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Hoshino, Masakazu ; Wakeman, Kevin C. ; Kato, Aki ; Kitayama, Taiju ; Sherwood, Alison R. ; Uwai, Shinya ; Kogame, Kazuhiro

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Taxonomic study of the polyphyletic *Dudresnaya* (Dumontiaceae, Florideophyceae) with descriptions of *Dudresnaya ryukyuensis* sp. nov. and two new genera, *Himehibirhodia* and *Nudresdaya*

Masakazu Hoshino,[1,2,3]* **Kevin C. Wakeman**,[4] **Aki Kato**,[5] **Taiju Kitayama**,[6] **Alison R. Sherwood**,[7] **Shinya Uwai**[1] **and Kazuhiro Kogame**[8]

[1]Research Center for Inland Seas, Kobe University, Kobe, Japan, [2]Department of Algal Development and Evolution, Max Planck Institute for Biology Tübingen, Tübingen, Germany, [3]Department of Natural History Sciences, Graduate School of Science, Hokkaido University, Sapporo, Japan, [4]Institute for the Advancement of Higher Education, Hokkaido University, Sapporo, Japan, [5]Fisheries laboratory, Blue Innovation Division, Seto Inland Sea Carbon-neutral Research Center, Hiroshima University, Hiroshima, Japan, [6]Department of Botany, National Museum of Nature and Science, Tsukuba, Japan, [7]School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, HI, USA and [8]Department of Biological Sciences, Faculty of Science, Hokkaido University, Sapporo, Japan

SUMMARY

The red algal genus *Dudresnaya* (Dumontiaceae, Gigartinales) has traditionally been a morphologically well-defined taxon, but its molecular phylogeny has rarely been studied. To examine the phylogenetic relationships among *Dudresnaya* species, we generated new partial sequences of mitochondrial *cox*1, chloroplast *rbc*L and nuclear 28S rRNA genes from an undescribed *Dudresnaya* species from Okinawa Island, Japan, alongside five additional described species. Our phylogenetic analyses show that *Dudresnaya* is genetically diverse and polyphyletic. Based on molecular phylogeny and morphological data, we describe the Okinawan *Dudresnaya* as a new species, *Dudresnaya ryukyuensis*, and transferred *Dudresnaya minima* and *Dudresnaya littleri*, which were phylogenetically and morphologically distinct from the genuine *Dudresnaya*, to the new genera *Himehibirhodia* and *Nudresdaya*, respectively. Our phylogenetic analyses also showed that the Dumontiaceae is not a monophyletic group including the Gainiaceae and Rhizophyllidaceae (DGR complex). Considering that the DGR complex exhibits female reproductive structures and their post-fertilization development that are similar to each other, the DGR complex appears to be recognized as the Dumontiaceae *sensu lato*.

Key words: *Dudresnaya ryukyuensis*, Dumontiaceae, Gainiaceae, *Himehibirhodia*, life cycle, molecular phylogeny, *Nudresdaya*, Rhizophyllidaceae.

INTRODUCTION

The family Dumontiaceae (Gigartinales, Rhodophyta) is characterized by (i) separated carpogonial branches and auxiliary cell branches that are distinctly differentiated from vegetative branches; (ii) fusion of the carpogonium with one or more cells of the carpogonial branch prior to formation of secondary connecting filaments to auxiliary cells; and (iii) the location of the auxiliary cell, which is mostly intercalary but rarely terminal in the auxiliary cell branch (Mitchell 1966; Shepley & Womersley 1983; Tai *et al*. 2001). *Dudresnaya* P.Crouan & H.Crouan *nom. et typ. cons.* is a dumontiacean genus, having erect thalli that are irregularly branched, lubricous, gelatinous, solitary or gregarious, which are attached by a small discoid crust. Anatomically, the erect thalli have a uniaxial construction, with axial cells producing a whorl of cortical fascicles and the inner cortical cells producing rhizoidal filaments that descend around the axial filaments. Currently, 20 species are recognized in *Dudresnaya*, although relatively large morphological variation has been reported. For example, (i) spermatangial mother cells can densely cover the outermost 3–6 cells of the spermatangial branches, forming corncob-like spermatangial structures, or can be arranged at the terminus of the outermost 1–2 cortical cells; (ii) the division of tetrasporangia can be zonate, cruciate or irregularly cruciate; and (iii) the life cycle can be either isomorphic or heteromorphic (Robins & Kraft 1985; Notoya 1988; Notoya & Aruga 1989; Tabares *et al*. 1997).

Although both the Dumontiaceae and *Dudresnaya* are morphologically well-defined groups, recent molecular phylogenetic studies do not support their monophyly. Dixon *et al*. (2015) showed that two dumontiacean algae, *Kraftia dichotoma* Shepley & Womersley and *Dasyphloea insignis* Montagne, form a clade with the Rhizophyllidaceae. Although *Dudresnaya* species have been rarely included in molecular

*To whom correspondence should be addressed.
Email: mhoshino.sci@gmail.com

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phylogenetic studies, Sherwood *et al.* (2010) showed that *Dudresnaya hawaiiensis* R.K.S. Lee and *Dudresnaya littleri* Abbott are distantly related in the Dumontiaceae, indicating that *Dudresnaya* is a complex of distinct lineages.

In the present study, we examined the phylogenetic relationships among *Dudresnaya* species with newly generated partial sequences of mitochondrial *cox*1, chloroplast *rbc*L and 28S ribosomal RNA genes from six *Dudresnaya* species: *Dudresnaya babbittiana* Abbott & K.J. McDermid, *Dudresnaya japonica* Okamura, *Dudresnaya kuroshioensis* Kajimura, *D. littleri*, *Dudresnaya minima* Okamura and one undescribed species from Okinawa Island, Japan. Our multigene phylogenetic analyses show that *Dudresnaya* is currently composed of three non-monophyletic lineages: *D. littleri*, *D. minima* and other species examined. To deal with this taxonomic problem, we propose taxonomic revisions for *Dudresnaya* and discuss taxonomic relationships between the Dumontiaceae and the related families, Gainiaceae and Rhizophyllidaceae.

MATERIALS AND METHODS

Specimen collection

In total, nine *Dudresnaya* plants were collected by skin diving at three localities in Okinawa Island (Fig. 1; see Supporting information, Table S1). Fragments of plants were preserved in 70% ethanol or salt for morphological observations, and in silica gel for DNA extraction. Pressed specimens were made and deposited in the Herbarium of Faculty of Science, Hokkaido University, Sapporo, Japan (SAP) or the National Museum of Nature and Science, Tsukuba, Japan (TNS). In addition to these specimens, pressed specimens of *D. babbittiana*, *D. japonica*, *D. kuroshioensis*, *D. minima* and *Dudresnaya okiensis* Kajimura housed in the herbarium SAP and TNS were morphologically examined and included in the molecular phylogenetic analyses (see Supporting information, Table S1) (herbarium acronyms follow Index Herbariorum; http://sweetgum.nybg.org/science/ih). The specimens of *D. japonica* and *D. minima* included those collected near the type localities (Fig. 1). The specimens of *D. kuroshioensis* and *D. okiensis* were the holotype specimens, and the specimens of *D. babbittiana* comprised the paratype specimens that were collected from the type locality, Midway Atoll (Fig. 1). The DNA samples of *D. littleri* that were used in Sherwood *et al.* (2010; specimen code: ARS00292 and ARS00359) were also used for molecular phylogenetic analyses; these were both collected from the island of O'ahu (Fig. 1), although not at the exact type locality. In addition to the *Dudresnaya* specimens, two specimens of the dumontiacean alga *Masudaphycus irregularis* (Masuda) Lindstrom were collected in Akkeshi, Hokkaido, Japan, for inclusion in the phylogenetic analyses.

Morphological observation and culture experiment

Morphological observations were conducted using a stereo, an inverted and a compound light microscope, and images were taken with Basler Aca2440-75uc (Basler, Ahrensburg, Germany), DFK 37AUX250 (The Imaging Source, Bremen, Germany) and Digital Sight DS-Fi1 (Nikon, Tokyo, Japan) digital cameras. Imaging software, comprising Basler Video Recording (Basler) and IC Capture (The Imaging Source), were used for the first two cameras, respectively. To observe internal anatomy, fragments of material were stained with cotton blue (lactic acid/phenol/glycerol/water 1:1:1:1), mounted in seawater or 50% glycerol/seawater on glass slides and squashed under a cover glass.

