



HOKKAIDO UNIVERSITY

| | |
|---------------------|---|
| Title | A morphological and phylogenetic study of the genus <i>Chondria</i> (Rhodomelaceae, Rhodophyta) |
| Author(s) | Sutti, Suttikarn |
| Degree Grantor | 北海道大学 |
| Degree Name | 博士(理学) |
| Dissertation Number | 甲第13264号 |
| Issue Date | 2018-06-29 |
| DOI | https://doi.org/10.14943/doctoral.k13264 |
| Doc URL | https://hdl.handle.net/2115/71176 |
| Type | doctoral thesis |
| File Information | Suttikarn_Sutti.pdf |



**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）の形態学および系統学的研究】

Suttikarn Sutti

Department of Natural History Sciences, Graduate School of Science

Hokkaido University

June 2018

CONTENTS

| | |
|--|----|
| Abstract..... | 2 |
| Acknowledgement..... | 5 |
| General Introduction..... | 7 |
| Chapter 1. Morphology and molecular phylogeny of the genus <i>Chondria</i> based on Japanese specimens..... | 14 |
| Introduction | |
| Materials and Methods | |
| Results and Discussions | |
| Chapter 2. <i>Neochondria</i> gen. nov., a segregate of <i>Chondria</i> including <i>N. ammophila</i> sp. nov. and <i>N. nidifica</i> comb. nov..... | 39 |
| Introduction | |
| Materials and Methods | |
| Results | |
| Discussions | |
| Conclusion | |
| Chapter 3. Yanagi nori—the Japanese <i>Chondria dasyphylla</i> including a new species and a probable new record of <i>Chondria</i> from Japan..... | 51 |
| Introduction | |
| Materials and Methods | |
| Results | |
| Discussions | |
| Conclusion | |
| References..... | 66 |
| Tables and Figures | |

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 (*rbcL*), small subunit of nuclear ribosomal rRNA (SSU rRNA) and mitochondrial cytochrome oxidase subunit 1 (*cox1*) gene sequences; new sequences were generated for 12 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 (*rbcL* 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 *rbcL* 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 (*rbcL*, SSU and *cox1*) 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, Pukyong 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 was considered 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, Pihelliales, Plocamiales, Rhodachlyales, Rhodogorgonales, Rhodymeniales, Sebdeniales, Sporolithales and Thoreaales (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, *Cladymania* Harvey, *Coeloclonium* J. Agardh, and *Husseyia* J. Agardh) and two parasitic genera (*Benzaitenia* Yendo, and *Ululania* K.E. Apt & K.E. Schleich) (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, subspherical 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 develop 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 although 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 (*rbcL*), small subunit of nuclear ribosomal rRNA (SSU rRNA) and mitochondrial cytochrome oxidase subunit 1 (*cox1*) 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 *rbcL*, SSU rRNA and *cox1* 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 dissimilarity 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, *Platycondria* 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 *et 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 *et 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* (Kützinger) 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 *rbcL*, SSU and *cox1* 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 (*cox1* and *rbcL*) 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 Chondriaceae 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 *rbcL* and SSU trees. *Polysiphonia pacifica* Hollenberg (KM254964) was used as an outgroup for the *cox1* 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 bearing 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* (Kützting) 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 dissimilarities from the British ‘*C. tenuissima*’, near its type locality. An examined specimen from Ireland [SAP115387, tetrasporophyte] was well corresponded with *C. capillaris* described 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 *Chondria* 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 repeatedly 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. magashimensis*, *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 bulbous 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 *rbcL* 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 *rbcL* 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 *rbcL*. 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 crassicaulis*.

Cox1 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, *cox1* 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 *rbcL* 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 spermatangial branches, by which Chondrieae is characterized, are plesiomorphy rather than apomorphy.

The genus *Chondria* was also not supported in the present molecular trees (*rbcL* 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 *rbcL* 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 *Platycondria*. 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 *rbcL* 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 *cox1* tree had lower resolution than *rbcL* and SSU trees probably due to the high evolutionary rate of the *cox1* 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 *Husseyia*) of the Chondrieae have not been included in molecular phylogenetic studies. Considering the results of the present molecular 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: *rbcL*= MG255065, *SSU*= MG272243, *cox1*= 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 \times 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 *rbcL* 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 *rbcL*. 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 *rbcL* 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 *cox1* tree, the tribe Chondrieae was not supported, due to a lower resolution than those of the *rbcL* 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 throughout 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 (*rbcL* 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, Pukyong 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 longish 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: *rbcL*= MG255062, *SSU*= MG272240, *cox1*= 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 \times 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 thickenings 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). Tetrasporangia 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 thickenings 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, sessile, 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* (Kützinger) 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. lancifolia*, *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 *rbcL* 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 *rbcL* 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 *et 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 *et 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 (*rbcL*, 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.

References

- ABE T., KURIHARA A., KAWAGUCHI S., TERADA R. & MASUDA M. 2006. Preliminary report on the molecular phylogeny of the *Laurencia* complex (Rhodomelaceae). *Coastal Marine Science* 30: 209–213.
- ABBOTT I.A. & HOLLENBERG G.J. 1976. *Marine algae of California*. Stanford University Press. California. 827 pp.
- ADL S.M., SIMPSON A.G.B., FARMER M.A., ANDERSEN R.A., ANDERSON O.R., BARTA J.R., BOWSER S. S., BRUGEROLLE G., FENSOME R.A., FREDERICQ S., JAMES T. Y., KARPOV S., KRUGENS P., KRUG J., LANE C.E., LEWIS L.A., LODGE J., LYNN D.H., MANN D.G., MCCOURT R.M., MENDOZA L., MOESTRUP Ø., MOZLEY-STANDRIDGE S. E., NERAD T.A., SHEARER C. A., SMIRNOV A.V., SPIEGEL F. W. & TAYLOR M.F.J.R. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *The Journal of Eukaryotic Microbiology* 52: 399–451.
- ADL S.M., LEANDER B.S., SIMPSON A.G.B., ARCHIBALD J.M., ANDERSON O.R., BASS D., BOWSER S.S., BRUGEROLLE G., FARMER M.A., KARPOV S., KOLISKO M., LANE C.E., LODGE D.J., MANN D.G., MEISTERFELD R., MENDOZA L., MOESTRUP Ø., MOZLEY-STANDRIDGE S. E., SMIRNOV A.V. & SPIEGEL F. 2007. Diversity, nomenclature, and taxonomy of protists. *Systematic Biology* 56: 684–689.
- ADL S.M., SIMPSON A.G.B., LANE C.E., LUKES J., BASS D., BOWSER S.S., BROWN M.W., BURKI F., DUNTHORN M., HAMPL V., HEISS A., HOPPENRATH M., LARA E., LE GALL L., LYNN D.H., MCMANUS H., MITCHELL E.A.D., MOZLEY-STANDRIDGE S.E., PARFREY L.W., PAWLAWSKI J., RUECKERT S., SHADWICK L., SCHOCH C.L., SMIRNOV

- A. & SPIEGEL F. W. 2012. The revised classification of eukaryotes. *The Journal of Eukaryotic Microbiology* 59: 429–493.
- AGARDH C.A. 1817. *Synopsis algarum Scandinaviae*. Berling. Lund. 135 pp.
- ATEWEBERHAN M. & PRUD'HOMME VAN REINE W.F. 2005. A taxonomic survey of seaweeds from Eritrea. *Blumea* 50: 65–111.
- BACCI D.S. 2005. *Estudos taxonômicos do gênero Chondria (Ceramiales, Rhodophyta) no litoral dos estados de São Paulo de Espírito Santo, Brazil. São Paulo*. Unpublished MS thesis. Instituto de Botânica da Secretaria de Estado do Meio Ambiente. São Paulo. 114 pp.
- BARBARA I., CHOI H.-G., SECILLA A., DIAZ-TAPIA P., GOROSTIAGA J.M., SEO T.-K., JUNG M.-Y. & BERECIBAR E. 2013. *Lampisiphonia iberica* gen. et sp. nov. (Ceramiales, Rhodophyta) based on morphology and molecular evidence. *Phycologia* 52: 137–155.
- BHATTACHARYA D & MEDLIN L. 1995. The phylogeny of plastids: a review based on comparisons of small subunit ribosomal RNA coding regions. *Journal of Phycology* 31: 489–498.
- Bold H.C. & Wynne M.J. 1985. *Introduction to the algae*. 2nd Edition. Prentice-Hall, Inc. Englewood Cliffs. New Jersey. 720 pp.
- BØRGESEN F. 1924. Marine algae from Easter Island. In: *The Natural History of Juan Fernandez and Easter Island* (Ed. by C. Skottberg) 2: 249–309.
- BUSTAMANTE D.E., WON B.Y., MILLER K.A. & CHO T.O. 2017. *Wilsonosiphonia* gen. nov. (Rhodomelaceae, Rhodophyta) based on molecular and morpho-anatomical characters. *Journal of Phycology* 53: 368–380.

- CASSANO V., OLIVEIRA M.C., GIL-RODIGUEZ C., SENTIEZ A., DIAZ-LARREA J. & FUJII M.T. 2012. Molecular support for the establishment of the new genus *Laurenciella* within the *Laurencia* complex (Ceramiales, Rhodophyta). *Botanica Marina* 55: 349–357.
- COLLINS F.S. & HERVEY A.B. 1917. The algae of Bermuda. *Proceedings of the American Academy of Arts and Sciences* 53: 1–195.
- CREED M., FUJII, M.T., BARRETO M.B. DE B., GUIMARÃES S.M.P. DE B., CASSANO V., PEREIRA S.M.B., CARVALHO M. DE F. DE O & KHADER S. 2010. Rhodophyceae. In: *Catálogo de plantas e fungos do Brasil. Vol. 1.* (Ed. by R.C. Forzza). Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Rio de Janeiro. 873 pp.
- DAWSON E.Y. 1963. Marine red algae of Pacific Mexico 8. *Nova Hedwigia* 6: 441–480.
- DAWSON E.Y. & TÖZÜN B. 1964. The structure and reproduction of the red alga *Chondria nidifica* Harvey. *Transactions of the San Diego Society of Natural History* 13: 285–300.
- DE JONG Y.S.D.M., HITIPEUW C. & PRUD'HOMME VAN REINE W. F. 1999. A taxonomic, phylogenetic and biogeographic study of the genus *Acanthophora* (Rhodomelaceae, Rhodophyta). *Blumea* 44: 217–249.
- DE TONI G.B. 1903. *Sylloge algarum omnium hucusque cognitarum. Vol. 4. Florideae. Sect. 3.* Padua. Italy. 775–1522 pp.
- DIAZ-TAPIA P., MAGGS C.A., WEST J.A. & VERBRUGGEN H. 2017. Analysis of chloroplast genomes and a supermatrix inform reclassification of the Rhodomelaceae (Rhodophyta). *Journal of Phycology* 53: 920–937.

- DICKINSON C.I. 1963. *British seaweeds*. The Kew Series. Eyre & Spottiswoode. London. 232 pp.
- Dixon P. S. 1973. *Biology of the Rhodophyta*. Oliver & Boyd. Edinburgh. 285 pp.
- FALKENBERG P. 1901. Die Rhodomelaceen des Golfes von Neapel und der angrenzenden Meeres-abschnitte. *Fauna und Flora des Golfes von Naepel und der angrenzenden Meeres-abschnitte* 25: 1–754.
- FRANCIS C., BOLTON J.J., MATTIO L., MANDIWANA-NEUDANI T. & ANDERSON R.J. 2017. Molecular systematics reveals increased diversity within the South African *Laurencia* complex (Rhodomelaceae, Rhodophyta). *Journal of Phycology* 53: 804–819.
- FRESHWATER D.W. & RUENESS J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33: 187–194.
- FRESHWATER D.W. 2000. Florideophyceae. Version 24 March 2000. <http://tolweb.org/Florideophyceae/21781/2000.03.24> in The Tree of Life Web Project, <http://tolweb.org>; searched on February 2018.
- GÓMEZ GARRETA A., GALLARDO T., RIBERA M.A., CORMACI M., FURNARI G., GIACCONE G. & BOUDOURESQUE C.-F. 2001. Checklist of the Mediterranean seaweeds. III. Rhodophyceae Rabenh. 1. Ceramiales Oltm. *Botanica Marina* 44: 425–460.
- GORDON-MILLS E. 1987. Morphology and taxonomy of *Chondria tenuissima* and *Chondria dasyphylla* (Rhodomelaceae, Rhodophyta) from European waters. *British Phycological Journal* 22: 237–255.
- GUIRY M. D. 2012. *A catalogue of Irish seaweeds*. A.R.G. Gantner Verlag, K.G., Ruggell. Liechtenstein. 250 pp.

- GUIRY M.D. & GUIRY G.M. 2018. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on February 2018.
- HARDY F.G. & GUIRY M.D. 2003. *A check-list and atlas of the seaweeds of Britain and Ireland*. British Phycological Society. London. 435 pp.
- HARVEY W.H. 1853. Nereis Boreali-Americana. Part II. Rhodospermeae. *Smithsonian Contributions to Knowledge* 5: 1–258, pls. XIII–XXXVI.
- HARVEY W.H. 1858. Nereis Boreali-Americana. Part III. Chlorospermeae. *Smithsonian Contributions to Knowledge* 10: 1–140, pls. XXXVII–L.
- HARVEY W.H. 1860. Characters of new algae, chiefly from Japan and adjacent regions, collected by Charles Wright in the North Pacific Exploring Expedition under Captain James Rodgers. *Proceedings of the American Academy of Arts and Sciences* 4: 327–335.
- HOLLENBERG G.J. 1945. New marine algae from southern California. *American Journal of Botany* 32: 447–451.
- HOLMES E.M. 1896. New marine algae from Japan. *Journal of the Linnean Society of London, Botany* 31: 248–260.
- HOMMERSAND M.H. 1963. The morphology and classification of some Ceramiaceae and Rhodomelaceae. *University of California Publications in Botany* 35: 165–366.
- HOOKE J.D. & HARVEY W.H. 1845. Algae novae zelandiae. *London Journal of Botany* 4: 521–551.
- HUDSON W. 1778. *Flora anglica*, ed. 2. Privately published. London. 690 pp.

- JOHN D.M. & AL-THANI R.F. 2014. Benthic marine algae of the Arabian Gulf: a critical review and analysis of distribution and diversity patterns. *Nova Hedwigia* 98: 341–392.
- JOHN D.M., PRUD'HOMME VAN REINE W.F., LAWSON G.W., KOSTERMANS T.B. & PRICE J.H. 2004. A taxonomic and geographical catalogue of the seaweeds of the western coast of Africa and adjacent islands. *Beihefte zur Nova Hedwigia* 127: 1–339.
- KOGAME K., UWAI S., ANDERSON R. J., CHOI H.-G. & BOLTON J. J. 2017. DNA barcoding of South African geniculate coralline red algae (Corallinales, Rhodophyta). *South African Journal of Botany* 108: 337–341.
- KUCERA H. & SAUNDERS G.W. 2012. A survey of Bangiales (Rhodophyta) based on multiple molecular markers reveals cryptic diversity. *Journal of Phycology* 48: 869–882.
- KURIHARA A., ABE T., TANI M. & SHERWOOD A.R. 2010. Molecular phylogeny and evolution of red algal parasites: A case study of *Benzaitania*, *Janczewskia*, and *Ululania* (Ceramiales). *Journal of Phycology* 46: 580–590.
- KYLIN H. 1928. Entwicklungsgeschichtliche Florideen studien. *Acta Universitatis Lundensis* 24: 1–127.
- KYLIN H. 1941. Californische Rhodophyceen. *Acta Universitatis Lundensis* 372: 1–51.
- KYLIN H. 1956. *Die Gattungen der Rhodophyceen*. Gleerups. Lund. 673 pp.
- LEE H. B. & OH Y. S. 1986. A summer algal vegetation in Youngil Bay, eastern coast of Korea. *Korean Journal of Phycology* 1: 225–240.
- LEE Y.-P. & YOON S.Y. 1996. Taxonomy of *Chondria* (Rhodophyta) in Korea. *Algae* 11: 107–139.
- LEE R.E. 1999. *Phycology*. 3rd Edition. Cambridge University Press. Cambridge. 614 pp.

