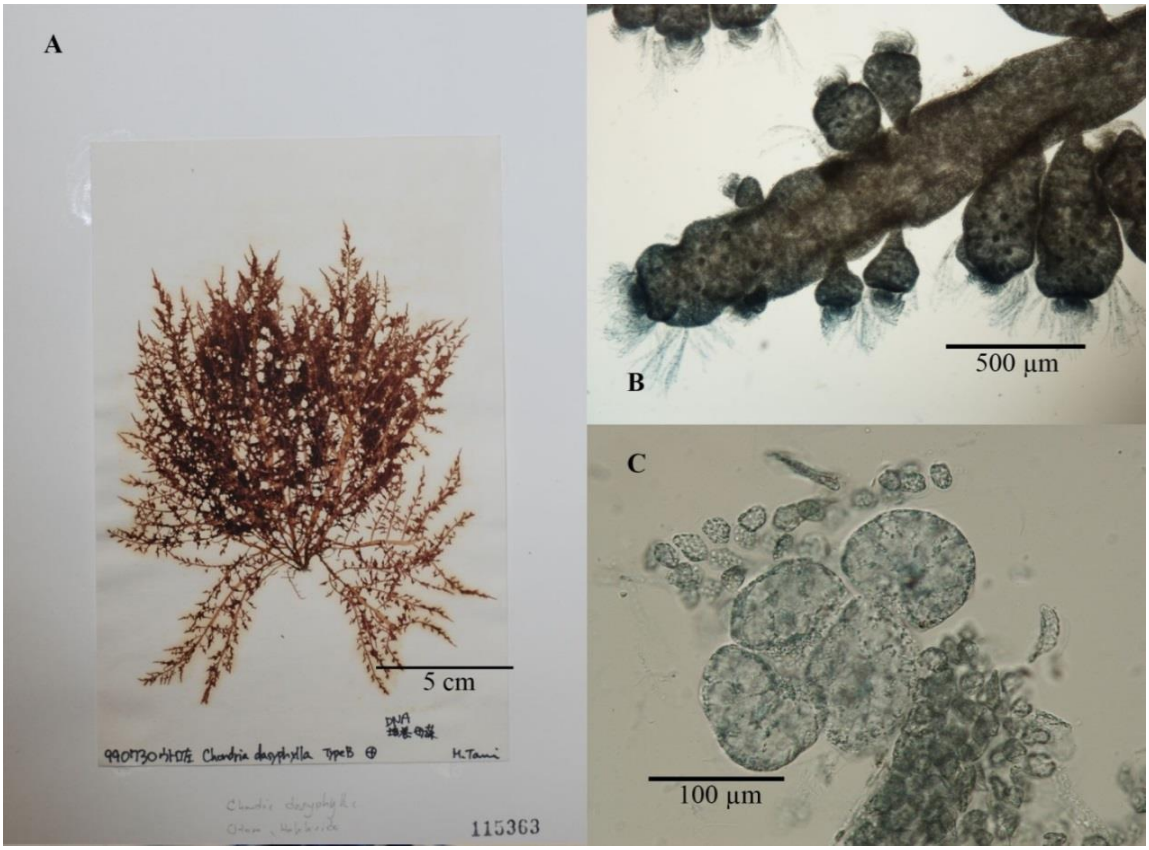

Fig. 18. Morphology of a tetrasporophyte of *Chondria acuminata* sp. nov. (= Japanese '*Chondria dasyphylla*')

A. *Chondria acuminata* (SAP115363, *rbc*L [MG255062], SSU [MG272240], *cox*1 [MG272237]) collected from Utoro, Hokkaido, Japan on 30 July 1999.