To examine the life cycle of the Okinawan *Dudresnaya*, carpospores from female plants collected at Kin (Fig. 1) on 11 March 2021 were cultured. Culture experiments were conducted using plastic petri dishes (90 × 20 mm) and quarter-strength Provasoli's enriched seawater medium (Provasoli 1968). Carpospores released from the female plant were isolated with a Pasteur pipette and each spore was transferred to a separate Petri dish. The carpospores were cultured in a 20°C long day condition (LD;

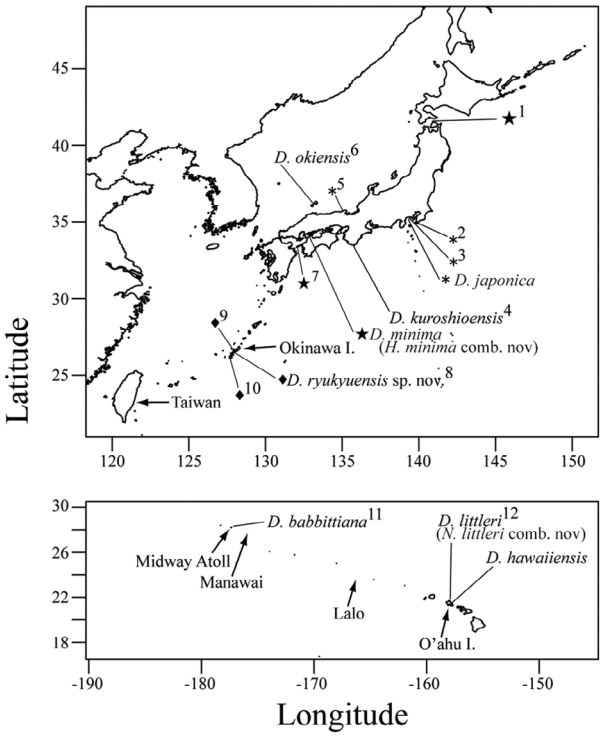

Fig. 1. Sampling sites in Japan and the Hawaiian Island chain in the present study. Species names are given for the type localities. Species collected from two or more sites are assigned symbols. For the sampling sites, from which specimens were examined in the present study, Arabic numerals (1–12) are given. 1, Ohma; 2, Sakata; 3, Chojagasaki; 4, Cape Shionomisaki; 5, Takeno; 6, Oki; 7, Cape Mimaisaki; 8, Kin; 9, Motobu; 10, Yamagusuku; 11, Midway Atoll; 12, O'ahu I. aux, auxiliary cell; auxb, auxiliary cell branch; ax, axial cell; b, basal cell of cortical lateral; cf., connecting filament; cp, carpogonium; cpb, carpogonial branch; fc, fusion complex; gi, gonimoblast initial; lb., lateral branch; nut, nutritive cell; pcf, primary connecting filament; rh, rhizoid; s, spermatia; scf, swollen part of secondary connecting filament; smc, spermatangial mother cell; tr, trichogyne; 2cf, secondary connecting filament.

light:dark = 16:8 h) and subsequently transferred to a 25°C short day condition (SD; light:dark = 10:14 h) to encourage tetrasporophyte maturation. Tetraspores released from the tetrasporophytes were isolated with a Pasteur pipette and each spore was transferred to a separate Petri dish. Gametophytes developed from the tetraspores were cultured in a 25°C LD (light:dark = 16:8 h). Fluorescent lighting was 30–50 μmol $m^{-2}s^{-1}$ photon flux density for all culture conditions. The morphology of the cultured thalli was observed as above, but without cotton blue staining.

Molecular phylogenetic analyses

Partial sequences of the mitochondrial *cox*1 (up to 664 bp), the chloroplast *rbc*L (up to 1310 bp) and nuclear 28S ribosomal RNA (up to 684 bp) genes were newly generated for inclusion in the phylogenetic analyses. Total genomic DNA was extracted using GenCheck DNA Extraction Reagent (FASMAC, Atsugi, Japan) in accordance with Hoshino *et al*. (2020) or a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Amplifications (PCRs) were performed using Tks Gflex DNA Polymerase (Takara Bio Inc., Otsu, Japan). The primers used were GazF1 and GazR1 (Saunders 2005) for *cox*1; F-57 (Freshwater & Rueness 1994), rbcL-Rh3 (Hanyuda *et al*. 2004), rbcL-Rh7 (Hanyuda *et al*. 2020), rbcLrevNEW (Saunders & Moore 2013), the newly designed DudrbcIF1 (5′-ATTATGCAGTTAAAGATACTG-3′), DudrbcIR1 (5′-AATAAC AGTACCAAGTTGC-3′), DudrbcIF2 (5′-ATGTATGAAAGAGCTGA G-3′) and DudrbcIR2 (5′-CACAAAGTCAGCTGTATC-3′) for *rbc*L; and D28S1F (5′-AGGTGTTGATTCATCGAGAC-3′) and D28S4R (5′-AACCCTTATCCCAAAGTTACG-3′) for 28S. The PCR program consisted of a denaturation step at 94°C for 1 min, followed by 35 cycles of 98°C for 10 s, 50°C for 15 s and 68°C for 30 s, and a final elongation step at 68°C for 7 min. For the pressed specimens collected before 2014, PCRs were performed using KOD FX Neo polymerase (TOYOBO, Osaka, Japan) and the primers listed in the Supporting information (Table S2). These primers were used to amplify regions of approximately 100–300 bp. The PCR program was as described in Hoshino *et al*. (2020). DNA sequencing was conducted as described in Kogame *et al*. (2015). The resulting sequences were deposited in GenBank (accession numbers are given in the Supporting information, Table S3).

The newly generated sequences were aligned with sequences downloaded from GenBank and two datasets were made for phylogenetic analysis. The first dataset was constructed to infer the phylogeny of dumontiacean species and consisted of 63 operational taxonomic units (OTUs) of the Dumontiaceae with its five phylogenetically close families (22 OTUs from the Gainiaceae, Rhizophyllidaceae, Kallymeniaceae, Etheliaceae, Ptilocladiopsidaceae) and *Polyides rotunda* (Polyidaceae) as the outgroup (see Supporting information, Table S3). The second dataset was used to infer the phylogenetic position of the Dumontiaceae in the Gigartinales and consisted of 32 families of Gigartinales and the Acrosymphytales as the outgroup (total 58 OTUs: up to four species per family; see Supporting information, Table S4). The *cox*1 and *rbc*L sequences were aligned using CLUSTAL W (Thompson *et al*. 1994) in MEGA version 7 (Kumar *et al*. 2016). The 28S sequences were aligned using MAFFT in the GUIDANCE2 Server (Landen & Graur 2008; Penn *et al*. 2010; Sela *et al*. 2015) and the positions in the alignment with a score below 0.93 (i.e. poorly aligned positions) were excluded. The alignments of *cox*1, *rbc*L and 28S were concatenated using Kakusan4 (Tanabe 2011). The final length of the alignments was 4676 bp for the first dataset (664 bp of *cox*1, 1363 bp of *rbc*L, 2649 bp of 28S) and 4804 bp for the second dataset (664 bp of *cox*1, 1358 bp of *rbc*L, 2782 bp of 28S).

For the phylogenetic analyses of the concatenated datasets, IQ-TREE, version 2.2.0.3 (Chernomor *et al*. 2016; Kalyaanamoorthy *et al*. 2017; Minh *et al*. 2020) were used for maximum likelihood (ML) analysis, and MrBayes version 3.2.7 (Huelsenbeck & Ronquist 2001; Ronquist *et al*. 2012) for Bayesian inference (BI) analysis. The ML analysis by IQ-tree was performed with 1000 of standard non-parametric bootstrap replicates and with the best partition and nucleotide models inferred by the flag '−m MFP + MERGE'. The BI analysis was performed with the partition and nucleotide models inferred by Kakusan4 based on the Bayesian Information Criterion (BIC; Schwarz 1978) and Monte Carlo Markov chains (MCMC) were run with the following parameters: two runs, four chains, sampling frequency of 100 and burn-in fraction of 0.25. Stationarity of the MCMC run was monitored using Tracer, version 1.7.1 (Rambaut *et al*. 2018), and the analyses were terminated after 7 253 000 generations for the first dataset and 30 000 000 generations for the second dataset. The ML and the Bayesian trees were visualized using FigTree, version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree). For the first dataset, ML analyses were also conducted for each gene, separately, following the same procedure as for the concatenated analyses. Uncorrected pairwise genetic distances (*p*-distances) for inter- and intra-species comparisons based on the *cox*1, *rbc*L and 28S datasets were calculated in MEGA, version 7.

RESULTS

Taxonomic observations

Here, we describe the *Dudresnaya* specimens from Okinawa Island as a new species, *Dudresnaya ryukyuensis*.

Dudresnaya ryukyuensis *M. Hoshino, Wakeman, Kitayama & Kogame sp. nov. (Figures 2–8)*

Description: Gametophytes are erect, yellow to wine-red in color, gelatinous and lubricous in texture, and up to 12 cm in height. The stipe is terete. The main branch is terete to slightly compressed, up to 14 mm in width, irregularly radially branched, sometimes gradually densely covered with branchlets towards terminal. Annulations absent even in terminal branchlets. The axial filament of the branchlet produces two to four cortical fascicles in a whorl, sometimes produces secondary axial filaments, terminates not with an apical cell but with cortical fascicles, whereas the position of the apical cell is unclear. The primary axial filaments and secondary axial filaments are not distinguishable except that secondary axial filaments usually have thinner axial cells. The apical cell is recognized only at the unbranched indeterminate axis

primordia. Hexagonal crystals are absent in all cells. The cortical fascicles are dichotomously branched, while their distal cells are cylindrical and lack hairs. The rhizoids arise from the basal cell of cortical fascicles, carpogonial branches and auxiliary cell branches, and develop downward along the axes, frequently branching but without producing cortical fascicles. The gametophytes are dioicous. The spermatangial branches arise laterally on cortical fascicles. The spermatangial mother cells are densely arranged to the outermost 2–7 cells of the spermatangial branches, forming corncob-like structures. The carpogonial branches and the auxiliary cell branches lack a mucilage coat; they are morphologically and spatially distinct (non-procarpic). The carpogonial branches are slightly reflexed, usually composed of six to nine cells including the basal cells, and are terminated by a carpogonium with a long trichogyne. The auxiliary cell branches are usually composed of 15–18 cells including the basal cell. The middle 7–10 cells of the branch are rounded, and the auxiliary cell is located at the center of these modified cells and is usually flattened and smaller than its neighboring cells. The mature carposporophytes are spherical to slightly ellipsoidal and reached 140 μm in diameter, and encircle the auxiliary cell branch with a narrow slit. The tetrasporophytes observed in culture condition are discoid, up to 5 mm in diameter, crimson in color, and mature with cruciately divided tetrasporangia. *Cox*1, *rbc*L and 28S sequences of the holotype specimen: GenBank accession LC577547, LC577557, LC632012, respectively.