- LE GALL L. & SAUNDERS G.W. 2010. DNA barcoding is a powerful tool to uncover algal diversity: a case study of the Phyllophoraceae (Gigartinales, Rhodophyta) in the Canadian flora. *Journal of Phycology* 46: 374–89.
- LEWIS J.E. & NORRIS J.N. 1987. A history and annotated account of the benthic marine algae of Taiwan. *Smithsonian Contributions to Marine Sciences* 29: 1–38.
- LUDWIG G. & SCHNITTLER M. 1996. Rote Liste gefährdeter Pflanzen Deutschlands. *Schriftenreihe für Vegetationskunde* 28: 1–744.
- MAGGS C.A. & HOMMERSAND M.H. 1993. *Seaweeds of the British Isles. Vol. 1 Rhodophyta, Part 3A Ceramiales*. The Natural History Museum. London. XV. 444 pp.
- MARTIN-LESCANNE J., ROUSSEAU F., REVIERS B.D., PAYRI C., COULOUX A., CRUAUD C. & LE GALL L. 2010. Phylogenetic analyses of the *Laurencia* complex (Rhodomelaceae, Ceramiales) support recognition of five genera: *Chondrophycus*, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua* stat. nov. *European Journal of Phycology* 45: 51–61.
- METTI Y., MILLAR A.J.K. & STEINBERG P. 2015. A new molecular phylogeny of the *Laurencia* complex (Rhodophyta, Rhodomelaceae) and a review of key morphological characters result in a new genus, *Coronaphycus*, and a description of *C. novus*. *Journal of Phycology* 51: 929–942.
- MILLAR A.J.K. & WYNNE M.J. 1992. *Chondria viticulosa* sp. nov. (Rhodomelaceae, Rhodophyta), a distinctly flattened species from south-eastern Queensland, Australia. *Australian Systematic Botany* 5: 421–429.
- Miller K.A. 2012. *Seaweeds of California. Updates of California Seaweed Species List*. University of California. Berkeley. 59 pp.

- MNTANGI M.J. & FARRAR J.F. 1978. Physiological ecology of the marine alga *Acrocystis nana*. *New Phytologist* 80: 199–208.
- MOREIRA D, LE GUYADER H. & PHILIPPE H. 2000. The origin of red algae and the evolution of chloroplasts. *Nature* 405: 69–72.
- NAM K.W., MAGGS C.A., MCIVOR L. & STANHOPE M.J. 2000. Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *Journal of Phycology* 36: 759–772.
- NAM K.W. 2006. Phylogenetic re-evaluation of the *Laurencia* complex (Rhodophyta) with a description of *L. succulenta* sp. nov. from Korea. *Journal of Applied Phycology* 18: 679–697.
- NETO A.I. 1994. Checklist of the benthic marine macroalgae of the Azores. *Arquipélago* 12A: 15–34.
- NORRIS R.E. 1988. Structure and tetrasporangial reproduction in *Acrocystis* (Rhodomelaceae, Rhodophyta), newly reported for South Africa. *South African Journal of Botany* 54: 633–635.
- NOZAKI H., MATSUZAKI M., TAKAHARA M., MISUMI O., KUROIWA H., HASEGAWA M., SHIN-IT., KOHARA Y., OGASAWARA N. & KUROIWA T. 2003. The phylogenetic position of red algae revealed by multiple nuclear genes from mitochondria-containing eukaryotes and an alternative hypothesis on the origin of plastids. *Journal of Molecular Evolution* 56:485–497.
- OKAMURA K. 1907. *Icones of Japanese algae*. Vol. 1. Tokyo. pp. 65–92, pl. 19-20.
- OKAMURA K. 1909. *Icones of Japanese algae*. Vol. 1. Tokyo. pp. 12–15, pl. 3.
- OKAMURA K. 1912. *Icones of Japanese algae*. Vol. 2. Tokyo. pp. 167–186, pl. 99.

- OKAMURA K. 1927. *Icones of Japanese algae*. Vol. 5. Tokyo. pp. 159–180, pl. 241–245.
- OKAMURA K. 1935. *Icones of Japanese algae*. Vol. 7. Tokyo. pp. 39–48, pl. 321–325.
- OKAMURA K. 1936. *Nippon Kaiso Shi*. Uchidarokakuho. Tokyo. 964 pp. (in Japanese).
- PERESTENKO L.P. 1980. *Vodorosli Zaliva Petra Velikogo* (The seaweeds of Peter the Great Bay). Leningrad. 147 pp.
- POSADA D. & CRANDALL K.A. 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50: 580–601.
- RAMÍREZ M.E. & SANTELICES B. 1991. Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica. *Monografías Biológicas* 5: 1–437.
- ROBBA L., RUSSELL S. J., BARKER G. L. & BRODIE, J. 2006. Assessing the use of the mitochondrial *cox1* marker for use in DNA barcoding of red algae (Rhodophyta). *American Journal of Botany* 93: 1101–1108.
- RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D., DARLING A., HOHNA S., LARGET B., LIU L., SUCHARD M. A. & HUELSENBECK J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- ROUSSEAU F., GEY D., KURIHARA A., MAGGS C.A., MARTIN-LESCANNE J., PAYRI C., REVIERS V.D., SHERWOOD A.R. & LE GALL L. 2017. Molecular phylogenies support taxonomic revision of three species of *Laurencia* (Rhodomelaceae, Rhodophyta), with the description of a new genus. *European Journal of Taxonomy* 269: 1–19.
- SAENGER P., DUCKER S.C. & ROWAN K.S. 1971. Two species of Ceramiales from Australia and New Zealand. *Phycologia* 10: 105–111.

- SAUNDERS G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society London B: Biological Sciences* 360: 1879–1888.
- SCHMITZ F. & FALKENBERG P. 1897. Rhodomelaceae. In: *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen unter Mitwirkung zahlreicher hervorragender Fachgelehrten, Teil 1, Abteilung 2*. Englemann. Leipzig. pp. 421–480.
- SCHNEIDER C.W. 2003. An annotated checklist and bibliography of the marine macroalgae of the Bermuda Islands. *Nova Hedwigia* 76: 275–361.
- SETCHELL W. A. & GARDNER N.L. 1924. New marine algae from the Gulf of California. *Proceeding of the California Academy of Science* 12: 695–949.
- SHIMADA S. 2000. *A systematic study of the order Gelidiales (Rhodophyta) from Japan*. Doctoral thesis. Hokkaido University. 83 pp.
- SILVA P.C. 1972. Remarks on algal nomenclature V. *Taxon* 21: 199–212.
- SILVA P.C., BASSON P.W. & MOE R. L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *University of California Publications in Botany* 79: 1–1259.
- STAMATAKIS A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- STEGENGA H., BOLTON J.J. & ANDERSON R.J. 1997. *Seaweeds of the southern African west coast*. Bolus Herbarium. University of Cape Town. Cape Town. 655 pp.
- SUÁREZ A.M. 2005. Lista de las macroalgas marinas Cubanas. *Revista de Investigaciones Marinas* 26: 93–148.

- SUTTI S., TANI M., YAMAGISHI Y., ABE T., MILLER K.A. & KOGAME K. 2018.
Neochondria gen. nov. (Rhodomelaceae, Rhodophyta), a segregate of *Chondria*, including *N. ammophila* sp. nov. and *N. nidifica* comb. nov. *Phycologia*: In press.
Accepted at 24 November 2017.
- TAMURA K., STECHER G., PETERSON D., FILIPSKI A. & KUMAR S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
- TANAKA T. 1965. Studies on some marine algae from southern Japan - VI. *Memoirs of the Faculty of Fisheries, Kagoshima University* 14: 52–71.
- TANI M. & MASUDA M. 2003. A taxonomic study of two minute species of *Chondria* (Ceramiales, Rhodophyta) from the north-western Pacific, with the description of *Chondria econstricta* sp. nov. *Phycologia* 42: 220–231.
- TANI M., YAMAGISHI Y., MASUDA M., KOGAME K., KAWAGUCHI S. & PHANG S.M. 2003. Taxonomic notes on marine algae from Malaysia. IX. Four species of Rhodophyceae, with the description of *Chondria decidua* sp. nov. *Botanica Marina* 46: 24–35.
- TAYLOR W.R. 1960. *Marine algae of the eastern tropical and subtropical coasts of the Americas*. University of Michigan Press. Ann Arbor. ix + 870 pp.
- TITLYANOV E.A. & TITLYANOV T.V. 2012. *Marine plants of the Asian Pacific region countries, their use and cultivation*. Dalnauka & A.V. Zhirmunsky Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences. Vladivostok. 376 pp.

- TSUDA R.T. & WALSH S.K. 2013. Bibliographic checklist of the marine benthic algae of Central Polynesia in the Pacific Ocean (excluding Hawai'i and French Polynesia). *Micronesica* 2013-02: 1–91.
- TURNER D. 1808. *Fuci sive Plantarum Fucorum*, Vol. 1. Mccreery. London. 164 pp.
- WEBER VAN BOSSE A. 1923. *Liste des Algues du Siboga*, II. Rhodophyceae, 2e partie. Ceramiales. Brill. Leiden. pp. 311–392.
- WITHERING W. 1796. *An arrangement of British plants*, ed. 3. Privately published. London. 402 pp.
- WOMERSLEY H.B.S. & BAILEY A. 1970. Marine algae of the Solomon Islands. *Philosophical Transactions of the Royal Society of London. B. Biological Sciences* 259: 257–352.
- WOMERSLEY H.B.S. 2003. *The marine benthic flora of southern Australia, Part IIID*. Australian Biological Resources Study, Canberra and the State Herbarium of South Australia. Canberra. 533 pp.
- WOODWARD T.J. 1794. Description of *Fucus dasyphyllus*. *Transactions of the Linnean Society of London* 2: 239–241.
- WYNNE M.J. 1986. Report on a collection of benthic marine algae from the Namibian coast (southwestern Africa). *Nova Hedwigia* 43: 311–355.
- WYNNE M.J. 1991. A change in the name of the type of *Chondria* C. Agardh (Rhodomelaceae, Rhodophyta). *Taxon* 40: 316–318.
- WYNNE M.J. 2011. A checklist of benthic marine algae of the tropical and subtropical western Atlantic: third revision. *Nova Hedwigia, Beihefte* 140: 1–166.

- YAMADA Y. 1935. Notes on some Japanese algae VI. *Scientific Papers of the Institute of Algological Research, Faculty of Science, Hokkaido Imperial University* 1: 27–35.
- YANG M.Y. & KIM M.S. 2015. Taxonomy of Grateloupia (Halymeniales, Rhodophyta) by DNA barcode marker analysis and a description of *Pachymeniopsis volvita* sp. nov. *Journal of Applied Phycology* 27:1373–1384.
- YOSHIDA T., NAKAJIMA Y. & NAKATA Y. 1990. Check-list of marine algae of Japan (revised in 1990). *The Japanese Journal of Phycology* 38: 269–320.
- YOSHIDA T. 1998. *Marine algae of Japan*. Uchida-Rokakuho Tokyo 1222 pp. (in Japanese).
- YOSHIDA T., SUZUKI M. & YOSHINAGA K. 2015. Checklist of marine algae of Japan (Revised in 2015). *The Japanese Journal of Phycology* 63: 129–189.

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 | <i>rbcL</i> | SSU | <i>cox1</i> |
|------------------------------|---|------------|------------------|-------------|----------|-------------|
| <i>Acrocystis nana</i> | Okinawa, Japan (2 March 1997) | Silica gel | SAP115399 | MG843864 | -- | MG843856 |
| <i>Chondria armata</i> | Malaysia (26 May 1999) | Silica gel | SAP115358 | MG255050 | -- | MG255068 |
| <i>Chondria armata</i> | Kagoshima, Japan (02 August 1997) | Silica gel | SAP115359 | MG255051 | -- | -- |
| <i>Chondria capillaris</i> | Finavarra, Ireland (17 August 2004) | Silica gel | SAP106295 | MG255052 | -- | -- |
| <i>Chondria crassicaulis</i> | Innoshima, Hiroshima, Japan (20 April 2015) | Silica gel | SAP115360 | MG255053 | -- | MG255069 |
| <i>Chondria crassicaulis</i> | Koinoura, Fukuoka, Japan (23 March 2015) | Silica gel | SAP115361 | MG255054 | -- | MG255070 |
| <i>Chondria crassicaulis</i> | Oshoro, Hokkaido, Japan (24 June 2015) | Silica gel | SAP115362 | MG255055 | MG272238 | MG255071 |

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

| | | | | | | |
|--|--|------------|-----------|----------|----------|----------|
| <i>Chondria acuminata</i> (Japanese ' <i>C. dasyphylla</i> ') | Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017) | Silica gel | SAP115390 | MG843866 | MG831940 | MG843858 |
| <i>Chondria acuminata</i> (Japanese ' <i>C. dasyphylla</i> ') | Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017) | Silica gel | SAP115391 | MG843867 | MG831941 | MG843859 |
| <i>Chondria cf. curdieana</i> (Japanese ' <i>C. dasyphylla</i> ') | Muroran, Hokkaido, Japan (21 August 2016) | Silica gel | SAP115392 | MG843868 | MG831942 | MG843860 |
| <i>Chondria cf. curdieana</i> (Japanese ' <i>C. dasyphylla</i> ') | Muroran, Hokkaido, Japan (22 August 2017) | Silica gel | SAP115395 | MG843869 | MG831943 | MG843861 |
| <i>Chondria cf. curdieana</i> (Japanese ' <i>C. dasyphylla</i> ') | Muroran, Hokkaido, Japan (22 August 2017) | Silica gel | SAP115396 | MG843870 | MG831944 | MG843862 |
| <i>Chondria</i> sp. 1 | Shiretoko, Hokkaido, Japan (21 August 2017) | Silica gel | SAP115397 | MG843871 | MG831945 | MG843863 |
| <i>Neochondria ammophila</i> (Japanese ' <i>C. capillaris</i> ') | Momonai, Otaru, Hokkaido (23 September 1996) | Silica gel | SAP115347 | MG255063 | MG272241 | -- |
| <i>Neochondria ammophila</i> (Japanese ' <i>C. capillaris</i> ') | Innoshima, Hiroshima, Japan (20 April 2015) | Silica gel | SAP115369 | MG255064 | MG272242 | MG272229 |

| | | | | | | |
|---|---|------------|-----------|----------|----------|----------|
| <i>Neochondria ammophila</i> (Japanese ' <i>C. capillaris</i> ') | Momonai, Hokkaido, Japan (29 June 2016) | Silica gel | SAP115370 | MG255065 | MG272243 | MG272230 |
| <i>Neochondria ammophila</i> (Japanese ' <i>C. capillaris</i> ') | Muroran, Hokkaido, Japan (26 July 2016) | Silica gel | SAP115371 | MG255066 | MG272244 | MG272231 |
| <i>Neochondria nidifica</i> (= <i>Chondria nidifica</i>) | 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 |
|-------------|---------------------|---------|-----------------------------|-----------------------------|
| <i>rbcL</i> | F8 | Forward | GGTGAATTCCATACGCTAAAATG | Abe <i>et al</i> (2006) |
| | R753 | Reverse | GCTCTTTCATACATATCTTCC | Freshwater & Rueness (1994) |
| | F605 | Forward | CCATTTTCATGCGTTGGAAAGAAAGAT | Shimada (2000) |
| | RH5 | Reverse | TAGAAACTCCAACAGCTTACGTTTAA | Abe <i>et al</i> (2006) |
| <i>cox1</i> | GazF1 | Forward | TCAACAAATCATAAAGATATTGG | Saunders (2005) |
| | GazR1 | Reverse | ACTTCTGGATGTCCAAAAAYCA | Saunders (2005) |
| SSU | SRrh1 | Forward | GCTTGTCTCAAAGACTAAGCC | This study |
| | SRrh5 | Reverse | GCCAAAATCCGACTACGAGC | This study |
| | SRrh4 | Forward | ACCAGCAGAGGGCAAGTCTG | This study |
| | SRrh9 | Reverse | CCTATTTAGCACGCCAGGT | This study |
| | SRrh8 | Forward | GGAAAACCTACCAGGTCCAG | This study |
| | SRrh12 | Reverse | CCTTCTGCAGGTTACCTAC | This study |