B. Young branch bearing tetrasporic branchlets.

C. High magnification of a segregated tetraspore.

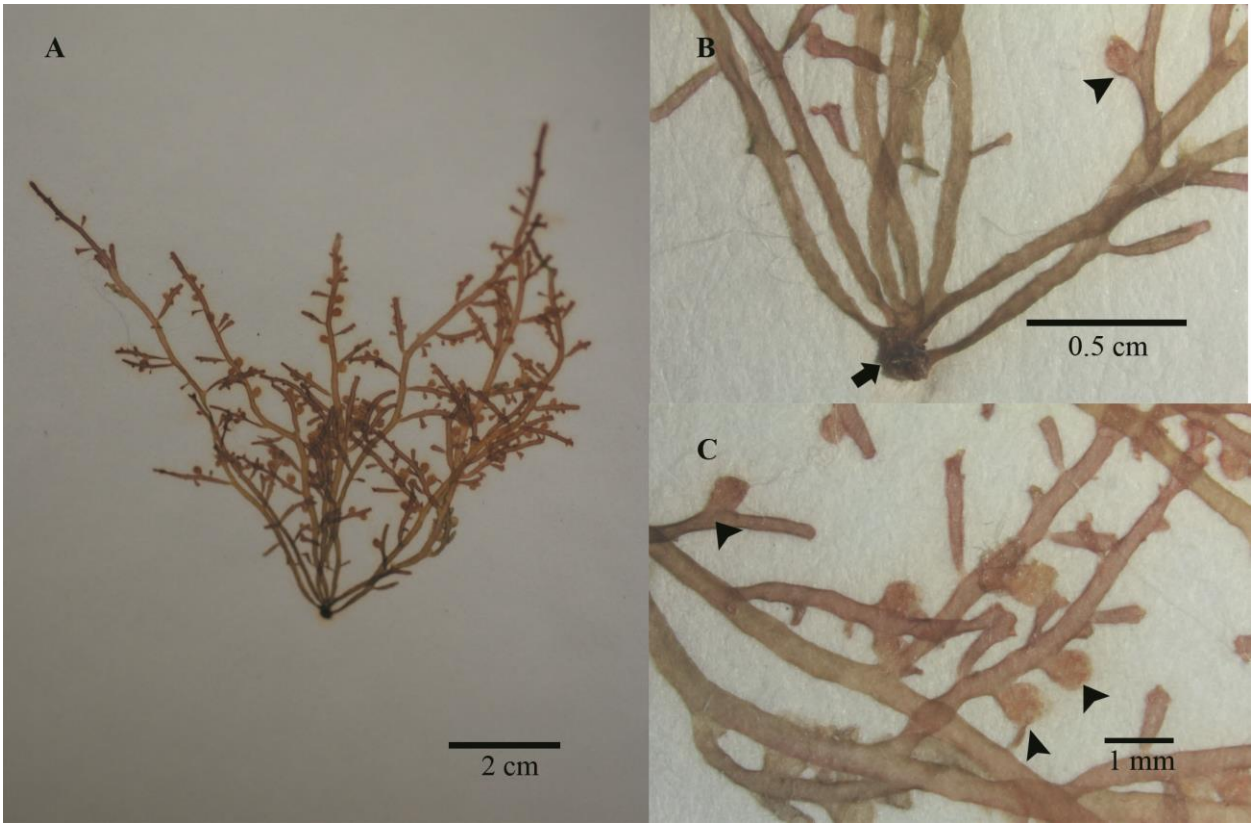

Fig. 19. Female gametophyte of *Chondria acuminata* sp. nov. (= Japanese '*Chondria dasyphylla*')

A. A specimen of *Chondria acuminata* (female gametophyte, SAP 115401) collected from Utoro, Hokkaido, Japan on 9 August 1998.

B. Basal part of a female thallus shows a discoid holdfast (arrow) sprouting several axes. Arrowhead indicates an urceolate cystocarp.