Holotype: SAP115580 (Fig. 2a), female, collected by M. Hoshino and K. C. Wakeman at Kin (26°26′51.6″N, 127°54′36.1″E), Okinawa Prefecture, Japan, on 1 March 2020, deposited in SAP.

Isotypes: SAP115581 (male) and TNS-AL213458 (female) collected by M. Hoshino and K. C. Wakeman at Kin, Okinawa Prefecture, Japan, on 1 March 2020, deposited in SAP and TNS.

Type locality: 26°26′51.6″N, 127°54′36.1″E; around 1 m depth, attached on rocks or corals; Kin, Okinawa Prefecture, Japan.

Etymology: This species is named after the Ryukyu Islands which includes its type locality, Okinawa I.

Japanese name: Shima-hibirodo (new name). 'Shima' means Ryukyu Is., and 'hibirodo' is the Japanese name of the genus *Dudresnaya* and *D. japonica* (Okamura 1908).

Paratypes: SAP115640 (one male and two females) and SAP115641 (female), collected by M. Hoshino and K. C. Wakeman at Kin, Okinawa Prefecture, Japan, on 11 March 2021; SAP115582 (female), collected by M. Hoshino and K. C. Wakeman at Gorilla Chop, Motobu, Okinawa Prefecture, Japan (26°38′10.0″ N, 127°52′55.9″ E), on 28 February 2020; TNS-AL152111 (female), collected by T. Kitayama at Yamagusuku, Itoman, Okinawa Prefecture, Japan (26°04′37.9″ N 127°41′25.1″ E), on 2 March 2002.

Morphological observation: In total, nine specimens from three localities of Okinawa I. (Fig. 1) were examined. Plants were irregularly and radially branched and reached 12 cm in height (Fig. 2a,b). Annulations were absent even in the young branches (Fig. 2c). Both primary and secondary axial filaments terminated with cortical fascicles and the position of their apical cells were unclear (Fig. 3a). Apical cells were recognized only at the unbranched indeterminate axis primordia (Fig. 3b). The axial cells produced whorls of two to four cortical fascicles, some of which seemed to develop later into secondary axial filaments (Fig. 6a). Hexagonal crystals reported in axial cells in some *Dudresnaya* species were absent (Fig. 3c). The cortical fascicles lacked hair cells at their tips. Rhizoids arose from the basal cell of cortical fascicles, carpogonial branches and auxiliary cell branches (Figs 3c, 5b, 6b).

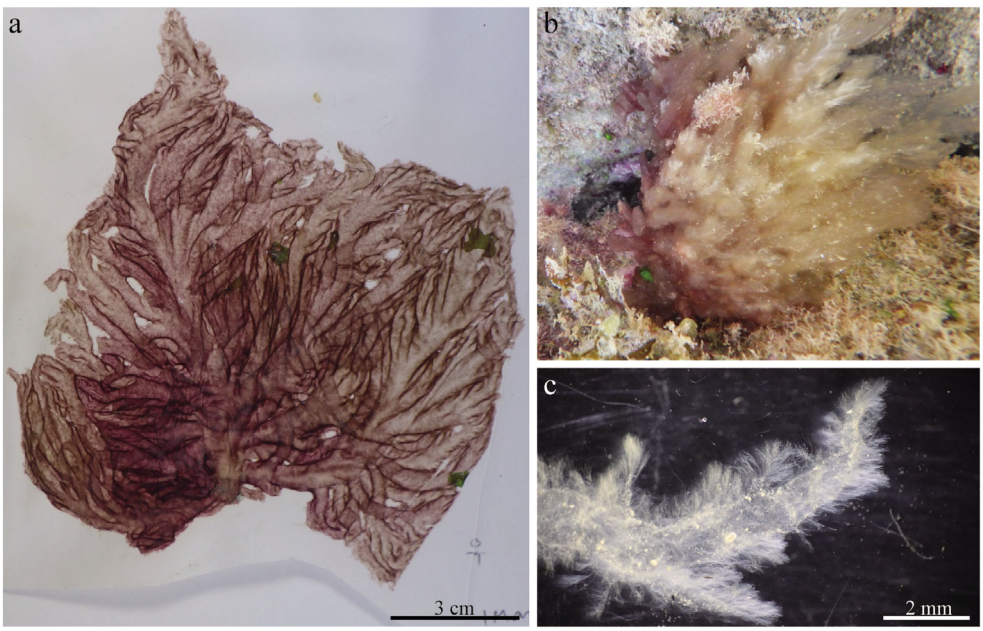

Fig. 2. *Dudresnaya ryukyuensis* sp. nov. External morphology. (a) Holotype specimen (SAP115580). (b) *In situ* habit of *D. ryukyuensis* from the type locality. (c) Young branch without annulations (SAP115582). Observed using a stereo light microscope.

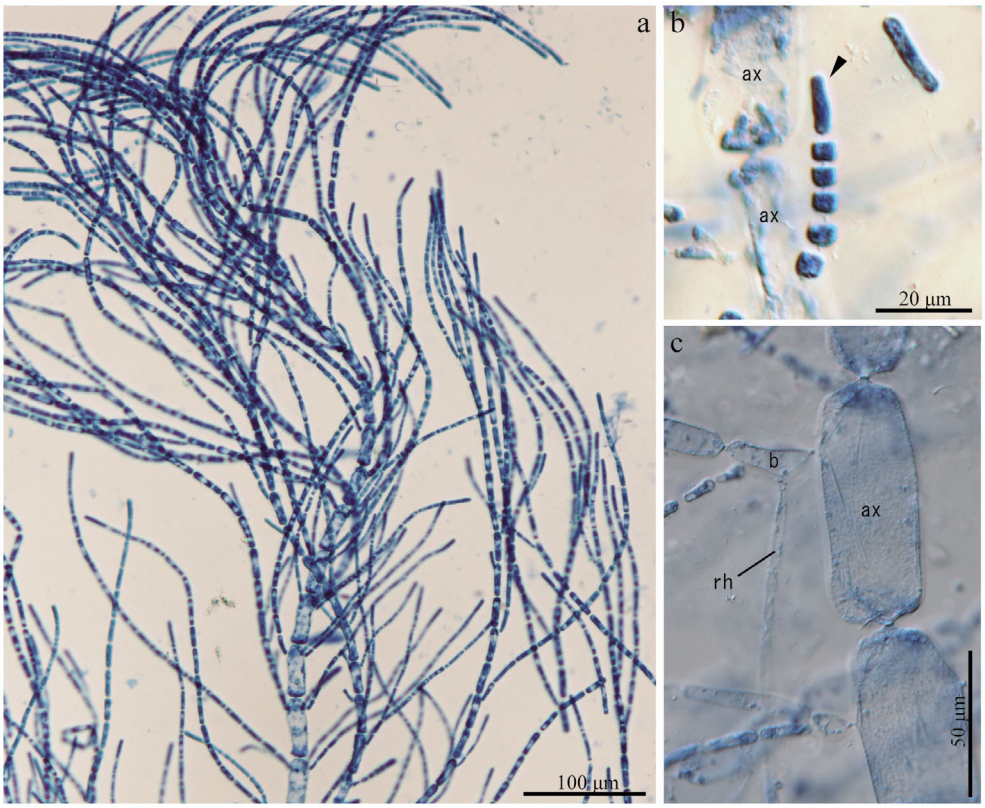

Fig. 3. *Dudresnaya ryukyuensis* sp. nov. Internal vegetative anatomy observed using a compound light microscope. (a) Branch tip with central axis not obviously percurrent (SAP115582). (b) Indeterminate axis primordium showing the apical cell (arrowhead) (SAP115581). (c) Rhizoid from basal cell of cortical lateral (SAP115582). aux, auxiliary cell; auxb, auxiliary cell branch; ax, axial cell; b, basal cell of cortical lateral; cf., connecting filament; cp, carpogonium; cpb, carpogonial branch; fc, fusion complex; gi, gonimoblast initial; lb., lateral branch; nut, nutritive cell; pcf, primary connecting filament; rh, rhizoid; s, spermatia; scf, swollen part of secondary connecting filament; smc, spermatangial mother cell; tr, trichogyne; 2cf, secondary connecting filament.

They were usually 4–6 μm (up to 10 μm) in diameter, developed downward along the axes, and frequently branched, but did not produce cortical fascicles.