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

| Species | Location; Collection date | GenBank accession number | | |
|----------------------------------|--|--------------------------|----------|-------------|
| | | <i>rbcL</i> | SSU | <i>cox1</i> |
| <i>Acanthophora pacifica</i> | USA, Hawaii; 24-Jan-2008 | | GU223750 | |
| <i>Acanthophora pacifica</i> | USA, Hawaii; -- | | | HQ422947 |
| <i>Acanthophora spicifera</i> | USA, Oahu; 11-Mar-2008 | | GU223753 | |
| <i>Acanthophora spicifera</i> | Japan, Okinawa; 03-Feb-2002 | | GU223763 | |
| <i>Acanthophora spicifera</i> | USA, HI, Kihei, Maui; 05-Apr-2006 | GQ252538 | | |
| <i>Acanthophora spicifera</i> | USA, Hawaii; -- | | | HQ422873 |
| <i>Acrocystis nana</i> | Japan, Nagasaki; 01-Jun-1999 | | GU223764 | |
| <i>Alsidium cymatophilum</i> | USA, Oahu; 19-Apr-2008 | | GU223765 | |
| <i>Benzaitenia yenoshimensis</i> | Japan, Chiba; 21-Apr-2008 | | GU223735 | |
| <i>Benzaitenia yenoshimensis</i> | Japan, Kashiwazaki; 03-May-2008 | | | GU223853 |
| <i>Benzaitenia yenoshimensis</i> | Japan, Kashiwazaki; 03-May-2008 | | | GU223854 |
| <i>Bostrychia moritziana</i> | Australia, Western Port Bay; 25-Oct-1986 | | AF203893 | |
| <i>Ceramium virgatum</i> | Spitsbergen, Kapp Thordsen; 11-Aug-2010 | | KP828754 | |

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

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

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

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

| | | |
|---------------------------------|-------------------------------------|----------|
| <i>Sonderella linearis</i> | Australia, Warrnambool; 12-Apr-1997 | AF203888 |
| <i>Tolypocladia glomerulata</i> | USA, Hawaii; 13-Apr-2008 | GU223799 |
| <i>Ululania stellata</i> | USA, Oahu, Hawaii; 11-Mar-2008 | GU223744 |
| <i>Ululania stellata</i> | 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 |
|--------------------------------|-------------------|-------------------------------------|-----------|
| <i>Chondria decipiens</i> | 26 September 1969 | Pacific grove, California, USA | UC1844102 |
| <i>Chondria decipiens</i> | 14 November 2012 | San Nicolas Island, California, USA | UC2025838 |
| <i>Chondria nidifica</i> | 27 January 1949 | Santa Rosa Island, California, USA | UC1022164 |
| <i>Chondria nidifica</i> | 20 February 1989 | Santa Rosa Island, California, USA | UC2036061 |
| <i>Chondria nidifica</i> | 12 December 2012 | Dana Point, California, USA | UC2026095 |
| <i>Chondria nidifica</i> | 6 January 2013 | San Nicolas Island, California, USA | UC2025809 |
| <i>Chondria capillaris</i> | 17 August 2004 | Frinavarra, Ireland | SAP115387 |
| ' <i>Chondria tenuissima</i> ' | 3 March 1932 | Himi, Toyama, Japan | SAP108861 |
| ' <i>Chondria tenuissima</i> ' | 18 May 1956 | Fukuoka, Japan | SAP094404 |
| ' <i>Chondria tenuissima</i> ' | 8 May 1962 | Noto, Ishikawa, Japan | SAP105508 |
| ' <i>Chondria tenuissima</i> ' | 21 July 1970 | Muroran, Hokkaido, Japan | SAP063400 |
| ' <i>Chondria tenuissima</i> ' | 20 July 1992 | Hokkaido, Japan | SAP097647 |
| ' <i>Chondria tenuissima</i> ' | 11 April 1993 | Hiroshima, Japan | SAP091695 |

| | | | |
|------------------------------|------------------|---------------------------|-----------|
| <i>'Chondria tenuissima'</i> | 21 May 2001 | Iyo, Ehime, Japan | SAP094422 |
| <i>'Chondria tenuissima'</i> | 14 July 2002 | Hakodate, Hokkaido, Japan | SAP110738 |
| <i>'Chondria tenuissima'</i> | 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 <i>‘C. capillaris’</i> (<i>Neochondria ammophila</i>) | <i>C. capillaris</i> ^{3,4} | <i>C. capensis</i> ⁵ | <i>C. decipiens</i> ^{1,6} | <i>C. nidifica</i> ^{1,2,6} (<i>Neochondria nidifica</i>) |
|--------------|--|---|---|--|--|
| 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 | <i>Neochondria</i> ⁹ | Chondrieae | | | | | |
|-------------------------------|--|---|---|--|-----------------------------------|--|---|
| | | <i>Chondria</i> ^{4,6,8,9} | <i>Acanthophora</i> ^{7,8} | <i>Acrocystis</i> ^{2,5} | <i>Cladhymenia</i> ^{1,3} | <i>Coeloclonium</i> ⁸ | <i>Husseyia</i> 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 pericentral cells | cut off from pericentral cells | cut off from pericentral cells | embedded in cortex | cut off from pericentral cells | cut off from pericentral 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 | = <i>Chondria acuminata</i> ; tetrasporophyte |
| Utoro, Hokkaido, Japan (9 August 1998) | SAP115401 | Pressed | = <i>Chondria acuminata</i> ; female gametophyte |
| Utoro, Hokkaido, Japan (30 July 1999) | SAP115402 | Pressed | = <i>Chondria acuminata</i> ; female gametophyte |
| Chashikotsu, Shiretoko, Hokkaido, Japan (11 November 2008) | SAP115389 | Pressed | = <i>Chondria acuminata</i> ; tetrasporophyte |
| Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017) | SAP115390 | Pressed | = <i>Chondria acuminata</i> ; tetrasporophyte |
| Shishi-iwa, Shiretoko, Hokkaido, Japan (21 August 2017) | SAP115391 | Pressed | = <i>Chondria acuminata</i> ; tetrasporophyte |

| | | | |
|--|-----------|---------|---|
| Muroran, Hokkaido, Japan (21 August 2016) | SAP115392 | Pressed | = <i>Chondria</i> cf. <i>curdieana</i> ; male and female gametophyte |
| Muroran, Hokkaido, Japan (21 August 2016) | SAP115394 | Pressed | = <i>Chondria</i> cf. <i>curdieana</i> ; male and female gametophyte |
| Muroran, Hokkaido, Japan (21 August 2017) | SAP115395 | Pressed | = <i>Chondria</i> cf. <i>curdieana</i> ; male gametophyte |
| Muroran, Hokkaido, Japan (21 August 2017) | SAP115396 | Pressed | = <i>Chondria</i> cf. <i>curdieana</i> ; female gametophyte |

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

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

| | | | |
|---------------------------------|-----------|--------------------------|------------|
| <i>'Chondria dasyphylla'</i> | 29-Jul-06 | Aburatsubo, Kanagawa | SAP103075 |
| <i>(Chondria acuminata)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 26-Aug-06 | Otaru, Hokkaido, Japan | SAP111274 |
| <i>(Chondria acuminata)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 25-Feb-09 | Mitoma, Fukuoka, Japan | SAP107367 |
| <i>(Chondria acuminata)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 5-Sep-33 | Akkeshi, Hokkaido, Japan | SAP112321 |
| <i>(Chondria cf. curdieana)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 5-Sep-33 | Akkeshi, Hokkaido, Japan | SAP112322 |
| <i>(Chondria cf. curdieana)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 5-Sep-33 | Akkeshi, Hokkaido, Japan | SAP112323 |
| <i>(Chondria cf. curdieana)</i> | | | |
| <i>'Chondria dasyphylla'</i> | 29-Jun-04 | Akkeshi, Hokkaido, Japan | SAP098589 |
| <i>(Chondria cf. curdieana)</i> | | | |
| <i>Chondria pellucida</i> | 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 | <i>C. acuminata</i> (Japanese ' <i>C. dasyphylla</i> ') | <i>C. cf. curdieana</i> (Japanese ' <i>C. dasyphylla</i> ') | <i>Chondria</i> sp. 1 | <i>Chondria chejuensis</i> | <i>Chondria curdieana</i> | <i>Chondria dasyphylla</i> | <i>Chondria pellucida</i> | <i>Chondria succulenta</i> |
|-------------------|--|---|--|--|--|-------------------------------|---|-------------------------------|
| 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 littoral zone | epiphytic on sea grass leaf | epilithic | epilithic in the lower tidal mark | epilithic or epiphytic on <i>Posidonia</i> 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 <i>C. dasyphylla</i>); pinnate laterals | tetrasporangia near ends of branchlets; on 2-3 pericentral cells per axial cells |
| Spermatangial organ | no data | discoïd; 1-2 marginal sterile layers with flattish edge | no data | discoïd with single marginal sterile layer | discoïd; 2 marginal sterile layers; sterile with flattish edge | discoïd 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 | discoïd 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 | <i>rbcL</i> , SSU, <i>cox1</i> | <i>rbcL</i> , SSU, <i>cox1</i> | <i>rbcL</i> , SSU, <i>cox1</i> | no data | no data | <i>rbcL</i> , 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 |

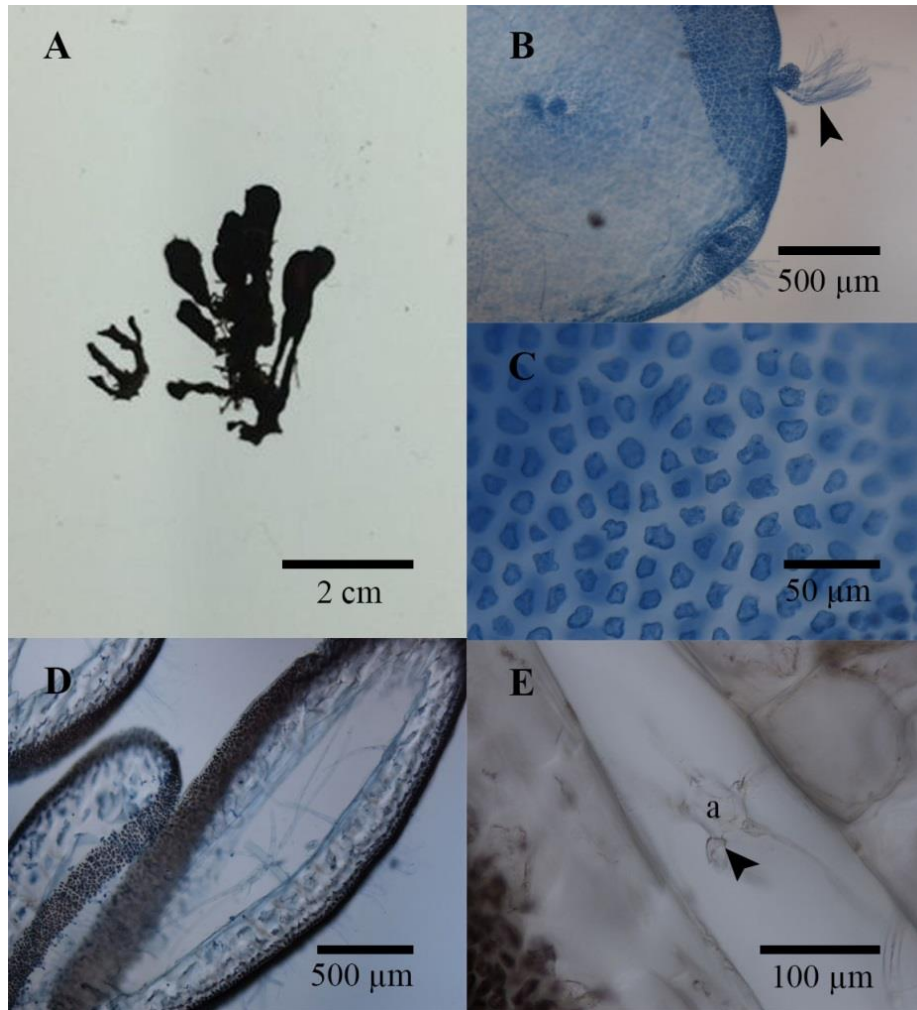


Fig. 1. Vegetative morphology of an examined *Acrocystis nana*.

A. *Acrocystis nana* (SAP115399, *rbcL* [MG843864], *cox1* [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.

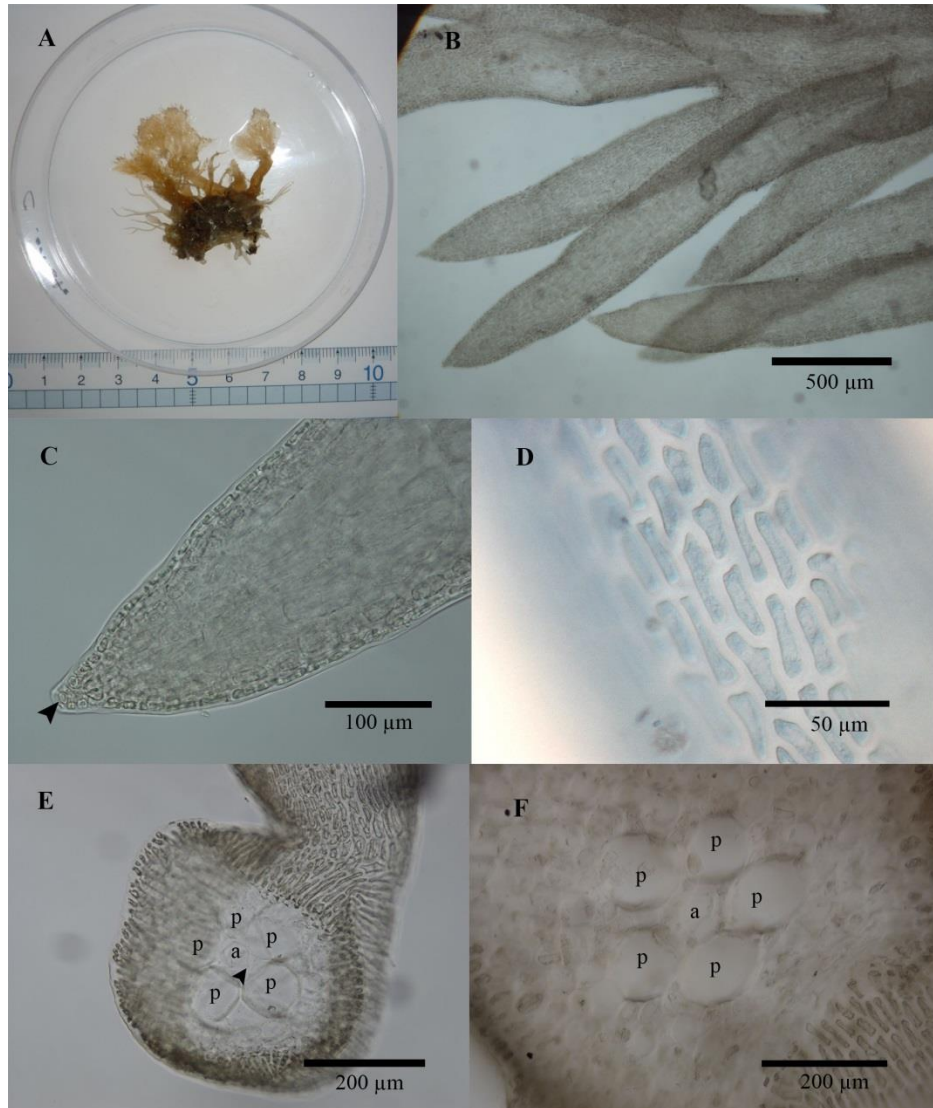


Fig. 2. Vegetative morphology of *Chondria armata*.