C. Branches bearing cystocarps. Arrowheads indicate urceolate cystocarps without markedly cystocarpic spurs.

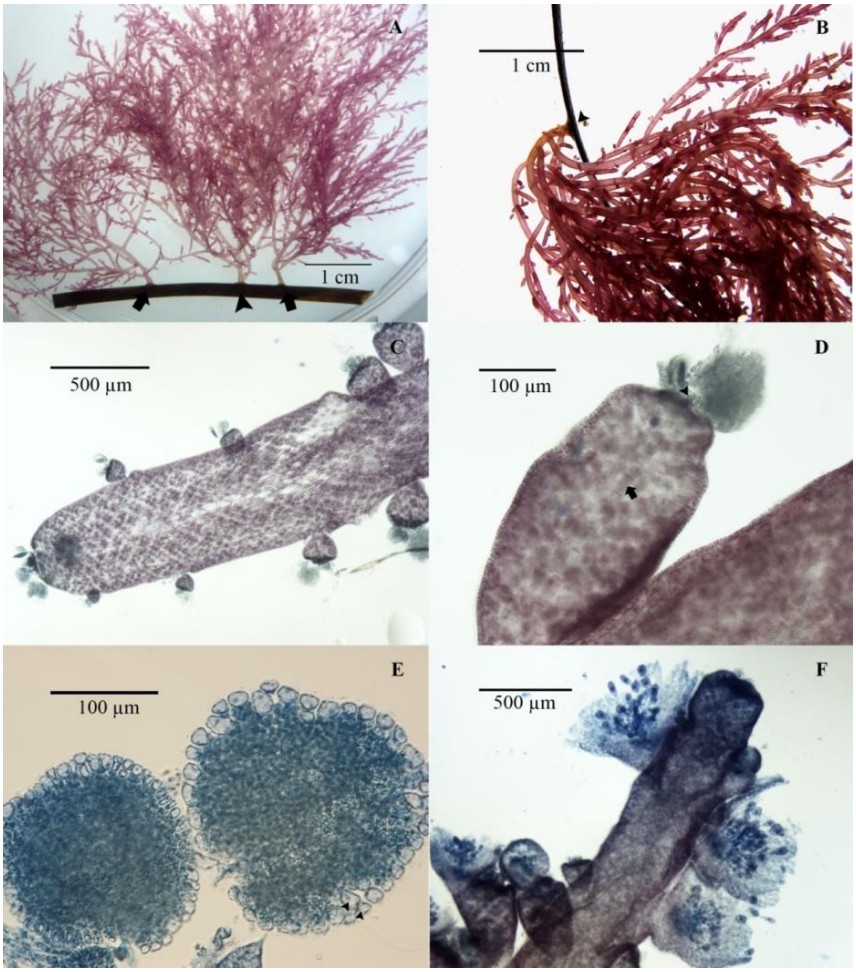

Fig. 20. Morphology of male and female gametophyte of *Chondria* cf. *curdieana* (= Japanese '*Chondria dasyphylla*')

A. *Chondria* cf. *curdieana* attached on a seagrass (*Phyllospadix iwatensis*). Arrowheads indicate male gametophytes and arrow indicates female gametophyte (SAP115394) collected from Hokkaido, Japan on 21 August 2016.

B. Basal part of a female thallus showing a discoid holdfast (arrowhead) attached on a seagrass leaf.

C. Distal branch of a male thallus bearing ultimate branchlets.

D. A branchlet bearing spermatangial plates. Arrowhead indicates apical cell cutting of 5 pericentral cell. Arrow indicates a row of connected axial cells.