Only gametophytes were collected from the field. These gametophytes were dioicous, two being male and seven being female. Spermatangial branches arose laterally on cortical fascicles (Fig. 4a), whereas the spermatangial mother cells were densely arranged to the distal 2–7 axial cells of the spermatangial branches, forming corncob-like structures (Fig. 4b). The carpogonial and auxiliary cell branches arose from the axial cells or basal part of cortical fascicles, replacing cortical fascicles/filaments (Figs 5a and 6a). A mucilage coat was not observed around the carpogonial and auxiliary cell branches. The mature carpogonial branches were slightly reflexed, usually composed of six to nine cells including the basal cells, and were terminated by a carpogonium with a long trichogyne (Fig. 5a,b). Rhizoids or sterile laterals often arose from the basal cell of the carpogonial branch (Fig. 5a,b). The mature auxiliary cell branches were usually composed of 15–18 cells including the basal cell (Fig. 6a–c), but sometimes had a long, terminal cortical filament, and composed of more than 25 cells. The middle 7–10 cells of the branch were modified (i.e. rounded and often darkly

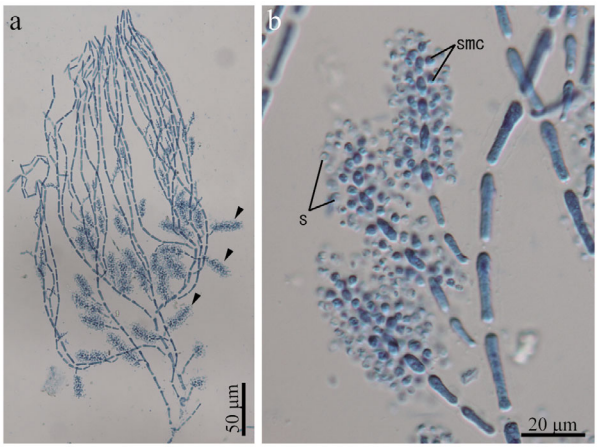

Fig. 4. *Dudresnaya ryukyuensis* sp. nov. Male reproductive structure observed using a compound light microscope. (a) Spermatangial branches (arrowheads) on the cortical fascicles (SAP115581). (b) Spermatangial mother cells densely covered the distal 3–5 cells of spermatangial branches, forming corncob-like spermatangial structures (SAP115581).

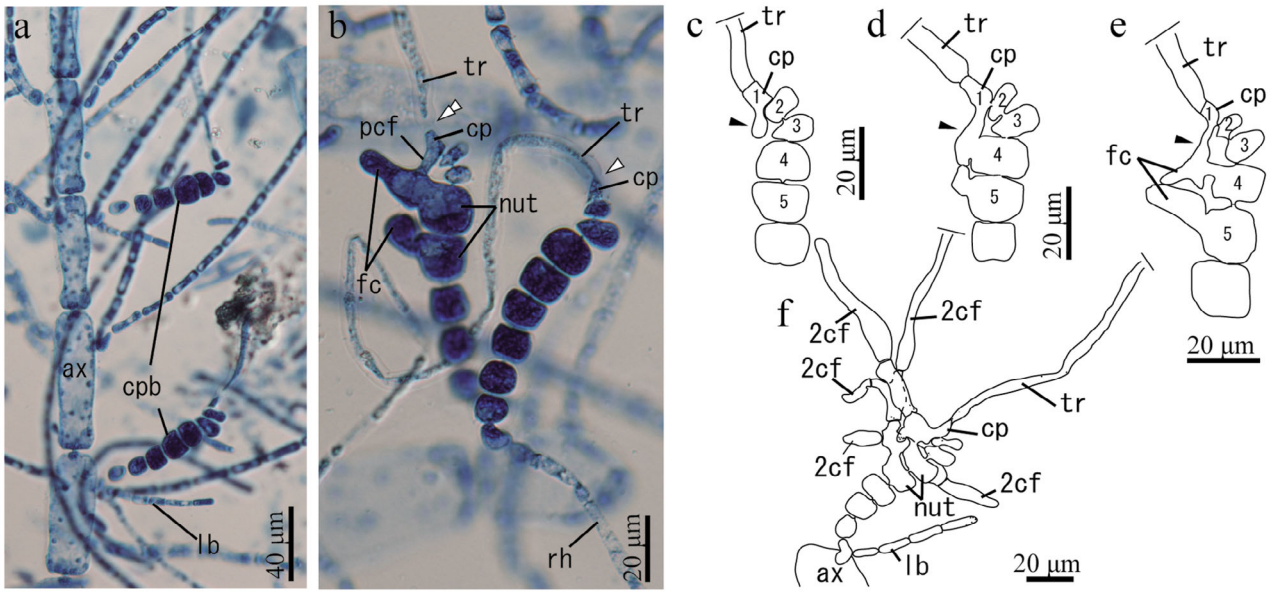

Fig. 5. *Dudresnaya ryukyuensis* sp. nov. Carpogonial branch and its post-fertilization development, observed using a compound light microscope. (a) Carpogonial branches on axial cells (SAP115582). (b) Unfertilized carpogonium showing unplugged trichogyne (arrowhead) and fertilized carpogonium showing basally plugged trichogyne (double arrowhead) and development of secondary connecting filaments from nutritive cells (SAP115582). (c–f) Post-fertilization development of carpogonium. Fertilized carpogonium extends primary connecting filament (c, arrowhead). The primary connecting filament (arrowhead) fuses with cells 4 and 5 of the carpogonial branches (nutritive cells; d) and forms fusion complexes (e). The fusion complexes subsequently cut off multiple secondary connecting filaments (f) (SAP115582).

stained) and the auxiliary cell was located at the center of these modified cells. The auxiliary cells are usually flattened and smaller than its neighboring cells (Fig. 6b). The auxiliary cells often had a slightly greater diameter at their distal end (Fig. 6b). Rhizoids sometimes arose from the basal cell of the auxiliary cell branch (Fig. 6b). No sterile laterals were observed on the basal cell of the auxiliary cell branch.

After fertilization, the trichogyne became plugged at its base (Fig. 5b) and the carpogonium extended a primary connecting filament that fused with the fourth and fifth cells (nutritive cells; Fig. 5c–f) of the carpogonial branch. From the resulting fusion complex, secondary connecting filaments, which fuse with the auxiliary cells, arose (Fig. 5f). Disappearance of the trichogyne during the post-fertilization development of the carpogonium, which was reported in *D. minima* and *D. littleri* (Kawashima 1959; Littler 1974), was not observed (Fig. 5b–f). After secondary connecting filaments laterally fused with the auxiliary cells (Fig. 6c), the fused portion of the connecting filament swelled and formed gonimoblast initials (Fig. 6d) and additional connecting filaments to other auxiliary cells. The mature carposporophyte was spherical to slightly ellipsoidal and reached 140 μm in diameter, which encircled the auxiliary cell branch with a narrow slit (Fig. 6e).

Life cycle in culture: Released carpospores were spherical and 10–12 μm in diameter ($n = 21$) (Fig. 7a). The first cell division divided the carpospores into two equal halves (Fig. 7b) and germinated unipolarly (Fig. 7c) or bipolarly. The germlings repeatedly branched and formed discoid plants (Fig. 7d). The plants often had fine hairs on the surface. The discoid thalli repeatedly underwent horizontal cell divisions and became thicker (Fig. 7e,f). In a 20°C LD, they did not produce any spore even after 8 months of cultivation, but some formed erect thalli from their edge (Fig. 7e). The erect thalli that developed in the tetrasporophyte generation did not form tetrasporangia but instead formed spermatangial branches. We did not examine whether the spermatia from the spermatangia were functional. Then, the discoid and erect thalli were transferred to a 25°C SD to encourage tetrasporophyte maturation. The discoid thalli transferred started to release tetraspores after 1 week of cultivation. The tetrasporangia formed on the surface of the discoid thalli without forming nemathecia and were larger than the vegetative cortical cells (Fig. 7g,h). The tetrasporangia were spherical, 9.6–18.9 μm in diameter (14.5 μm on average; $n = 16$) and cruciately-divided (Fig. 7g). The released tetraspores were spherical, 8.1–9.6 μm in diameter (9.1 μm on avg.; $n = 13$), and were connected by fine and sticky threads (Fig. 7i).

In the 25°C SD, the released tetraspores first divided into two equal halves and subsequently germinated in a unipolar or bipolar direction (Fig. 8a,b). The germlings repeatedly branched and formed compact discs with fine hairs (Fig. 8c). These discs started to form erect thalli in 2 weeks. The erect thalli were separately isolated into new Petri dishes and cultured in the 25°C LD (Fig. 8d). After 7 weeks of cultivation, erect thalli formed either female reproductive structures (carpogonial and auxiliary cell branches) (Fig. 8e) or spermatangial branches (Fig. 8f). The male and female gametophyte strains were deposited in the Kobe University Macro-Algal Culture Collection (strain codes: KU-3451–3453). In summary, our culture experiments indicated that *D. ryukyuensis* has a heteromorphic life cycle with erect dioicous gametophytes and discoid tetrasporophytes.