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

B. Unstricted, 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).

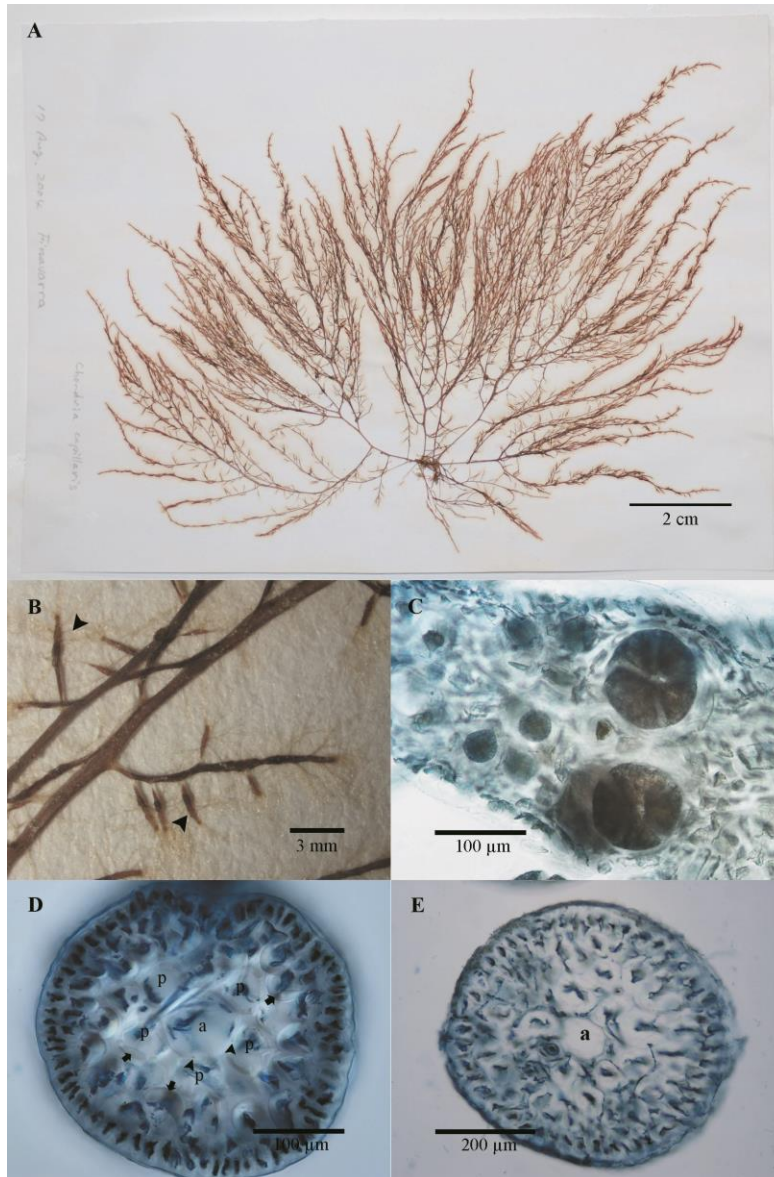


Fig. 3. Morphology of tetrasporophyte of an examined *Chondria capillaris*.

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.

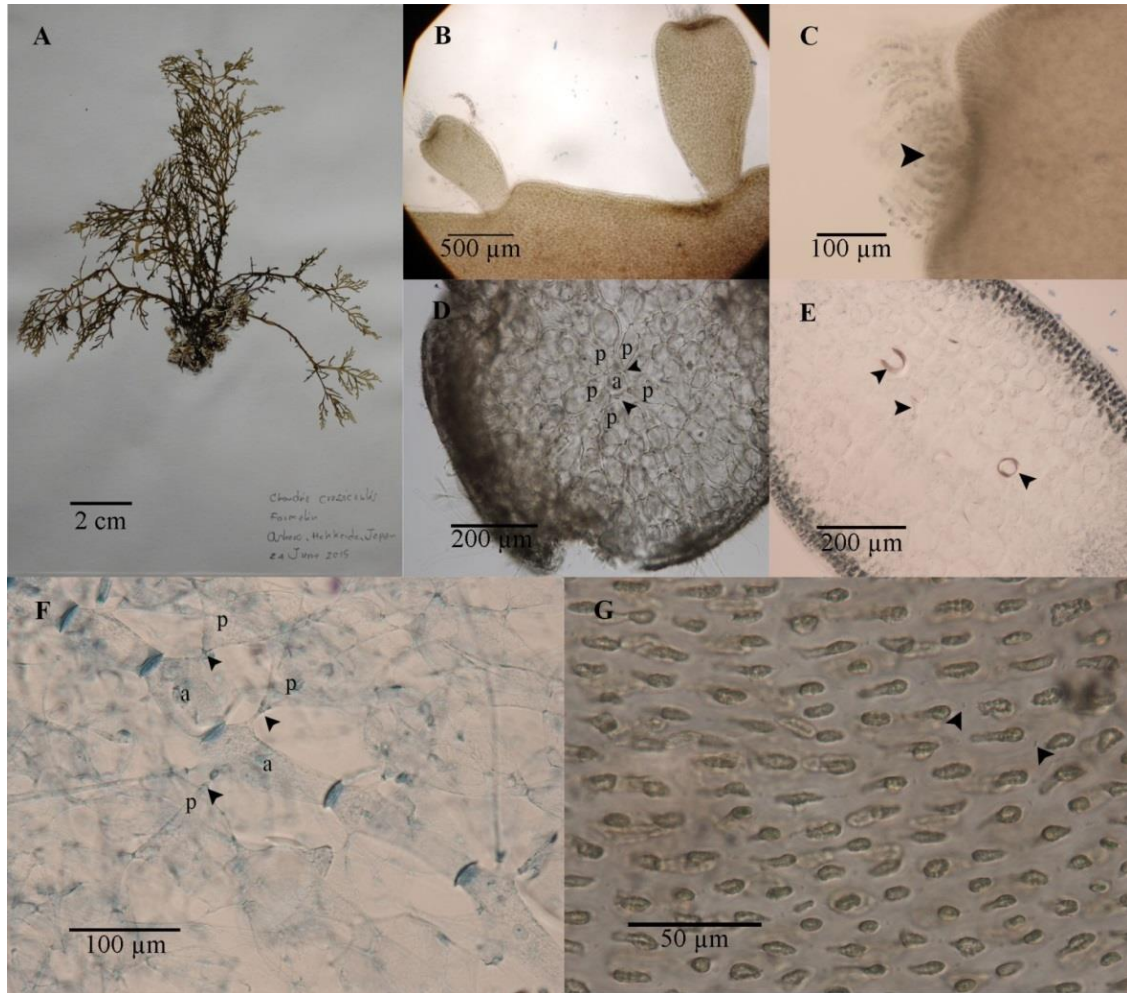


Fig. 4. Vegetative morphology of an examined *Chondria crassicaulis*.

A. *Chondria crassicaulis* (SAP115362, *rbcl* [MG255055], SSU [MG272238], *cox1* [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).

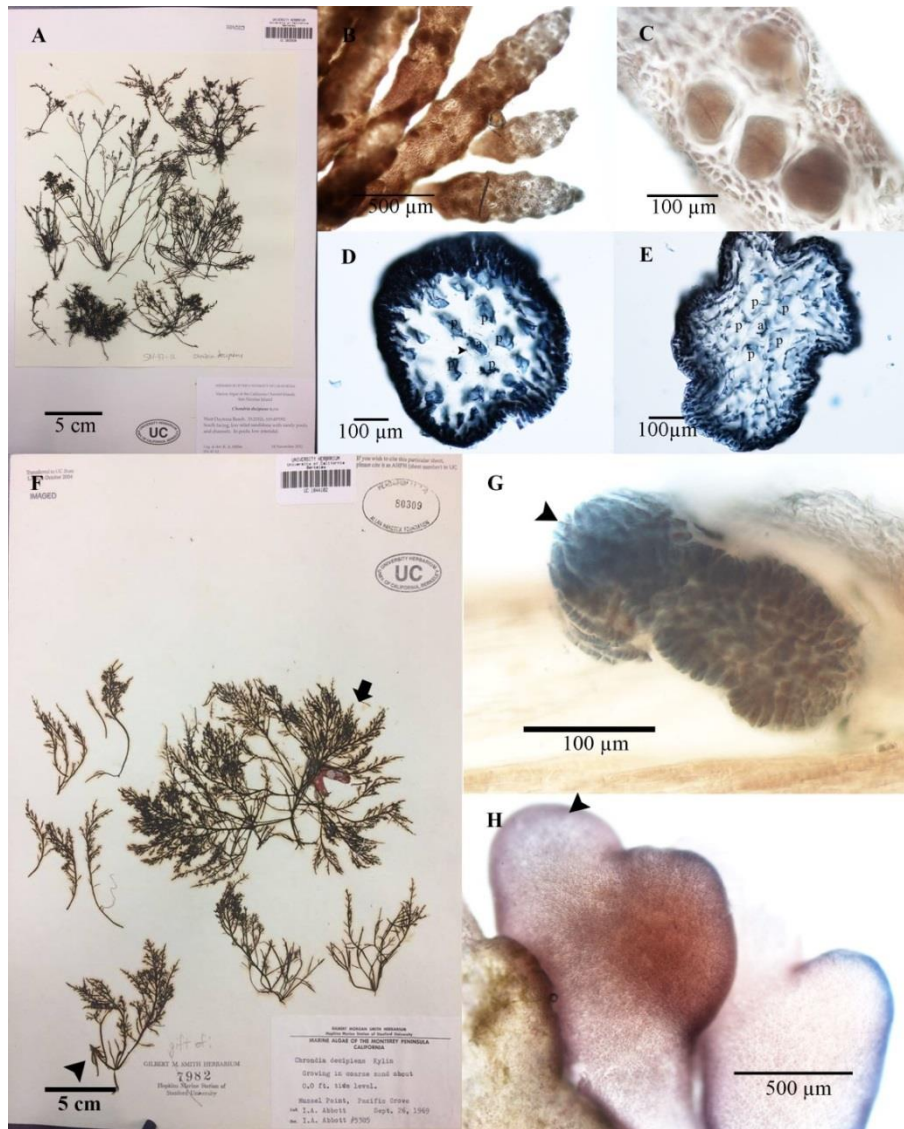


Fig. 5. Morphology of the examined *Chondria decipiens*.

A. *Chondria decipiens* (UC2025838, *rbcl* [MG255056], *cox1* [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.

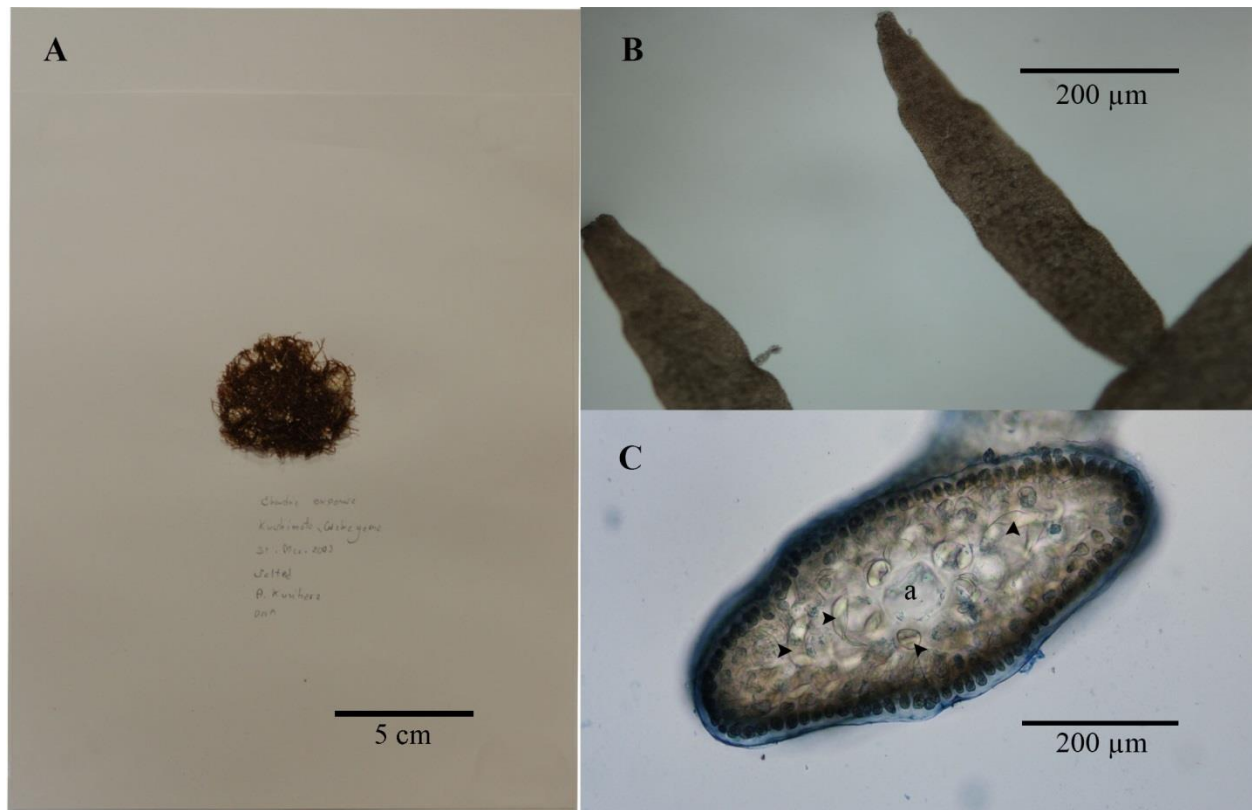


Fig. 6. Vegetative morphology of *Chondria expansa*.