E. High magnification of spermatangial plates. Arrowheads show two overlapping rows of sterile marginal cells.

F. Young branch bearing ovoid cystocarps without markedly cystocarpic spur at the base.

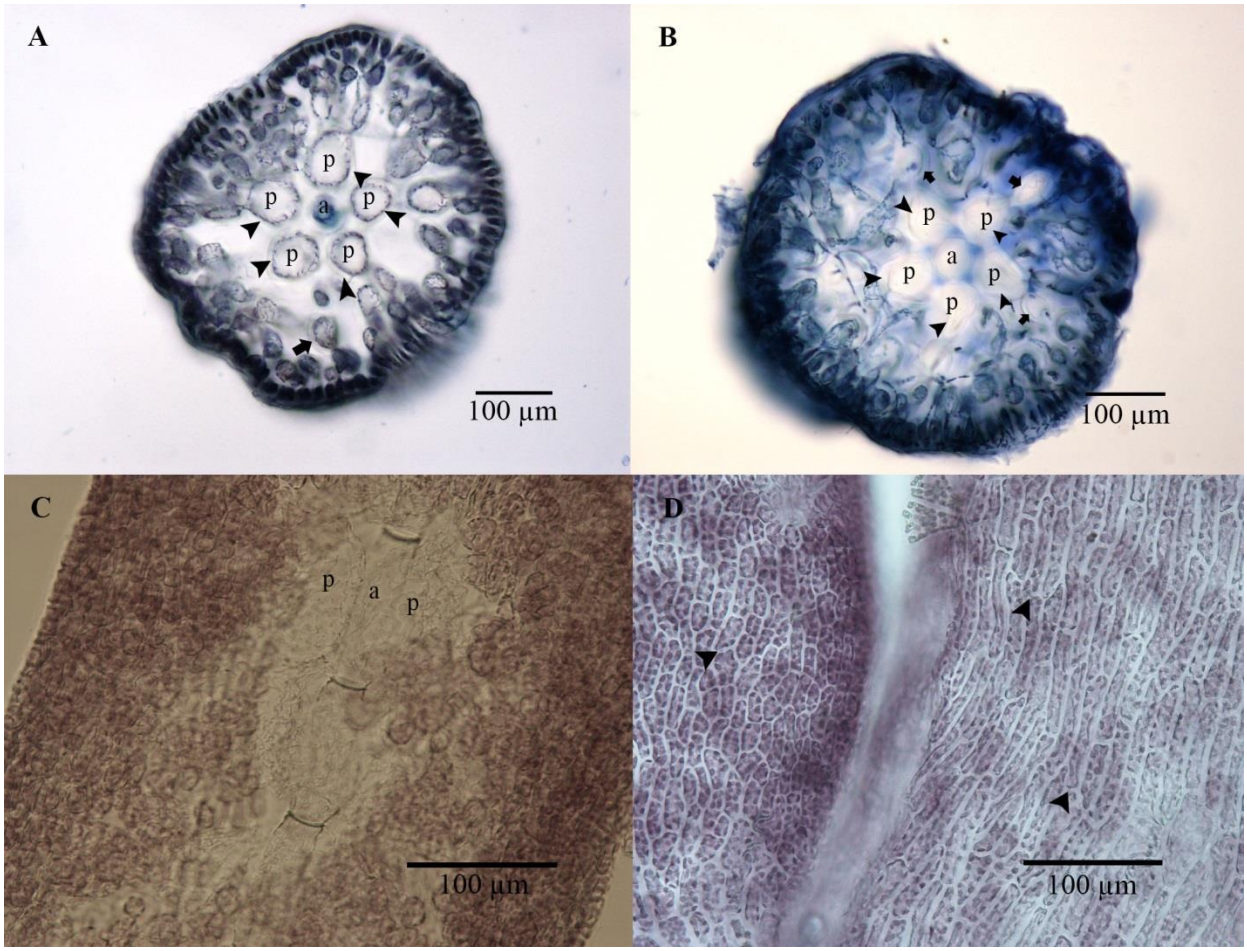

Fig. 21. Axial cell issuing pericentral cell and epical cells arrangement of *Chondria* cf. *curdieana* (= Japanese '*Chondria dasyphylla*')

A. Cross-section of a branchlet showing an axial cell (a) issuing 5 pericentral cells (p). Arrowheads indicate cell wall thickenings appearing in pericentral cells. Arrow indicates cell wall thickening in sub-cortical cell.

B. Cross-section of a median portion of an axis showing an axial cell (a) issuing 5 pericentral cells (p). Arrowheads indicate cell wall thickenings appearing in all pericentral cells. Arrows indicate cell wall thickening in sub-cortical cell.

C. Longitudinal section of a median axis showing arrangement of axial cells (A) and pericentral cells (P).

D. Epidermal cell arrangement in a brachlets (left) and median portion of an axis (right). Arrowheads indicate pit connection.