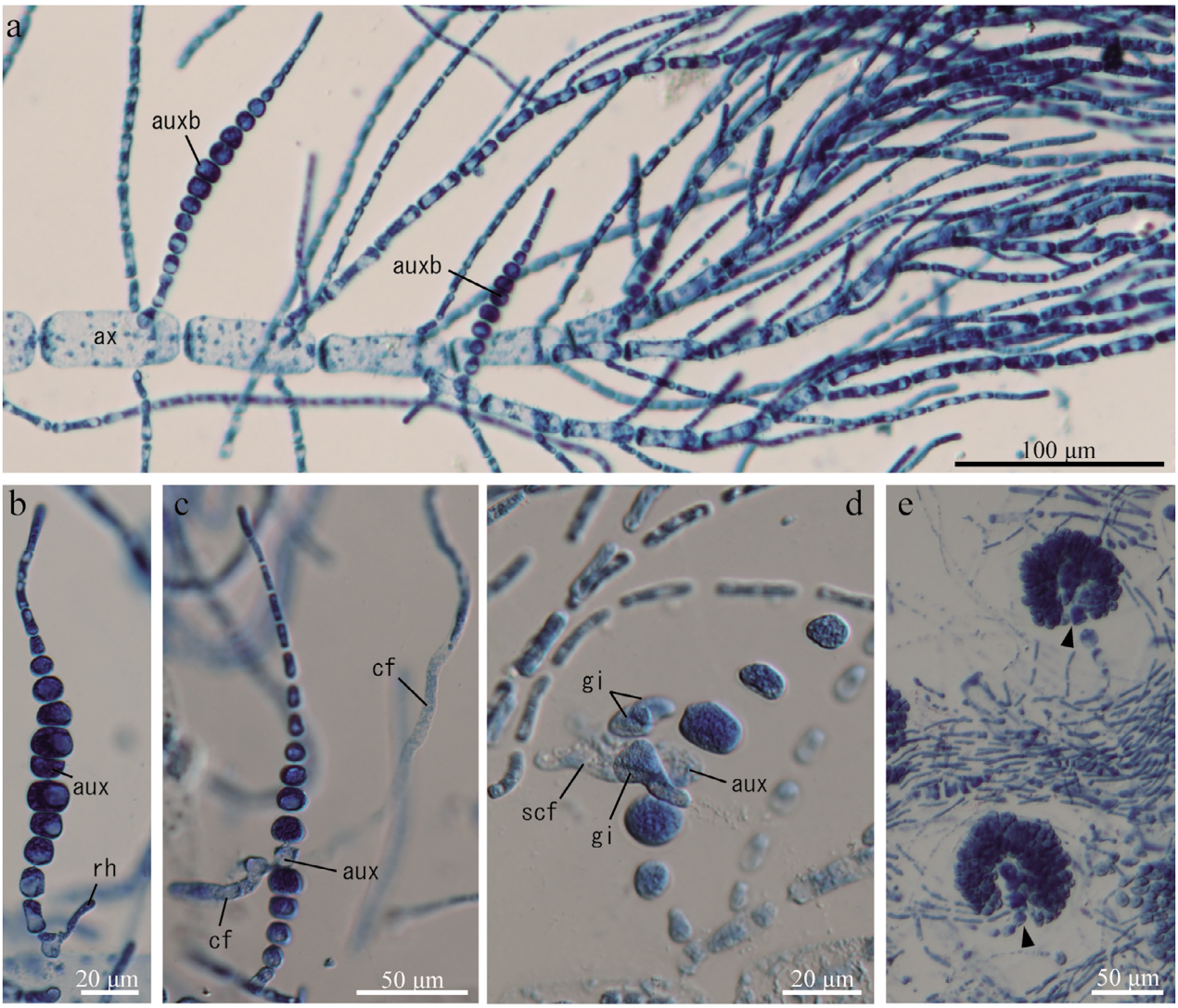

Fig. 6. *Dudresnaya ryukyuensis* sp. nov. Auxiliary cell branch and its post-fertilization development, observed using a compound light microscope. (a) Auxiliary cell branches developed on axial cells (SAP115582). (b) Mature auxiliary cell branch showing slightly flattened auxiliary cell (SAP115582). (c) Auxiliary cell branch showing lateral fusion of connecting filament with auxiliary cell (SAP115582). (d) Gonimoblast initials on swollen part of connecting filament developed on auxiliary cell (SAP115582). (e) Mature carposporophytes with a narrow slit (arrowhead) (TNS-AL152111).

Molecular phylogenetic analyses

In total, 32 sequences were generated (14 for *cox*1, 11 for *rbc*L and seven for 28S) (see Supporting information, Table S3). Although we could not generate any sequences from *D. okiensis*, the phylogenetic position of five *Dudresnaya* species (*D. babbittiana*, *D. japonica*, *D. kuroshioensis*, *D. minima* and *D. ryukyuensis*) and *M. irregularis* were examined for the first time in this study.

Our phylogenetic analyses demonstrate that the family Dumontiaceae is not monophyletic, nesting the families Gainiaceae and Rhizophyllidaceae in it (DGR complex) (Fig. 9; see also Supporting information, Fig. S1). The monophyly of the DGR complex was supported by our concatenated dataset [bootstrap proportion (BP) = 85, posterior probability (PP) = 1.0] (Fig. 9). The DGR complex can be further divided into well-supported five clades (A–E), although phylogenetic relationships among the five subgroups were unresolved (Fig. 9): clade A consisting of 'genuine' *Dudresnaya* (including the generitype), clade B consisting of *Rhodopeltis* Harvey, clade C consisting of *Gibsmithia* Doty, clade D consisting of the 13 genera distributed mainly in cold temperate waters of the Northern hemisphere, and clade E consisting of two species of *Dudresnaya*, *Dasyphloea*, *Kraftia*, *Gainia mollis* (Gainiaceae) and the Rhizophyllidaceae species. These five clades were recovered by single-marker-based phylogeny, although the statistical support for each clade was not always high (see Supporting information, Figs S2–S4).

The genus *Dudresnaya* was polyphyletic, as mentioned above. The genuine *Dudresnaya* belongs to clade A (BP = 100, PP = 1.0) (Fig. 9), whereas *D. littleri* and *D. minima* belonged to clade E with strong support (BP = 100,

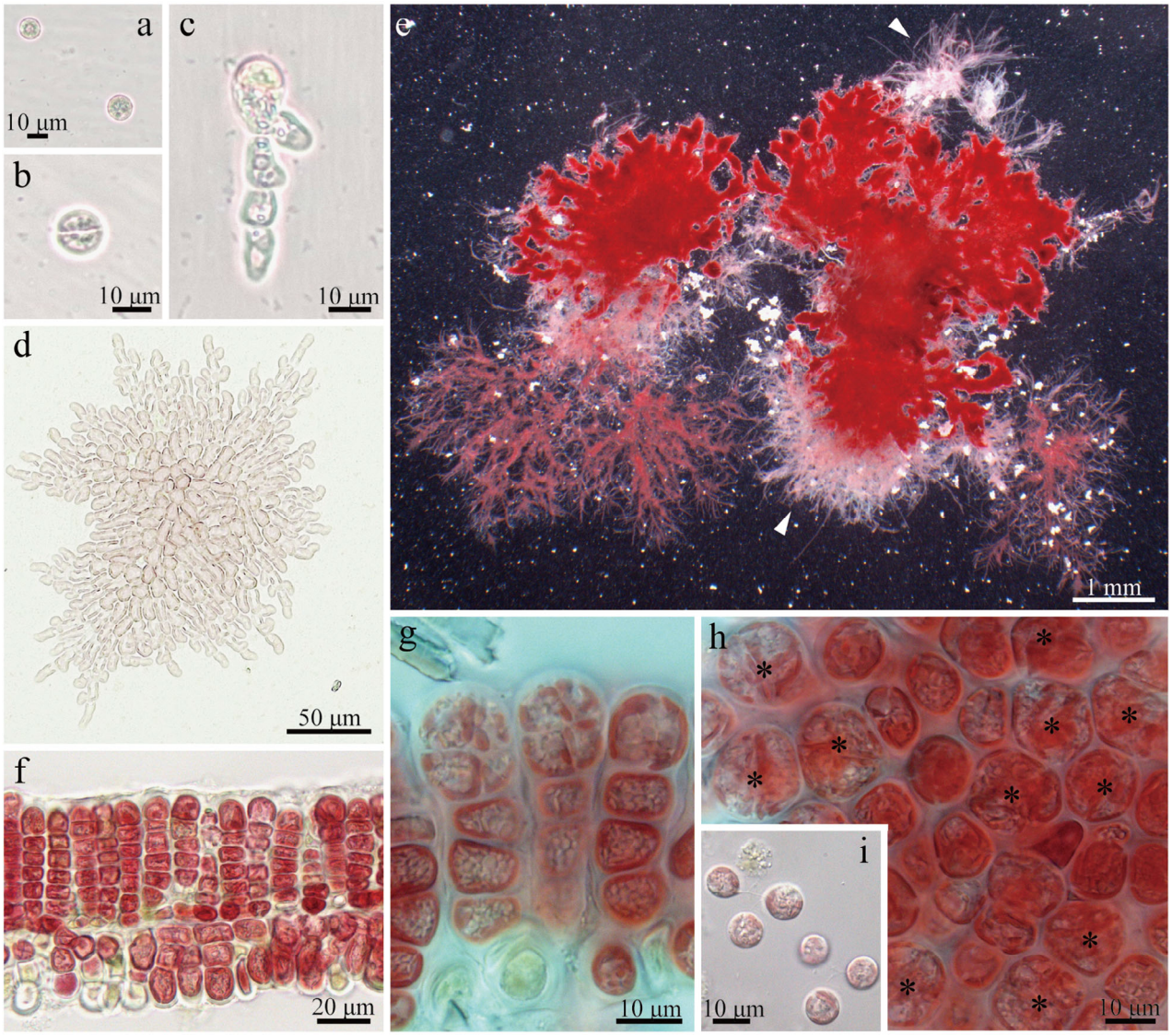

Fig. 7. Development of the carpospores of *Dudresnaya ryukyuensis* sp. nov. under culture conditions. (a) Released carpospores. Observed using an inverted light microscope. (b) Carpospores after the first cell division. Observed using an inverted light microscope. (c) Germling of the carpospore. Observed using an inverted light microscope. (d) Three-week-old discoid germling with several layers of cells in the central region. Observed using an inverted light microscope. (e) Mature discoid tetrasporophyte. Erect thalli (arrowheads) developed from the margin of the disc. Observed using a stereo light microscope. (f) Cross-section of immature part of a discoid tetrasporophyte. Observed using a compound light microscope. (g) Cross-section of mature part of a discoid tetrasporophyte, showing cruciately divided tetrasporangia. Observed using a compound light microscope. (h) Surface view of a discoid tetrasporophyte, showing tetrasporangia (asterisks). Observed using a compound light microscope. (i) Tetraspores released from a discoid tetrasporophyte. The spores are connected by fine threads. Observed using a compound light microscope.