A. *Chondria expansa* (SAP115365, *rbcL* [MG255057], *cox1* [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.

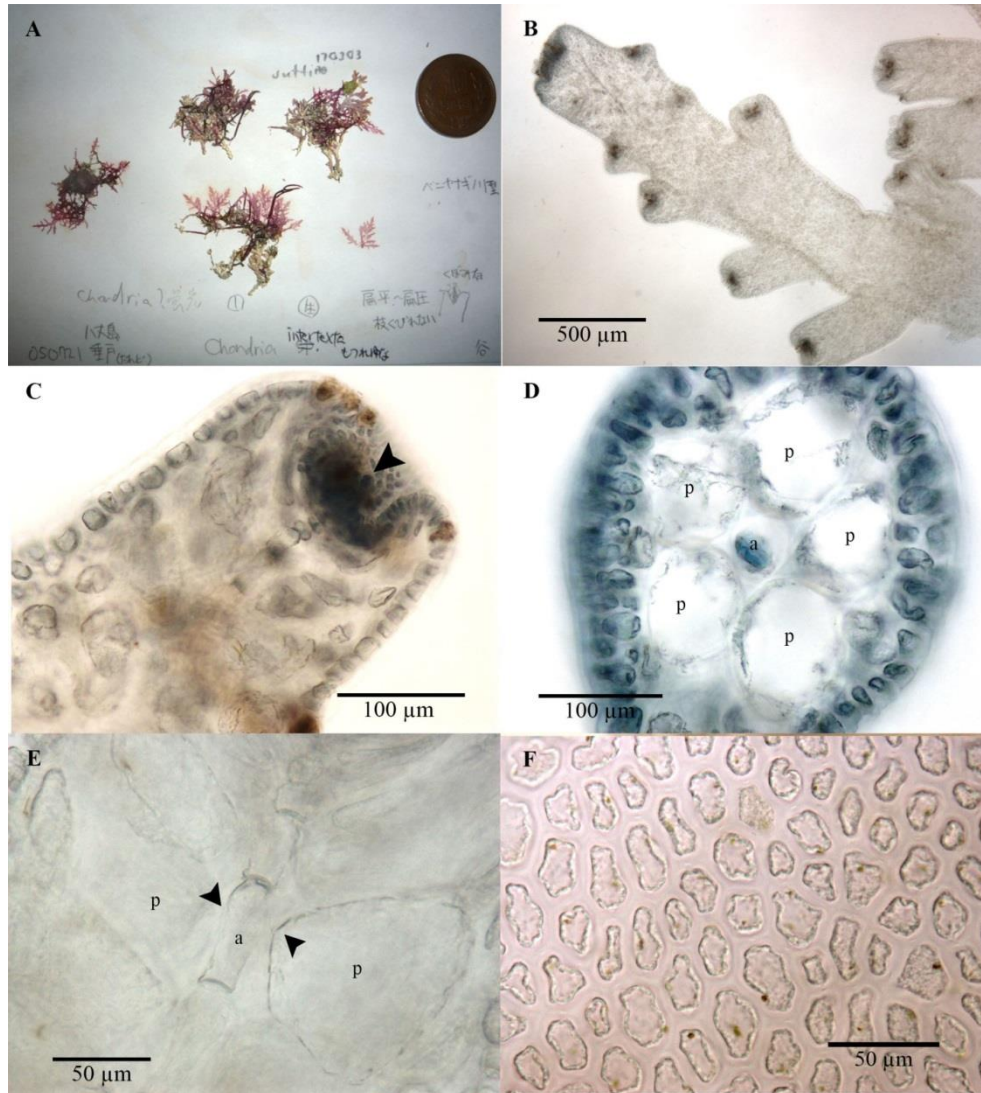


Fig. 7. Vegetative morphology of *Chondria intertexta*.

A. *Chondria intertexta* (SAP115364, *rbcL* [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.

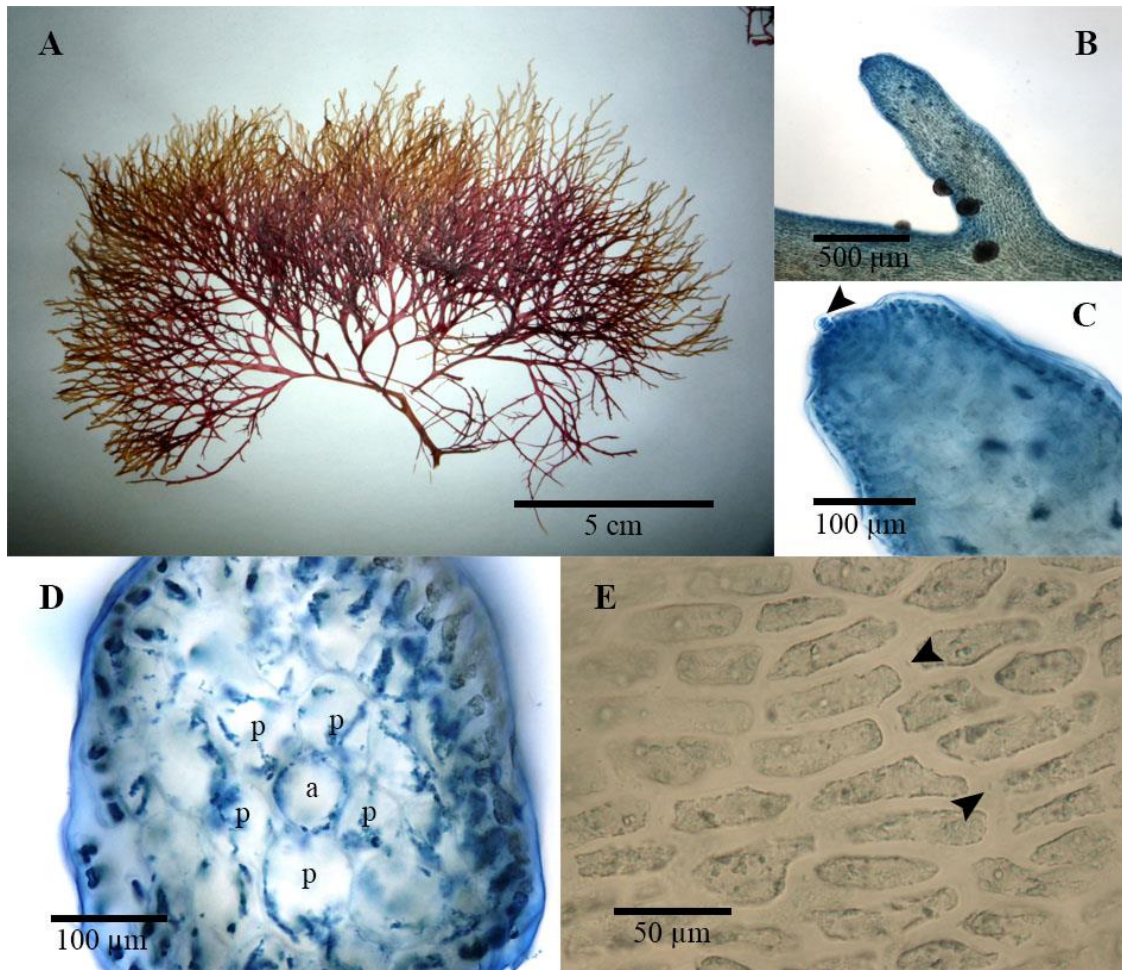


Fig. 8. Vegetative morphology of *Chondria mageshimensis*.

A. *Chondria mageshimensis* (SAP115367, *rbcL* [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.

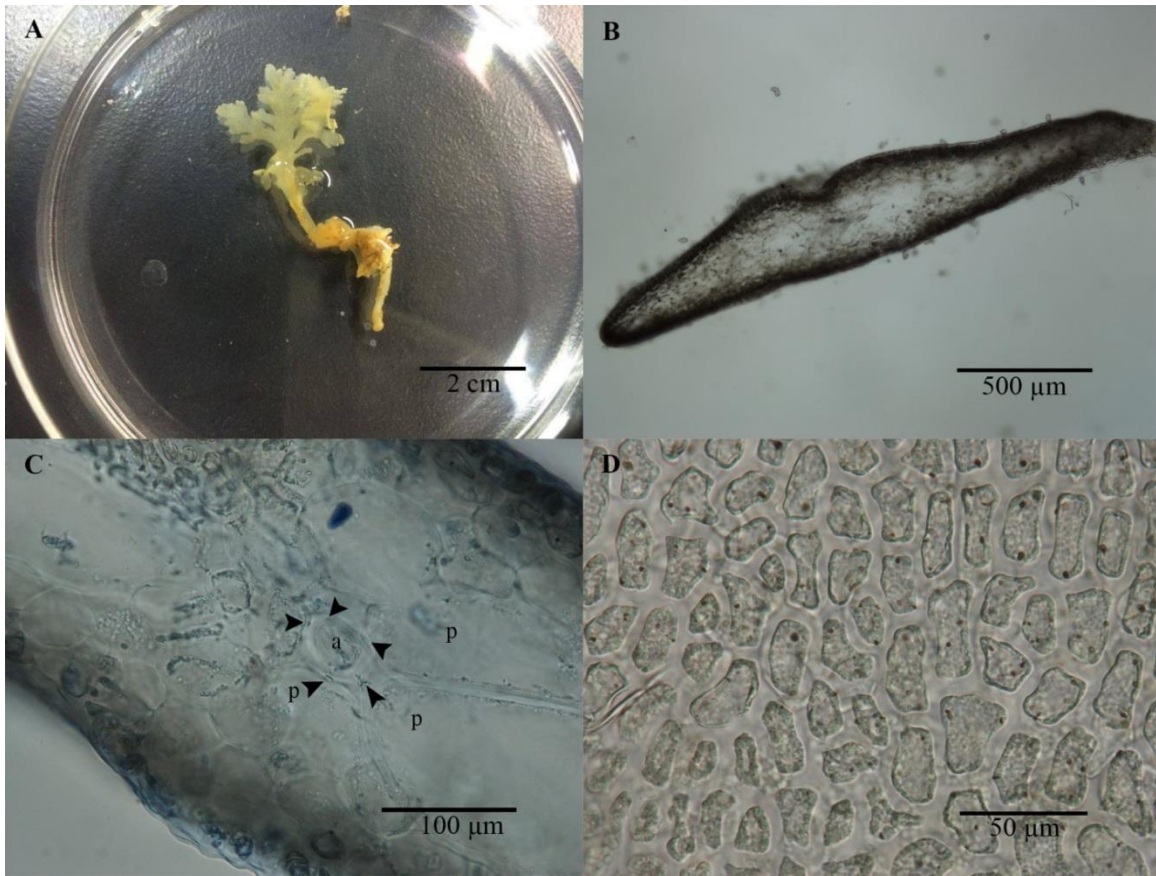


Fig. 9. Vegetative morphology of *Chondria ryukyuensis*

A. *Chondria ryukyuensis* (SAP115368, *rbcL* [MG255061], *cox1* [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.

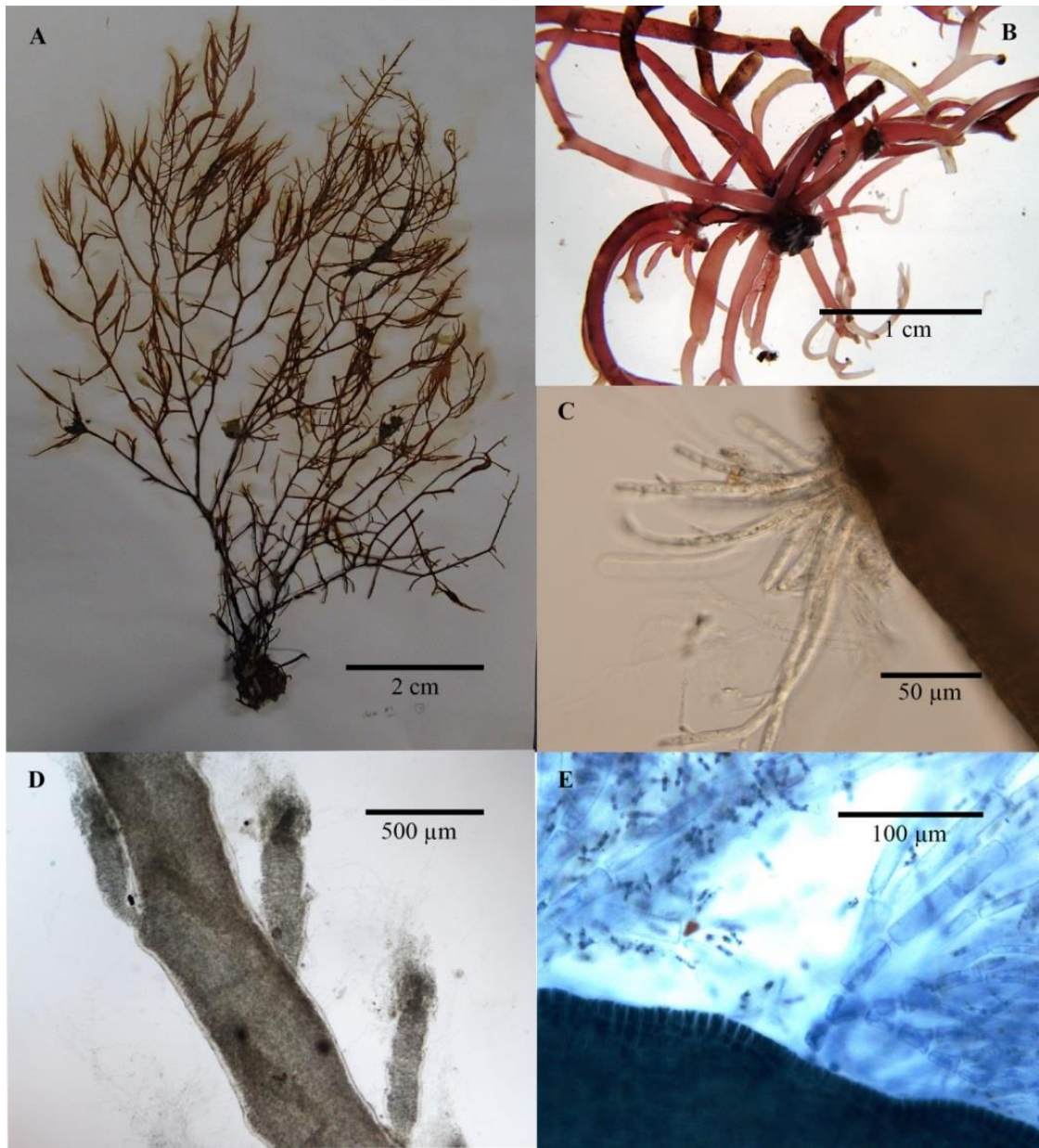


Fig. 10. Vegetative morphology of *Neochondria ammophila* sp. nov.

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.