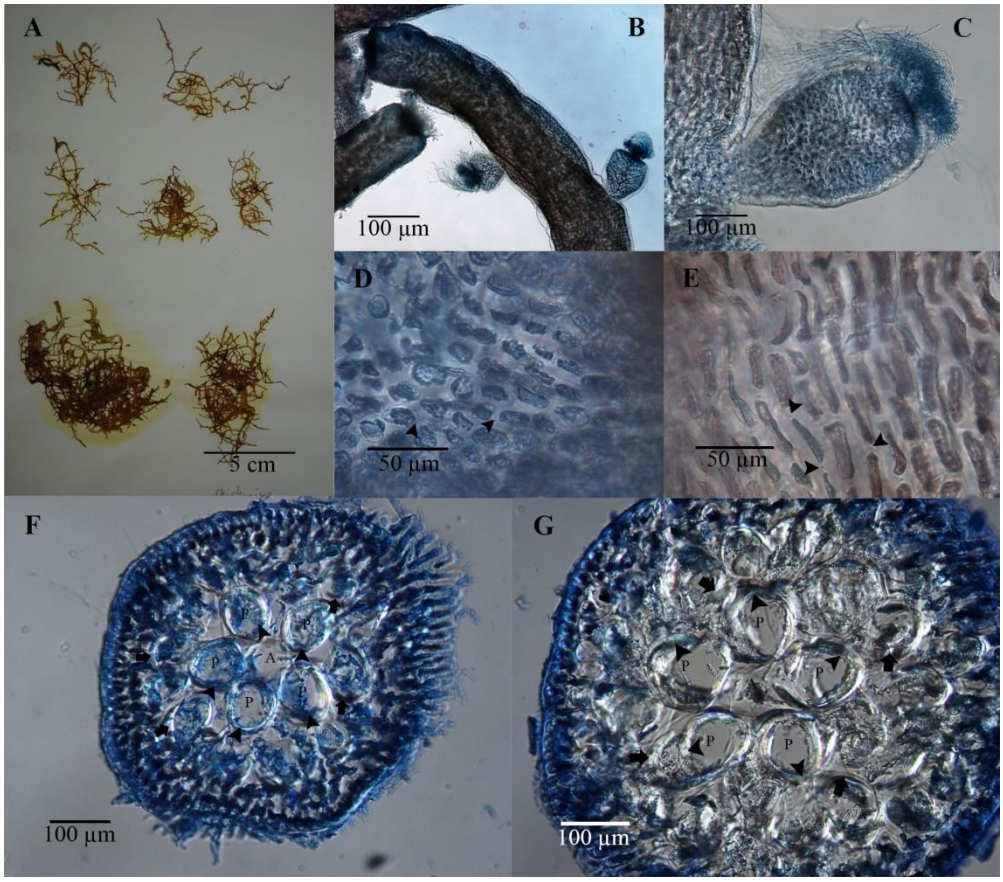

Fig. 22. Vegetative morphology of an examined *Chondria* sp. 1

A. *Chondria* sp. 1 (SAP115397, *rbc*L [MG843871], SSU [MG831945], *cox*1 [MG843863]) collected from Shiretoko, Hokkaido, Japan on 20 August 2017.

B. Young branch sparsely bearing branchlets.

C. High magnification of a clavate branchlet with depressed apex.

D. Rounded to oval epidermal cell of a young branch.

E. Rectangular epidermal cell of a median portion of an axis.

F. Cross-section of a young branch showing an axial cell (a) issuing 5 pericentral cells (p). Arrowheads indicate cell wall thickenings appearing in all pericentral cells. Arrows indicate cell wall thickening in sub-cortical cell.

G. Cross-section of a median portion of an axis showing an axial cell (a) issuing 5 pericentral cells (p). Arrowheads indicate cell wall thickenings appearing in all pericentral cells. Arrows indicate cell wall thickening in sub-cortical cell.