PP = 1.0), although the phylogenetic relationship between the two species was not well resolved (Fig. 9). In clade A, at least three lineages were recognized (lineages i–iii); well-supported lineage i (BP = 97, PP = 1.0) and weakly supported lineage ii (BP = 76, PP = 1.0) were sister (BP = 100, PP = 1.0), with lineage iii sister to them (Fig. 9). Our phylogenetic analyses showed that *D. hawaiiensis*, *D. japonica* and *Dudresnaya verticillata* (Withering) Le Jolis were not monophyletic. This indicates that some sequences deposited in GenBank may have an incorrect taxonomic name or suggests the existence of cryptic species. We considered the *D. hawaiiensis* lineage including the topotypes (Sherwood *et al*. 2022) to be the true *D. hawaiiensis* and that our *D. japonica* specimens, collected near the type locality, were the true *D. japonica* (Fig. 9). For the generitype *D. verticillata*, there were at least two lineages in lineage ii and one in lineage iii (Fig. 9). Considering that its type locality is in the U.K. (exact type locality is unknown; Withering 1796; Irvine 1983), true *D. verticillata* would be represented by either the specimens from the UK and Spain belonging to

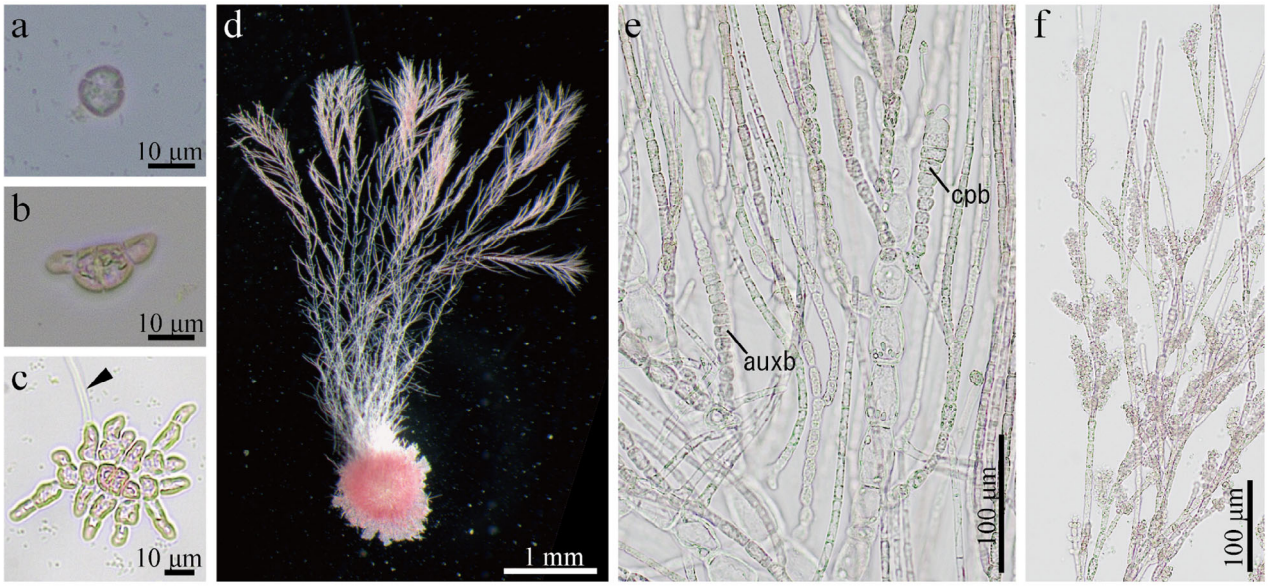

Fig. 8. Development of the tetraspores of *Dudresnaya ryukyuensis* sp. nov. under culture condition. (a) Tetraspore after the first cell division. Observed using an inverted light microscope. (b) Tetraspore germinated bipolarly. Observed using an inverted light microscope. (c) Six-day-old germlings of a tetraspore showing a hair (arrowhead). Observed by an inverted light microscope. (d) Young erect thalli (gametophyte) growing on a disc originated from a tetraspore. Observed using a stereo light microscope. (e) Carpogonial and auxiliary cell branches on a mature female gametophyte. Observed using an inverted light microscope. (f) Spermatangial branches on a mature male gametophyte. Observed using an inverted light microscope.

lineage ii, or from Ireland belonging to lineage iii, and probably not from Taiwan belonging to lineage ii (Fig. 9). *Dudresnaya* cf. *babbittiana*, which was first reported in the Hawaiian Islands outside the type locality Midway Atoll by Sherwood *et al*. (2022), was shown to be clustered with the paratype from the type locality (Fig. 9), indicating that this species is widely distributed in the Hawaiian Island chain (Figs 1 and 9). The new species *D. ryukyuensis* belonged to lineage i with *D. hawaiiensis* and *D. japonica*. The intraspecific genetic distance (*p*-distance) of *D. ryukyuensis* was up to ~1.96% in *cox*1, ~0.76% in *rbc*L and 0.0% (no polymorphism) in 28S. The genetic distance between *D. ryukyuensis* and its genetically closest described species, *D. hawaiiensis*, was ~8.6% in *cox*1, ~4.2% in *rbc*L and ~0.17% in 28S, and the intra-specific genetic distance of *D. hawaiiensis* was ~1.79% in *cox*1, ~1.72% in *rbc*L and 0.0% in 28S.

DISCUSSION

New *Dudresnaya* species from Japan

Based on morphological and molecular phylogenetic analyses, we describe a new species, *D. ryukyuensis*. This is the fifth *Dudresnaya* species from Japan and morphologically distinguishable from other four species, namely, *D. minima*, *D. okiensis*, *D. japonica* and *D. kuroshioensis*. The first two species differ from *D. ryukyuensis* in having annulations on their branches (Okamura 1932; Kajimura 1993). *Dudresnaya japonica* tends to exhibit more or less di/trichotomous and sparse branching (Okamura 1908), whereas *D. ryukyuensis* exhibits radial and dense branching. *Dudresnaya kuroshioensis* may exhibit a similar external morphology to *D. ryukyuensis* (i.e. radial branching, branches without annulations, and wide branches more than 0.5 cm), but it differs in having hexagonal crystals in the axial cells (Kajimura 1994).

Outside of Japan, several species may resemble *D. ryukyuensis* in external morphology: *Dudresnaya canariensis* Tabales, Afonso-Carrillo, Sansón & Reyes, *Dudresnaya capricornica* Robins & Kraft, *Dudresnaya abbottiae* Afonso-Carrillo & Tabares and *D. hawaiiensis* (Robins & Kraft 1985; Tabares *et al*. 1997; Afonso-Carrillo & Tabares 2004). *Dudresnaya canariensis* and *D. capricornica* exhibit hexagonal crystals in the axial cells and are distinct from *D. ryukyuensis* (Robins & Kraft 1985; Tabares *et al*. 1997), whereas *D. abbottiae* differs from *D. ryukyuensis* in that the central axis is percurrent at the branch top (Afonso-Carrillo & Tabares 2004). *Dudresnaya hawaiiensis* is morphologically identical to *D. ryukyuensis*, except that *D. hawaiiensis* is monoicous (Lee 1963) and *D. ryukyuensis* is dioicous. Because these sexual states co-exist in some red algal species, dioicy may not be a stable diagnostic character, whereas the genetic distance between *D. ryukyuensis* and *D. hawaiiensis* supports their independence. Phylogenetically, *D. ryukyuensis* was closest to *D.* '*japonica*' from Taiwan. The label '*D. japonica*' has been used in Taiwan since Shen and Fan (1950). Although the detailed morphology of *D.* '*japonica*' from Taiwan has likely not been reported so far, an image of a *D.* '*japonica*' specimen from Taiwan (catalog number: NCU-A-0010791; https://macroalgae.org/portal) resembles *D. hawaiiensis* and *D. ryukyuensis*, rather than *D. japonica*. Detailed observation of the Taiwanese specimens is necessary to discuss their taxonomic relationships with *D. ryukyuensis*.