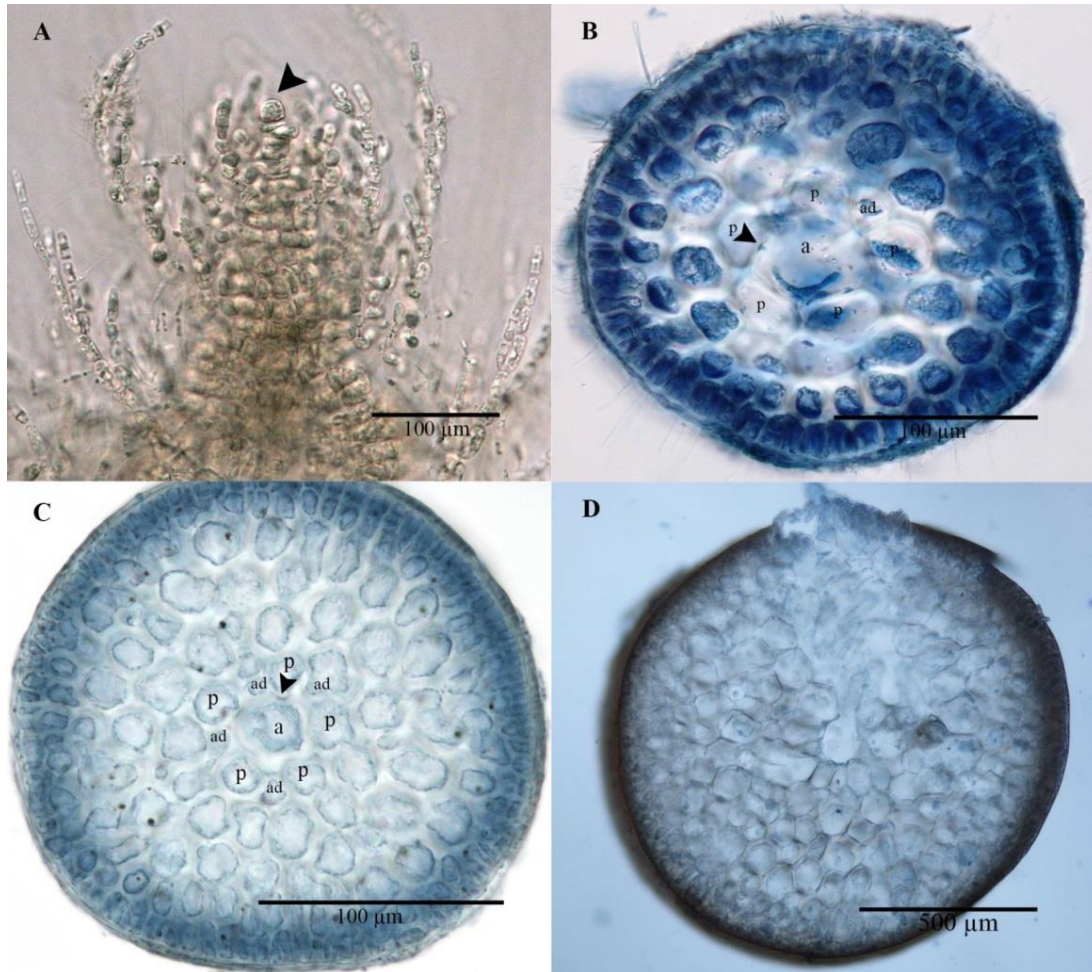


Fig. 11. Morphology of apical cell, axial cell, pericentral cell and adventitious elongate cells of *Neochondria ammophila* sp. nov.

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.

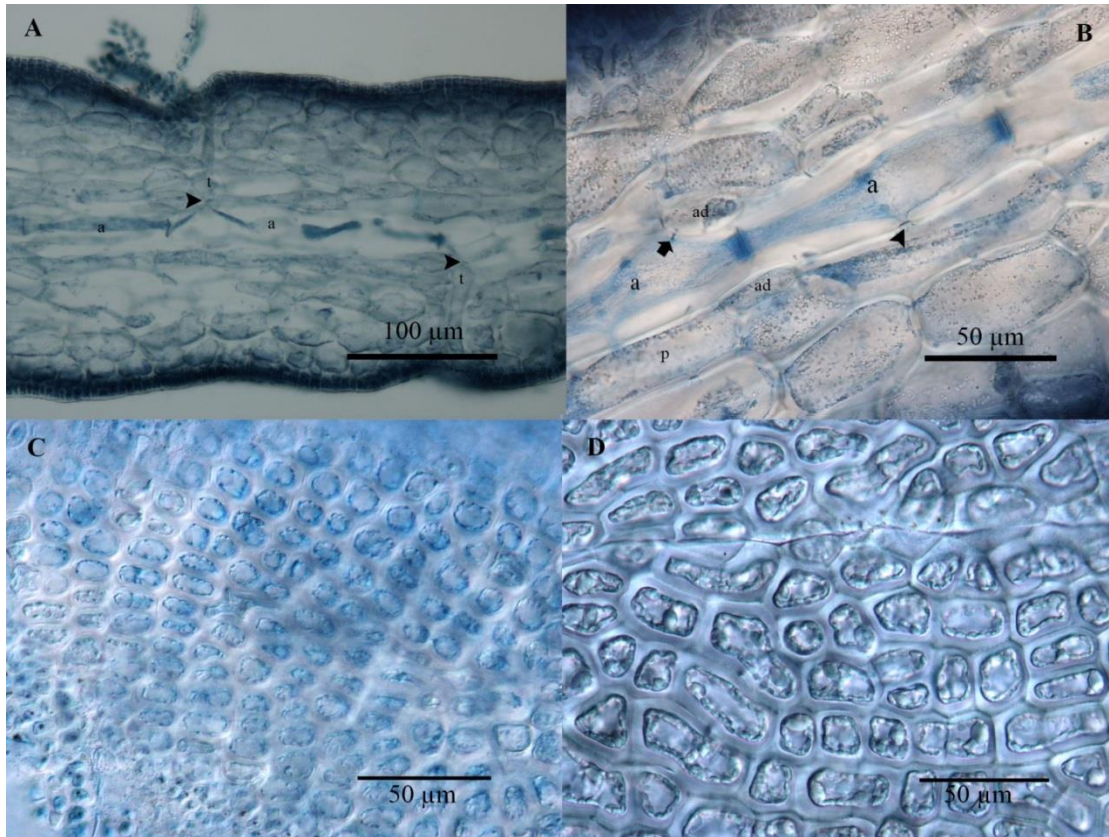


Fig. 12. Morphology of axial cells, pericentral cells, adventitious cells, trichoblast basal cells in longitudinal sections and epidermal cell arrangement of *Neochondria ammophila* sp. nov.

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.

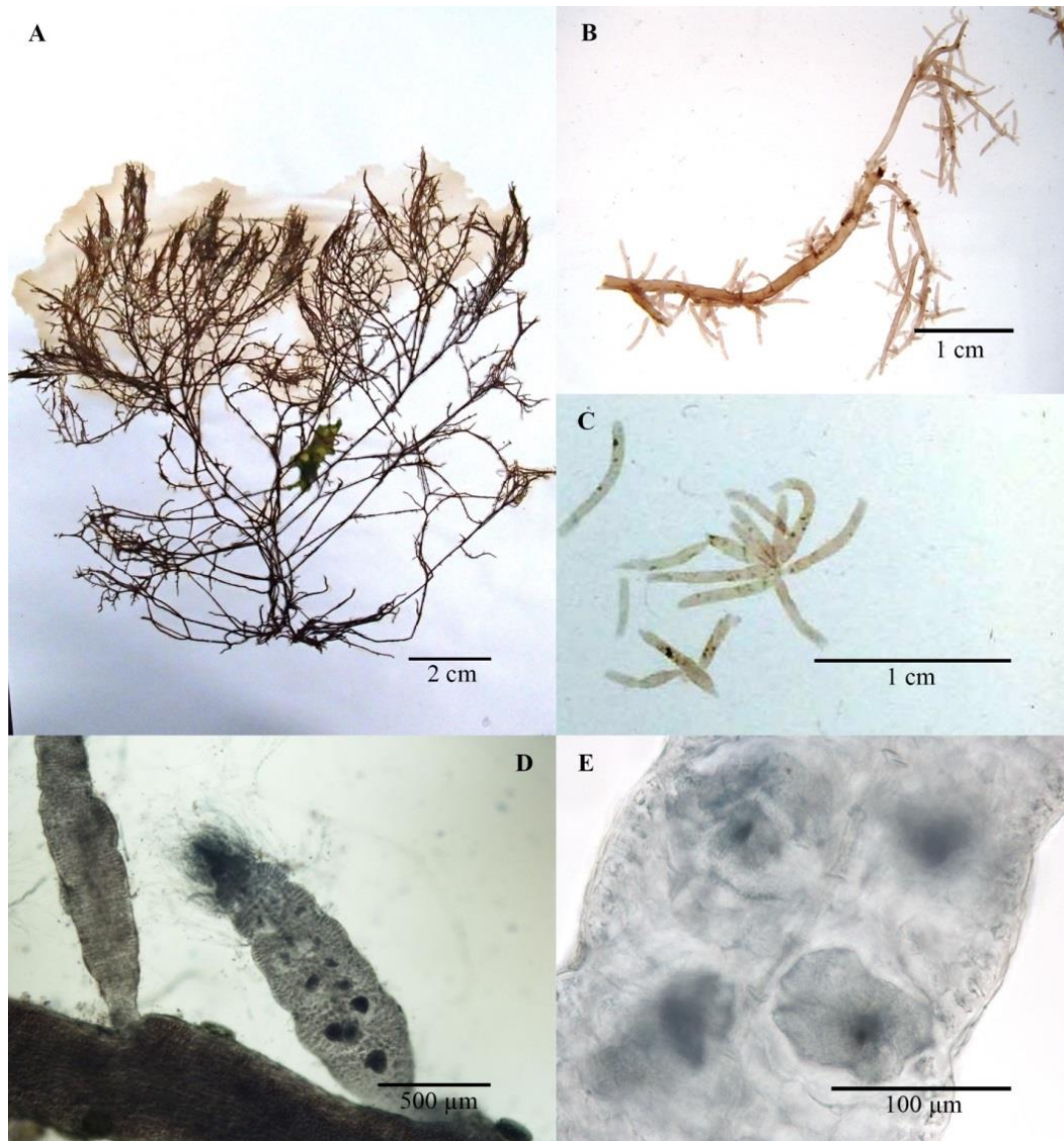


Fig. 13. Morphology of tetrasporophyte of *Neochondria ammophila* sp. nov.

A. Holotype specimen of *Neochondria ammophila* (tetrasporophyte, SAP115370, *rbcL* [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.

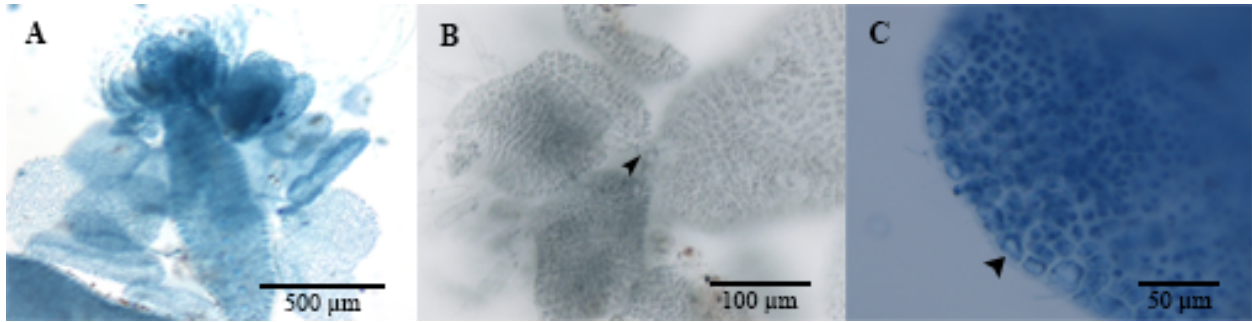


Fig. 14. Male gametophyte of *Neochondria ammophila* sp. nov. collected from Muroran, Hokkaido, Japan on 28 July 1999 (formalin preserved specimen, SAP115349).

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).

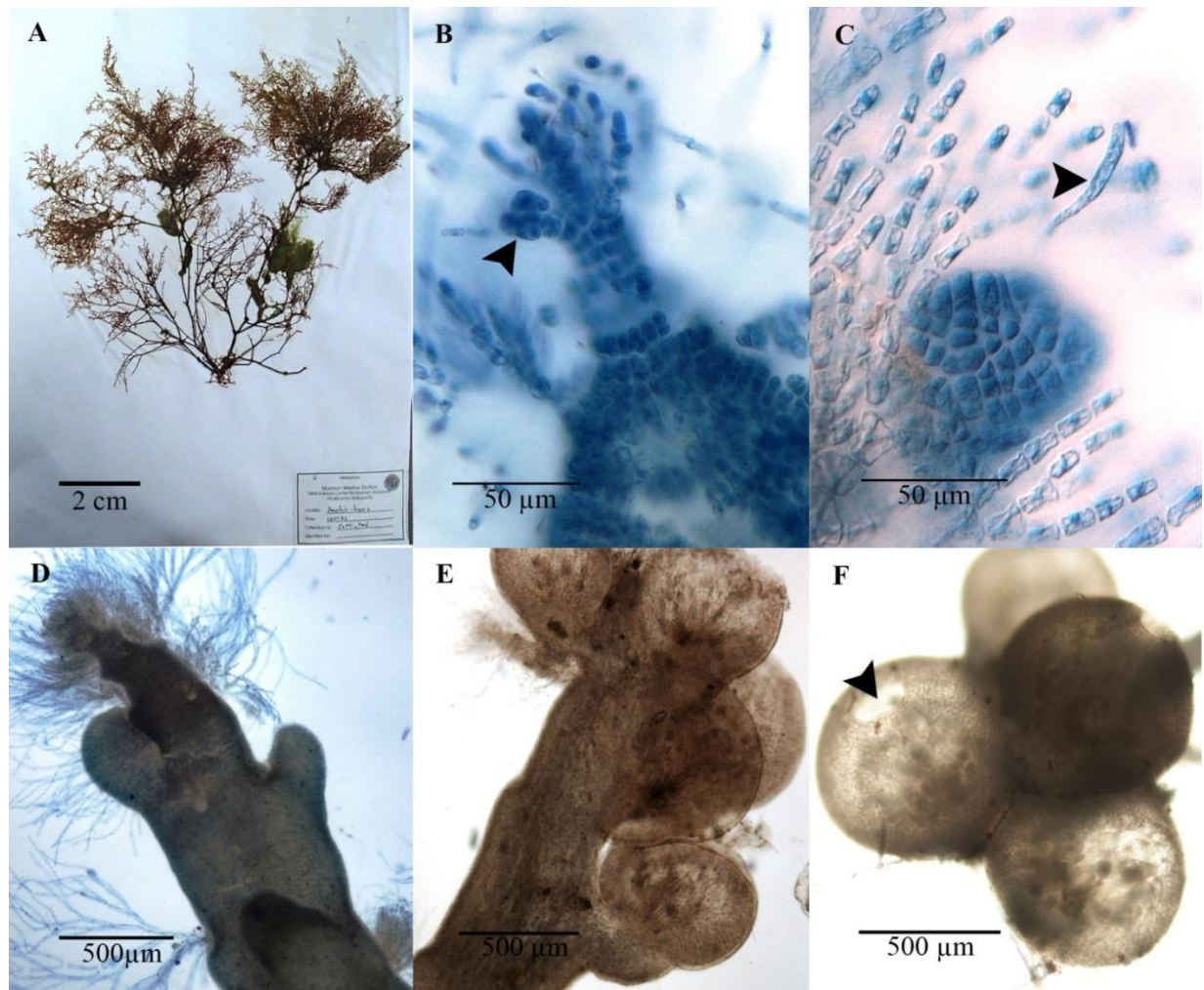


Fig. 15. Female gametophyte of *Neochondria ammophila* sp. nov.

A. Specimen of *Neochondria ammophila* (female gametophyte, SAP115371, *rbcL* [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.