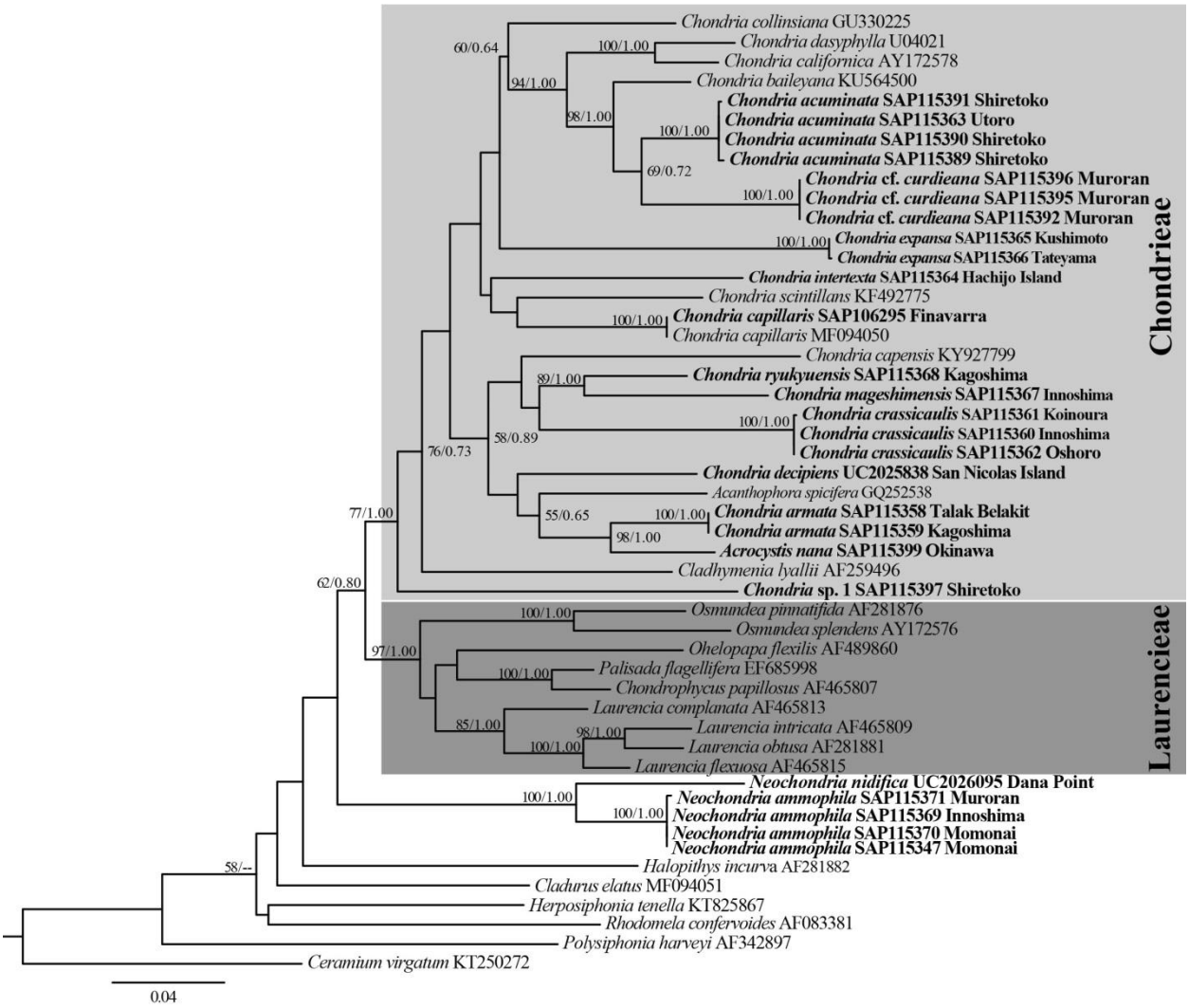

Fig. 23. Maximum likelihood tree generated from partial *rbc*L sequences representing 49 Rhodomelaceae and a Ceramiaceae sequence (KT250272) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities. Only bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.60 are shown.