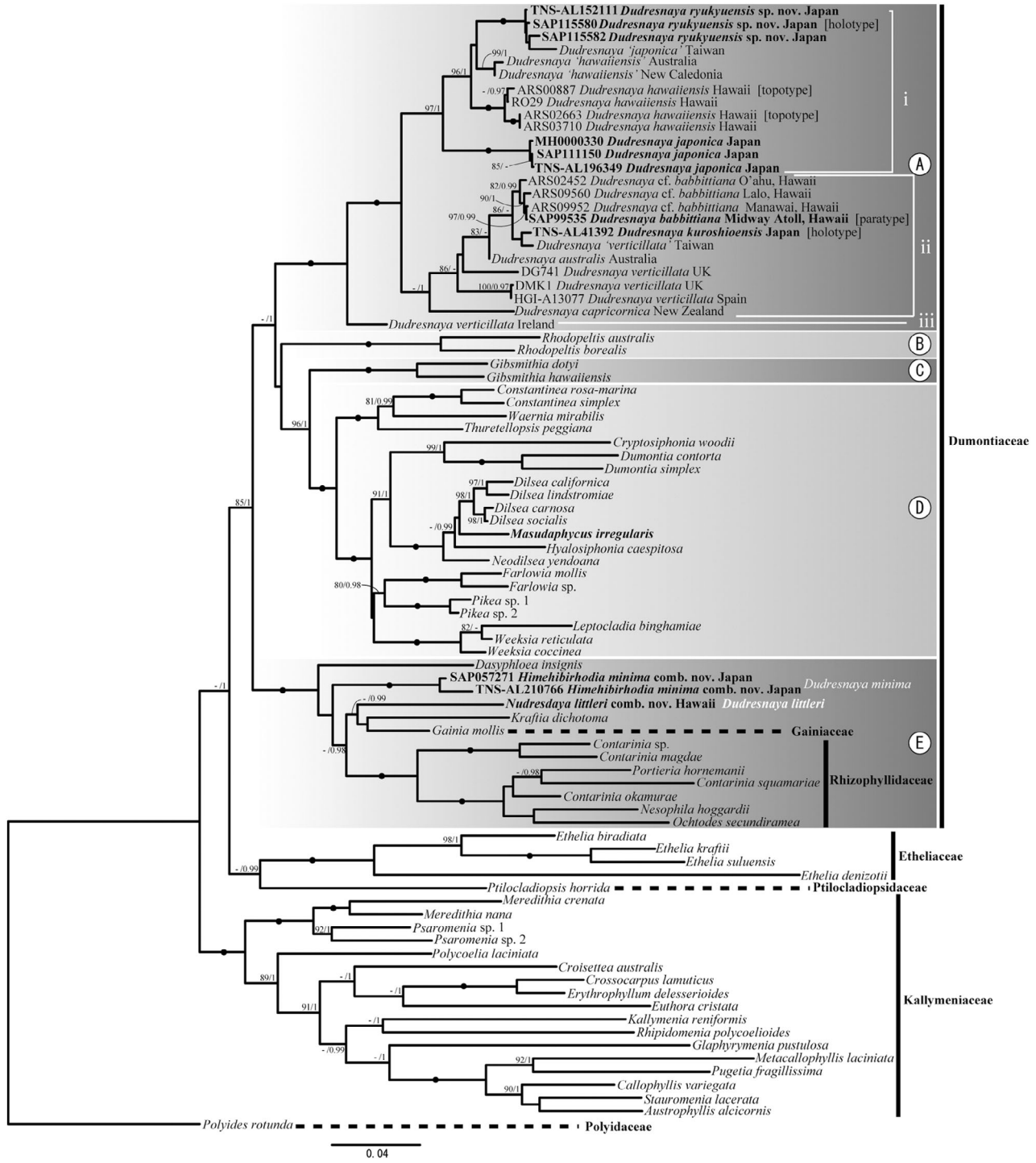

Fig. 9. Maximum likelihood tree based on the first dataset showing the phylogeny of the species of the Dumontiaceae and its related families: 86 OTUs, concatenated DNA sequences of mitochondrial *cox*1 and chloroplast *rbc*L and nuclear 28S ribosomal RNA genes (total 4676 bp). Numbers on branches indicate bootstrap values from ML analysis (left) and posterior probabilities from BI analysis (right). The black circles indicate branches with full support (100/1.0). Only bootstrap values >80 and posterior probabilities >0.95 are shown. Possible species misidentifications are indicated by single quoted species names (for details, see the Results section). The taxa synonymized in the present study are indicated by white text. Newly sequenced samples are indicated by bold text. Scale bar = the number of nucleotide substitutions per nucleotide site.

Transfer of *D. minima* and *D. littleri* to new genera

Our molecular phylogenetic analyses demonstrated that the genus *Dudresnaya* is polyphyletic as previously shown in Sherwood *et al.* (2010). Here, we propose the transfer of *D. minima* and *D. littleri* to the new genera *Himehibirhodia* and *Nudresdaya*, respectively (see taxonomic summary below). *Himehibirhodia* (*H. minima*) and *Nudresdaya* (*N. littleri*) are phylogenetically and morphologically distinct from *Dudresnaya* (Table 1). The trichogyne of *Himehibirhodia* and *Nudresdaya* disappears during post-fertilization development (Kawashima 1959; Littler 1974, as *Dudresnaya lubrica* Littler), whereas that of *Dudresnaya* does not (Okamura 1908; Robins & Kraft 1985; Kajimura 1994; present study), as shown in the generitype *D. verticillata* (Bornet & Thuret 1876; Kylin 1928; Robins & Kraft 1985). Furthermore, the distal end of the auxiliary cell branches of *Himehibirhodia* is terminated with rounded modified cells (Hasegawa 1949; Kawashima 1959; Kitayama 1989), whereas the auxiliary cell branches of *Dudresnaya* and *Nudresdaya* are usually terminated with cortical filaments (Littler 1974; Robins & Kraft 1985; present study). Furthermore, in *H. minima*, the tetrasporangia exhibit an irregularly cruciate division (Notoya 1988), whereas, in *Dudresnaya* and *Nudresdaya*, they exhibit a zonate division (Robins & Kraft 1985; Kajimura 1994; Abbott 1999) and, in some *Dudresnaya*, a cruciate pattern is observed (Notoya & Aruga 1989; present study).

As indicated by the fact that the heterotypic synonym of *H. minima* was established in *Thuretellopsis* Kylin (*T. japonica* Segawa & Ichiki; Segawa & Ichiki 1958), *H. minima* resembles *Thuretellopsis peggiana* Kylin, which is the sole species recognized in *Thuretellopsis*, in external morphology (i.e. soft and gelatinous thalli, crimson in color, and branches with annulations) (Lindstrom & Scagel 1987). However, *T. peggiana* differs from *H. minima* by possessing monosporangia in both gametophytes and sporophytes (Richardson & Dixon 1970), a feature that has never been reported in *Himehibirhodia*, *Nudresdaya* and *Dudresnaya* (Table 1). Our molecular phylogenetic analyses also support *Thuretellopsis* as distinct from the three other genera.

Himehibirhodia and *Nudresdaya* exhibit similar post-fertilization development of the carpogonial branches yet differ in life cycle (Table 1). Notoya (1988) cultured the carpospores of *H. minima* and reported a heteromorphic life cycle with a crustose tetrasporophyte and tetrasporangia that are irregularly cruciately divided. By contrast, *N. littleri* has an isomorphic life cycle with the isomorphic tetrasporophyte having zonately divided tetrasporangia (Abbott 1999; Abbott & McDermid 2001). Although the phylogenetic relationship between *Himehibirhodia* and *Nudresdaya* is unclear, the differences in life cycle strongly support the distinction of the two genera.

Genetic and morphological variation in *Dudresnaya*

After the exclusion of *H. minima* and *N. littleri*, the genus *Dudresnaya* can be defined by the persistence of trichogynes even after post-fertilization development, in addition to the traditional characters of *Dudresnaya* (Robins & Kraft 1985). However, this newly defined *Dudresnaya* still exhibits significant morphological diversity (Table 1). The most significant trait is the variation in life cycle: *Dudresnaya* includes species with an isomorphic life cycle and species with a heteromorphic life cycle (Table 1). We observed that the immature discoid tetrasporophyte of *D. ryukyuensis* developed erect thalli

Table 1. Morphological comparison of *Dudresnaya*, *Thuretellopsis* and the newly established genera, *Himehibirhodia* and *Nudresdaya*

	Dudresnaya lineage i	*Dudresnaya* lineage ii, iii	*Thuretellopsis*	*Himehibirhodia* gen. nov.	*Nudresdaya* gen. nov.
Branching pattern of erect thalli	Usually irregularly branched	Usually irregularly branched	Pinnately branched	Irregularly dichotomously branched	Irregularly branched
Annulations on young branch of erect thalli	Absent	Present/Absent	Present	Present	Absent
Hexagonal crystal in axial cells	Absent	Present	?	Absent	Absent
Disappearance of trichogyne during post-fertilization development	Absent	Absent	?	Present	Present
Shape of auxiliary cell	Flattened and smaller than its neighboring cells of the auxiliary cell branch	Flattened and smaller than its neighboring cells of the auxiliary cell branch	Same shape and size as its neighboring cells of the auxiliary cell branch	Rounded and the same size as its neighboring cells of the auxiliary cell branch	Rounded as its neighboring cells of the auxiliary cell branch
Tetrasporophyte	Crustose, but reported to be erect in *D. hawaiiensis*	Erect	Crustose	Crustose	Erect
Division of tetrasporangia	Cruciate, but reported to be zonate in *D. hawaiiensis*	Zonate	Cruciate	Irregularly cruciate	Zonate
Monosporangia	Absent	Absent	Present	Absent	Absent

bearing spermatangia. The erect thalli probably did not originate from tetraspores that had formed unnoticed because, if tetraspores had formed, both male and female gametophytes would have developed, but no erect thalli bearing carpogonial branches were observed. Occurrence of both sexual organs and tetrasporangia on a single plant has occasionally been reported in the Rhodophyceae (West & Norris 1966; Edelstein & McLachlan 1967; Rueness & Rueness 1985). However, we speculate that this phenomenon in *D. ryukyuensis* was an abnormal occurrence as a result of laboratory conditions, because these erect thalli formed after the long cultivation in the culture condition that is unsuitable for tetrasporophyte maturation (i.e. 20°C LD). Thus, we consider *D. ryukyuensis* to have a heteromorphic life cycle with erect gametophytes and discoid tetrasporophytes.