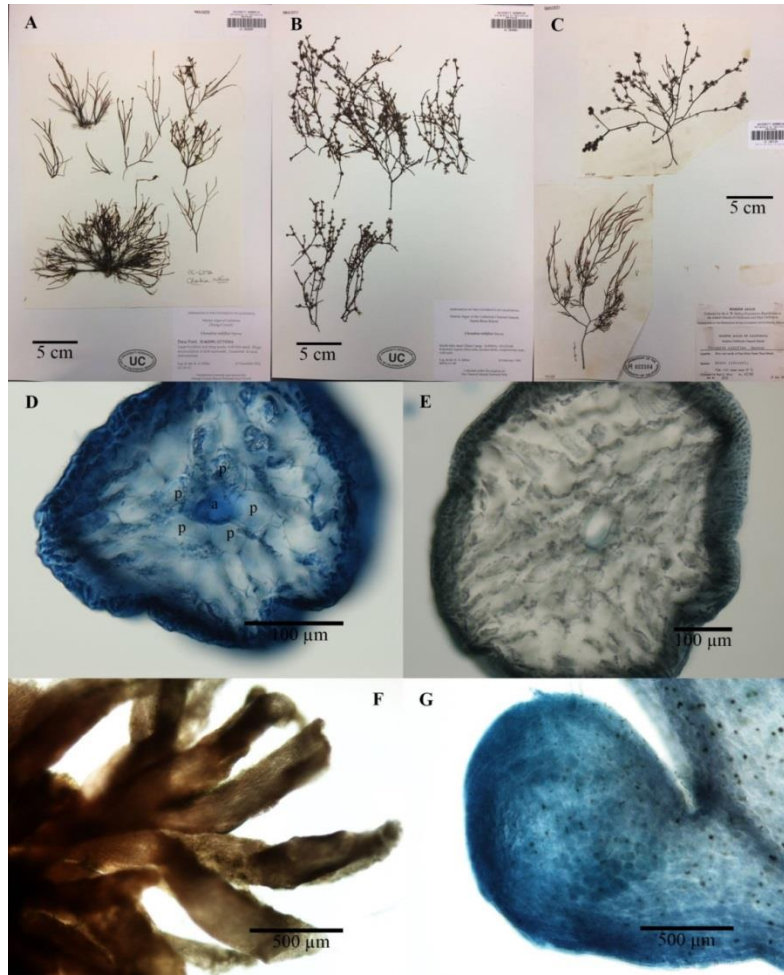


Fig. 16. Morphology of *Neochondria nidifica* comb. nov. (= *Chondria nidifica*)

A. *Chondria nidifica* (UC2026095, tetrasporophyte, *rbcL* [MG255067], SSU [MG272245], *cox1* [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.

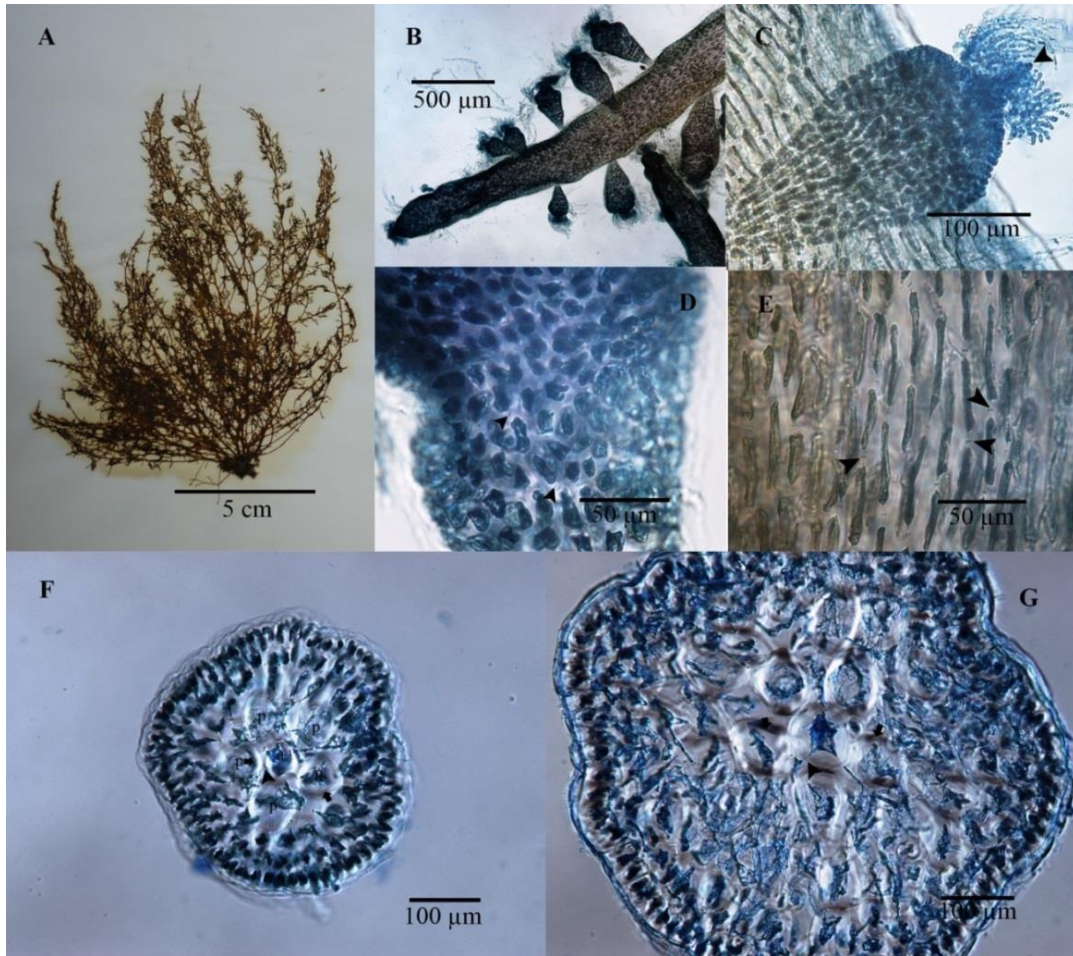


Fig. 17. Vegetative morphology of *Chondria acuminata* sp. nov. (= Japanese '*Chondria dasyphylla*')

A. *Chondria acuminata* (SAP115391, *rbcL* [MG843867], SSU [MG831941], *cox1* [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.

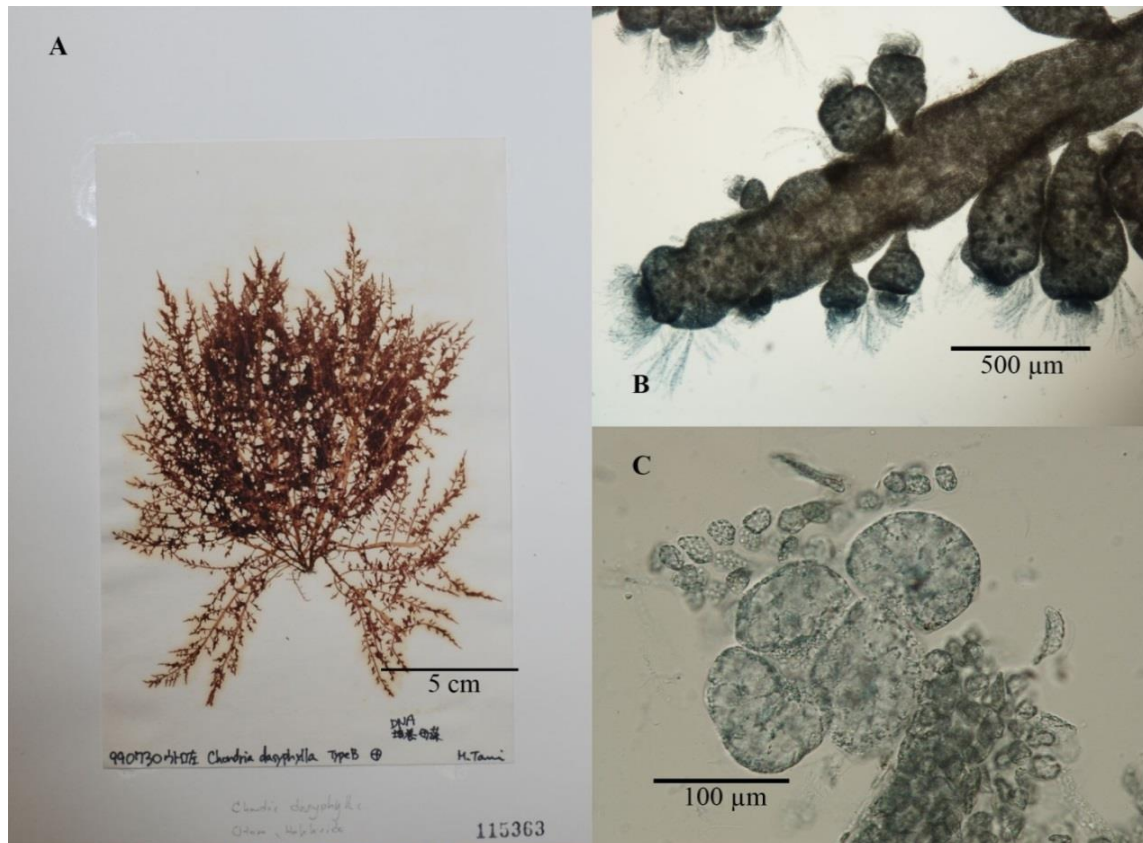


Fig. 18. Morphology of a tetrasporophyte of *Chondria acuminata* sp. nov. (= Japanese ‘*Chondria dasyphylla*’)

A. *Chondria acuminata* (SAP115363, *rbcL* [MG255062], SSU [MG272240], *cox1* [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.

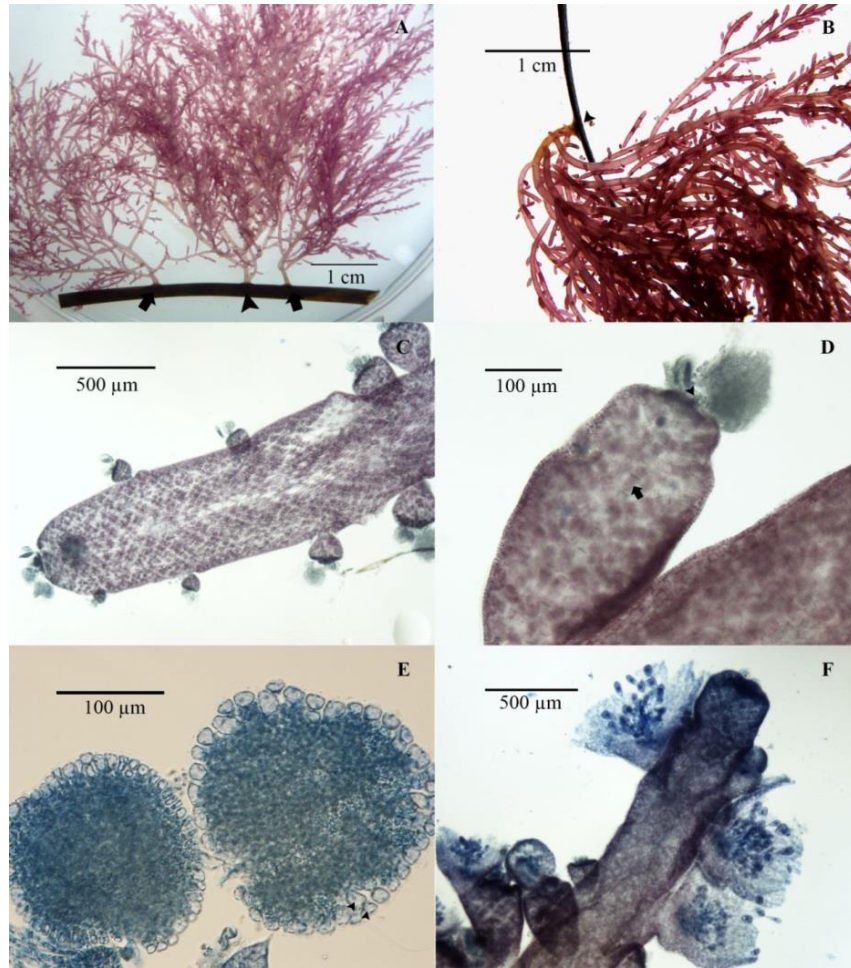


Fig. 20. Morphology of male and female gametophyte of *Chondria* cf. *curdieana* (= Japanese '*Chondria dasyphylla*')

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.

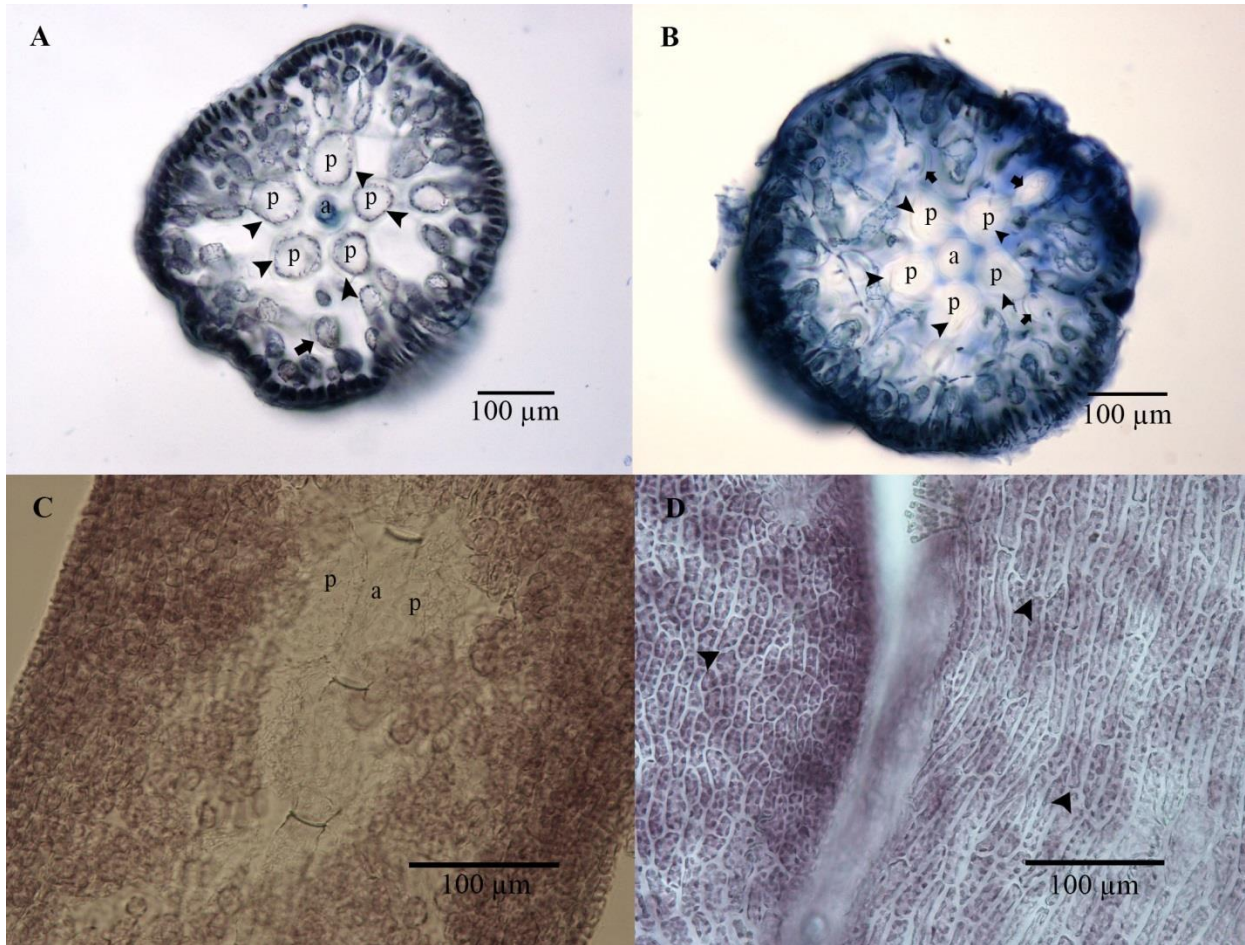


Fig. 21. Axial cell issuing pericentral cell and epical cells arrangement of *Chondria* cf. *curdieana* (= Japanese '*Chondria dasyphylla*')

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.