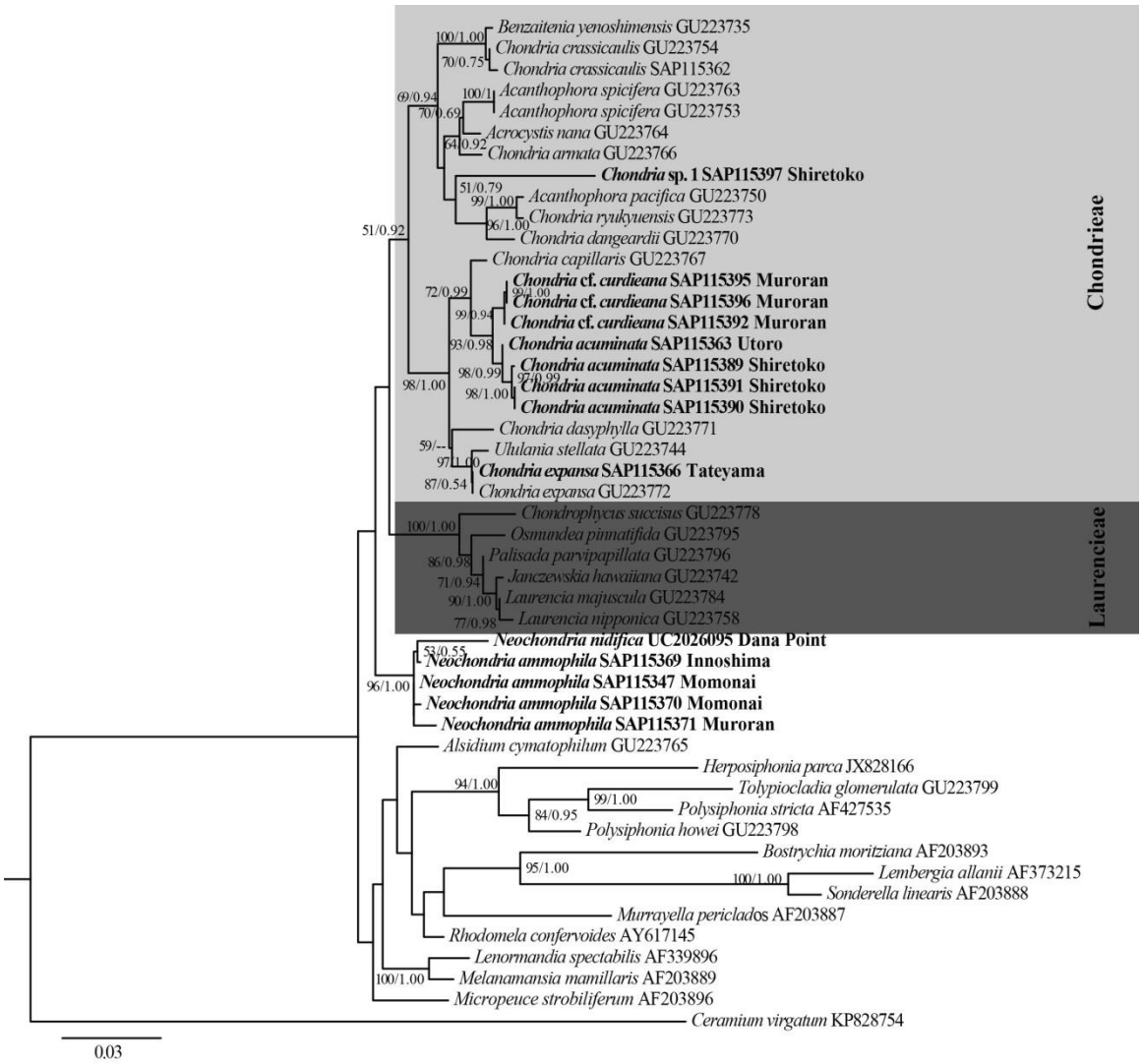

Fig. 24. Maximum likelihood tree generated from partial SSU rRNA gene sequences representing 47 Rhodomelaceae and a Ceramiaceae sequence (KP828754) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities. Only bootstrap values ≥ 0.50 and Bayesian posterior probabilities ≥ 0.50 are shown.