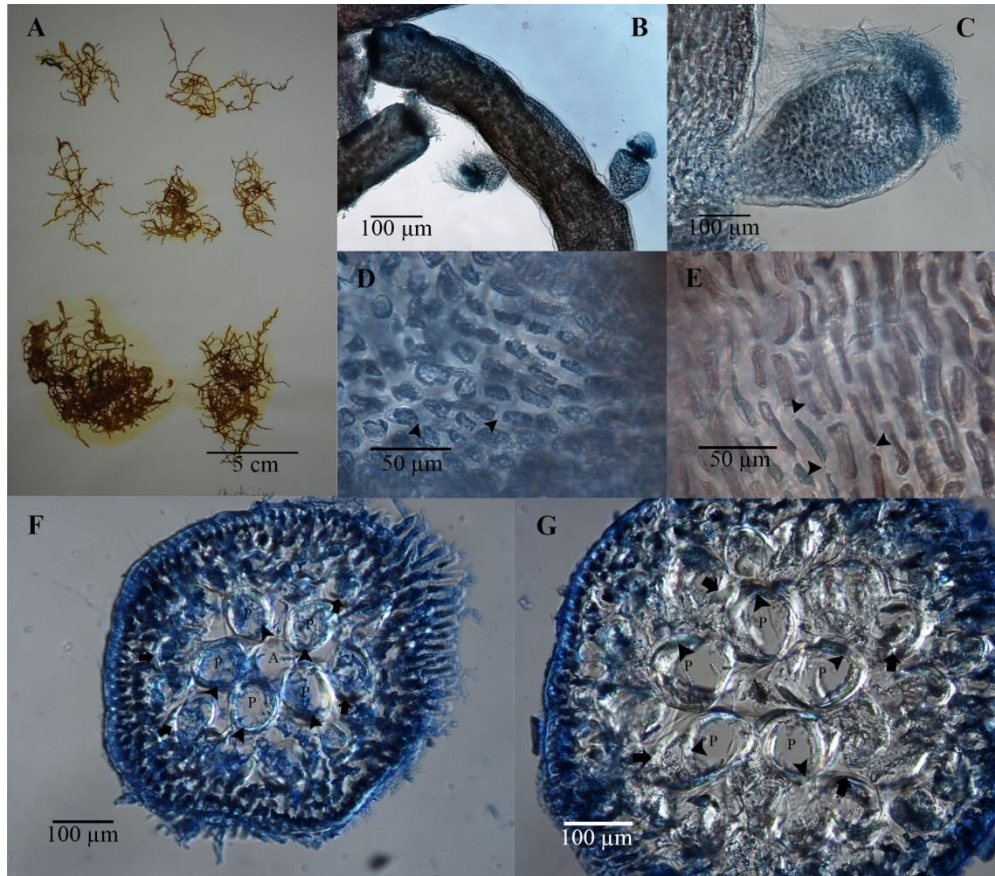


Fig. 22. Vegetative morphology of an examined *Chondria* sp. 1

A. *Chondria* sp. 1 (SAP115397, *rbcL* [MG843871], SSU [MG831945], *cox1* [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.

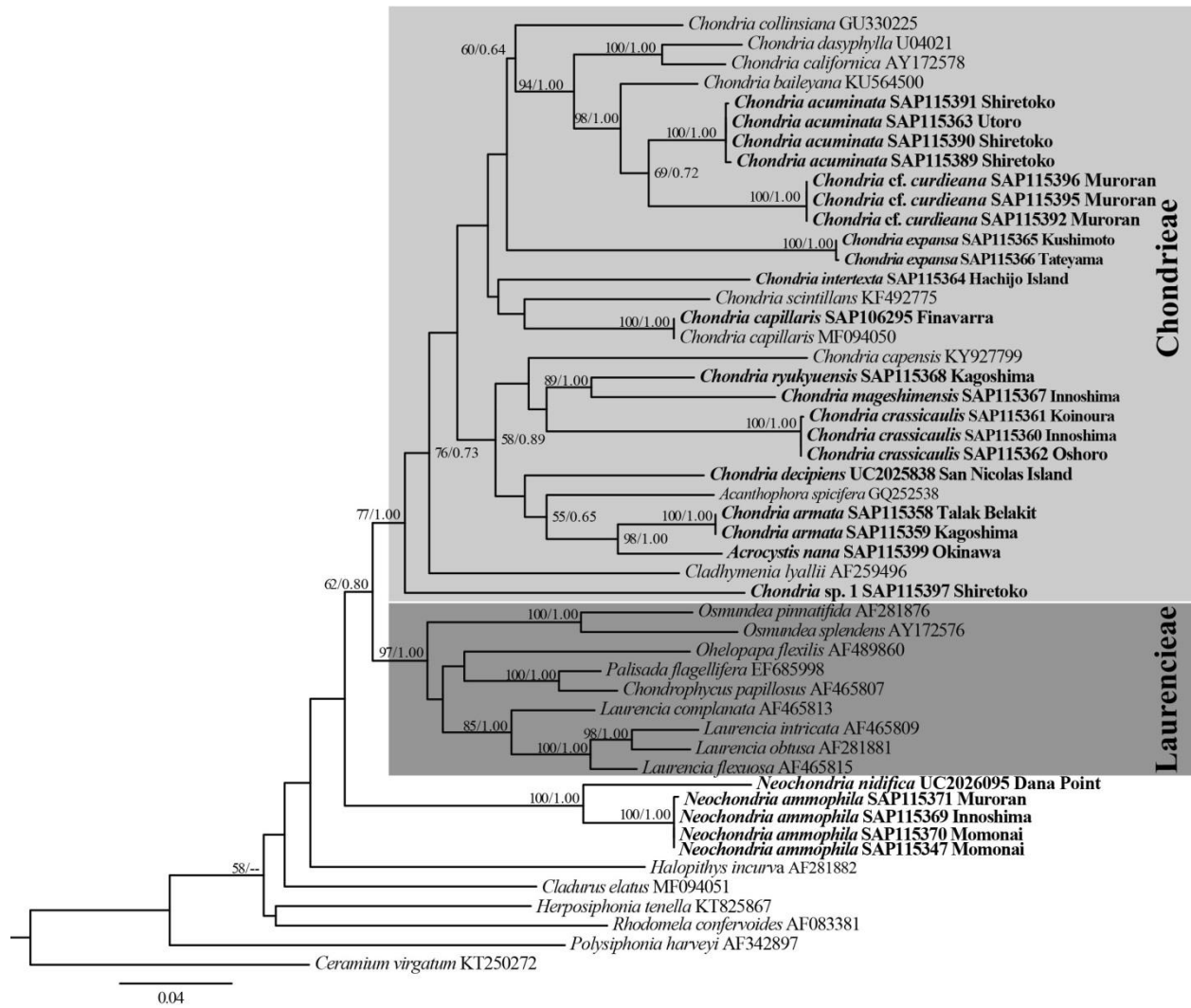


Fig. 23. Maximum likelihood tree generated from partial *rbcL* sequences representing 49 Rhodomelaceae and a Ceramiaceae sequence (KT250272) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities. Only bootstrap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

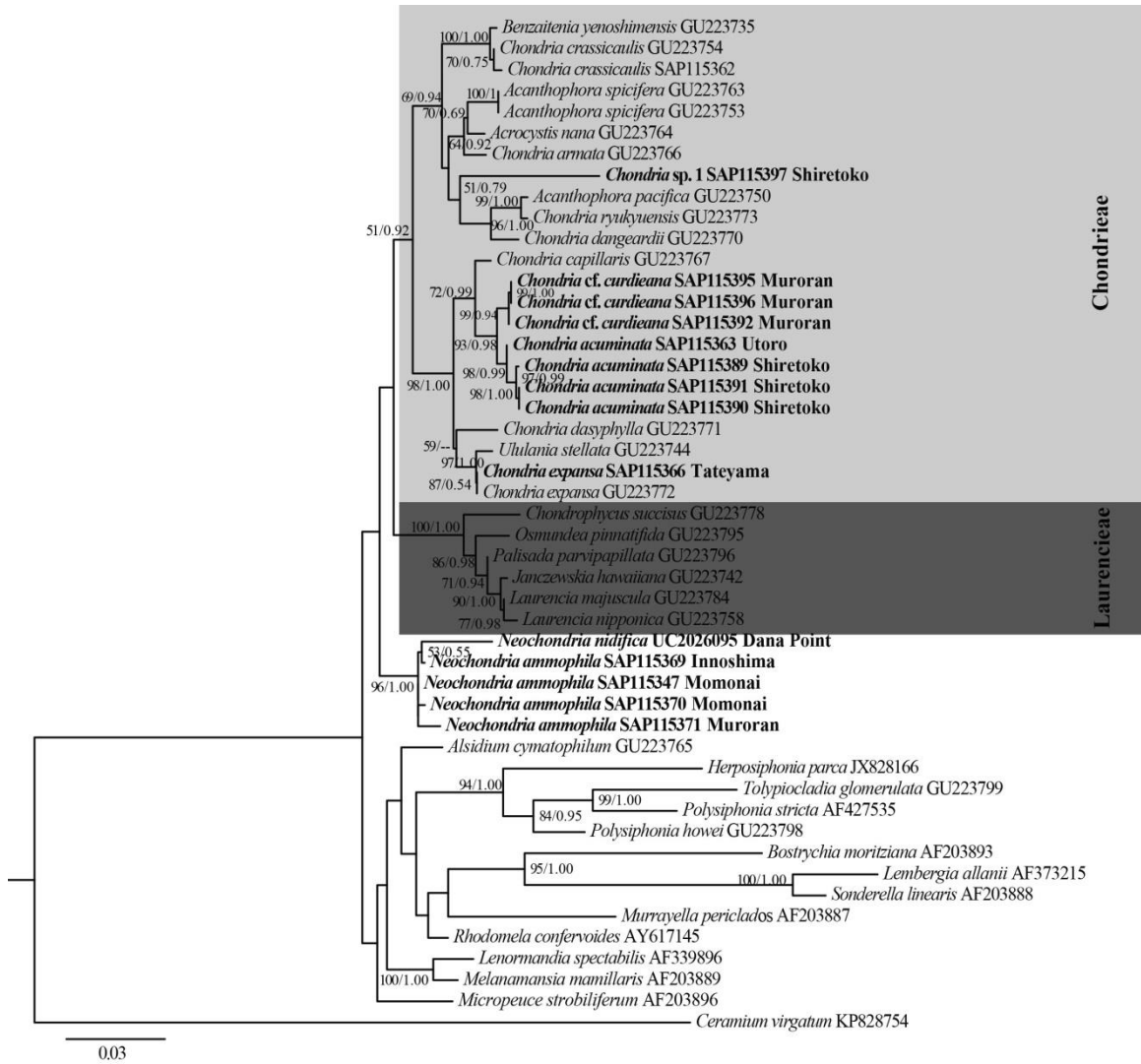


Fig. 24. Maximum likelihood tree generated from partial SSU rRNA gene sequences representing 47 Rhodomelaceae and a Ceramiaceae sequence (KP828754) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities.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

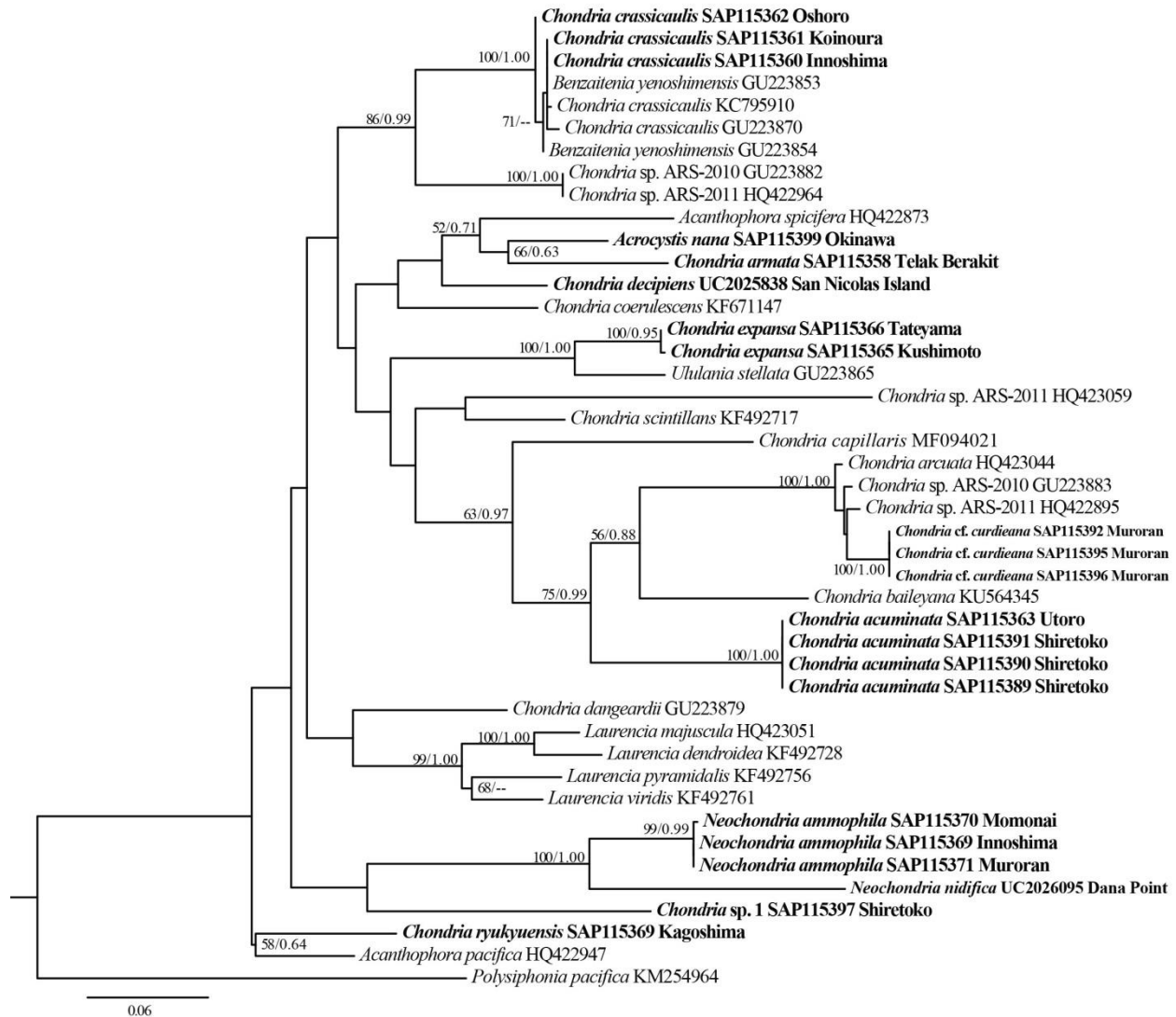


Fig. 25. Maximum likelihood tree generated from *cox1* sequences representing 43 Chondrieae-Laurencieae species and a *Polysiphonia* sequence (KM254964) as an outgroup. Boldface indicates sequences newly generated in this study. Support values of the branches are ML bootstrap /BI posterior probabilities. Only bootstrap values > 50 and Bayesian posterior probabilities ≥ 0.60 are shown.