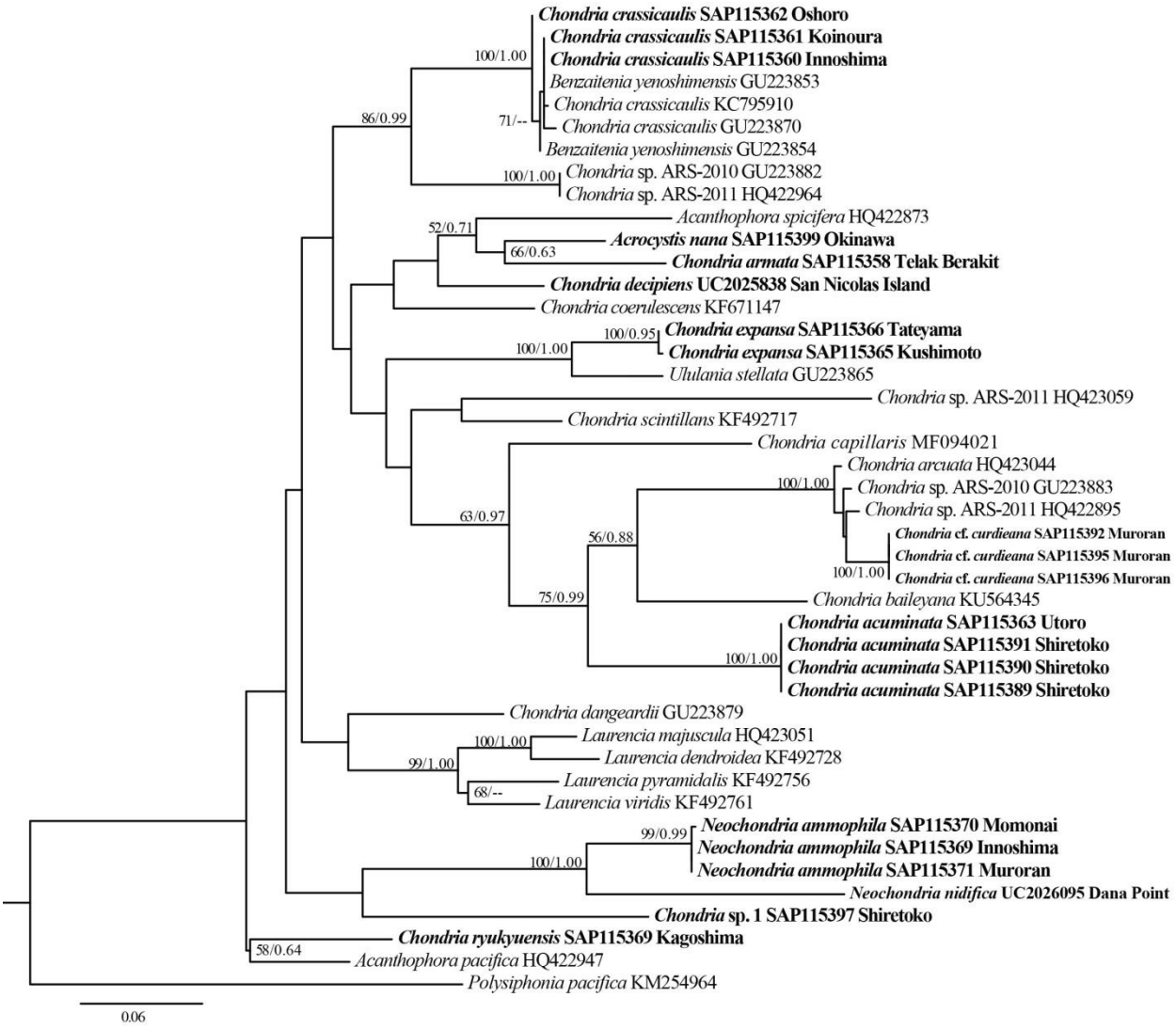

Fig. 25. Maximum likelihood tree generated from *cox*1 sequences representing 43 Chondrieae-Laurencieae species and a *Polysiphonia* sequence (KM254964) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities. Only bootstrap values > 50 and Bayesian posterior probabilities ≥ 0.60 are shown.