Besides *D. ryukyuensis*, *D. japonica* is known to have discoid tetrasporophytes (Notoya & Aruga 1989). In the molecular phylogenetic tree, *D. japonica* and *D. ryukyuensis* belong to lineage i with *D. hawaiiensis* and *D.* '*hawaiiensis*' from Australia and New Caledonia. In lineage i, *D. ryukyuensis*, *D. hawaiiensis* and *D.* '*hawaiiensis*' form a clade, and *D. japonica* is sister to the clade. Considering this topology, lineage i is expected to have discoid tetrasporophytes; however, Abbott (1999) and Abbott and McDermid (2001) reported erect tetrasporophytes in *D. hawaiiensis*. These reports should be re-examined because, except for these, tetrasporophytes have never been found in *D. hawaiiensis* (Lee 1963) and *D. hawaiiensis* from Australia (Robins & Kraft 1985; Huisman 2018). It is noteworthy that Robins and Kraft (1985) examined over 100 specimens of *D. hawaiiensis* from Australia, which is probably the same species as *D.* '*hawaiiensis*' from Australia in Fig. 9, but could never find tetrasporophytes. Furthermore, none of the *D. hawaiiensis* specimens, from which the sequence data that we used were generated, were morphologically tetrasporophytes. These facts raise the possibility that *D. hawaiiensis* has discoid tetrasporophytes, and that Abbott misidentified tetrasporophytes of other species as those of *D. hawaiiensis*. Indeed, a species showing an isomorphic life cycle, *D. babbittiana*, described by Abbott and McDermid (2001) as an endemic to Midway Atoll, was shown to be distributed around the type locality of *D. hawaiiensis* (Sherwood *et al*. 2022; present study).

By contrast to lineage i, lineage ii and iii include species with an isomorphic life cycle (Robins & Kraft 1985; Kajimura 1994; Abbott & McDermid 2001). Their sporophytes exhibit zonately divided tetrasporangia, whereas those of lineage i (*D. japonica* and *D. ryukyuensis*) exhibit cruciately divided tetrasporangia. Furthermore, hexagonal crystals are commonly observed in axial cells in lineages ii and iii (Robins & Kraft 1985; Kajimura 1994; Abbott & McDermid 2001), but never in lineage i.

Considering the genetic and morphological variations, *Dudresnaya* could arguably be separated into multiple genera. Although the phylogenetic position of the generitype *D. verticillata* was unclear because of its paraphyly, true *D. verticillata* probably belongs to lineage ii or iii because the lectotype of *D. verticillata* is an erect tetrasporophyte with zonately divided tetrasporangia (Lindstrom 1985). Therefore, at minimum, lineage i could be recognized as a new genus. Nevertheless, in the present study, we do not make further taxonomic revisions on *Dudresnaya* because of insufficient molecular data and a lack of information on the life cycle of *Dudresnaya* species. Out of 19 described *Dudresnaya* species, reliable molecular data (i.e. data from type specimens or specimens collected close to the type locality) have been obtained for only six species: *D. ryukyuensis*, *D. hawaiiensis*, *D. japonica, D. babbittiana*, *D. kuroshioensis* and *Dudresnaya australis*. Tetrasporophyte morphology is not known in some species, probably because some of them have small discoid tetrasporophytes that require culture experiments to observe. For some species, a different morphology and/or habitat information have been reported in the original description and in subsequent reports (e.g. *D. verticillata*, *D. australis*, *D. capricornica*, *D. hawaiiensis* and *Dudresnaya crassa* M.A.Howe). Molecular phylogenetic studies on more species/specimens with detailed descriptions of morphology and life cycle are necessary to solve the taxonomic problem of *Dudresnaya*.

Non-monophyletic dumontiaceae

Our molecular phylogenetic analyses showed that the Dumontiaceae is not monophyletic and includes the Gainiaceae and Rhizophyllidaceae (DGR complex), as shown in other studies (Tai *et al*. 2001; Dixon *et al*. 2015). The Gainiaceae is a monotypic family for the Antarctic alga *Gainia mollis* Moe and was established based on its non-calcareous crustose habit, isomorphic life cycle and nemathecial development of reproductive structures (Moe 1985). The Rhizophyllidaceae consists of four genera: *Contarinia* Zanardini, *Ochtodes* J. Agardh, *Portieria* Zanardini and *Nesophila* Nelson & Adams. Although these genera are mostly different from each other in vegetative morphology, they share large prominent gland cells and nemathecial development of reproductive structures as the synapomorphy (Wiseman 1975; Nelson & Adams 1996; Payo *et al*. 2011). In our opinion, the distinction among the Dumontiaceae, Gainiaceae and Rhizophyllidaceae is ambiguous. Most of the diagnostic traits of the Gainiaceae and Rhizophyllidaceae are also seen in dumontiacean taxa. Non-calcareous crustose habit and isomorphic life cycle have been reported in *Wearnia* (Wilce *et al*. 2003), and nemathecial development of reproductive structures has been reported in *Wearnia* and *Rhodopeltis* (Nozawa 1970; Itono & Yoshizaki 1992; Wilce *et al*. 2003).

The DGR complex shares (i) separate carpogonial branches and auxiliary cell branches (non-procarpic) that are more than two-celled and distinct from vegetative branches and (ii) fusion of the carpogonium with other cells of the carpogonial branch prior to formation of the secondary connecting filaments (Wiseman 1977; Shepley & Womersley 1983; Moe 1985; Payo *et al*. 2011). In the Florideophyceae, these features are also observed in the Peyssonneliales and Acrosymphytales (Kylin 1925; Lindstrom 1987; Kato & Masuda 2000), but, within the Gigartinales, they are unique to the DGR complex. Among the Gigartinales, the Ptilocladiopsidaceae, Kallymeniaceae and Etheliaceae were phylogenetically close to the DGR complex. Although the reproductive structure of the Etheliaceae is unknown, the female reproductive structures and their post-fertilization development of the former two families are generally similar to those of the DGR complex. However, the Ptilocladiopsidaceae differs from the DGR complex in that its auxiliary cell branches are two-celled (Rodríguez-Prieto *et al*. 2014). In the non-procarpic taxa of the Kallymeniaceae,

the carpogonia are located in the carpogonial branch system and the auxiliary cells in the auxiliary cell system (Rodríguez-Prieto & Hommersand 2009; D'Archino *et al*. 2016), which are distinct from those of the DGR complex. Thus, the female reproductive structures and their post-fertilization development mentioned above might be the synapomorphy of the DGR complex in the Gigartinales.

To solve the taxonomic problem of the DGR complex, two proposals can be considered: (i) integrating the Gainiaceae and Rhizophyllidaceae into the Dumontiaceae based on the potential synapomorphy mentioned above or (ii) splitting the DGR complex into multiple families in a manner consistent with the molecular phylogeny. For the second proposal, assuming the current topology of the phylogenetic tree is correct, one possible approach would be to either divide clade E and the others into two families or, in an extreme case, to split each of clade A to E into separate families. However, either case is challenging because there are currently no apparent diagnostic characters for these potential families, except the monogeneric ones. Thus, at present, the first proposal appears to be reasonable. We advocate for the Gainiaceae and Rhizophyllidaceae to no longer be recognized and consider the DGR complex as the Dumontiaceae *sensu lato*.

Taxonomic summary

Dumontiaceae Bory emend. M.Hoshino & Kogame

Description: The thalli are erect or crustose, uniaxial or multiaxial, non-calcareous or calcareous. The reproductive structures are sometimes in nemathecia. The female reproductive structure is non-procarpic. The carpogonial branches are at least four-celled, usually unbranched, distinctly differentiated from the vegetative branches; the carpogonium terminal on the carpogonium branch. The auxiliary cell branches are at least three-celled, usually unbranched, somewhat to distinctly differentiated from the vegetative branches; the auxiliary cell mostly intercalary in the auxiliary cell branch, but rarely terminal. The fertilized carpogonium generally fuses with one or more cells of the carpogonial branch itself prior to production of secondary connecting filaments that fuse with the auxiliary cells. The carposporophyte develops from the fusion complex of the auxiliary cell and the connecting filament; the gonimoblast filaments radiate, almost all the gonimoblast cells convert to carposporangia, resulting in a globular cluster of carposporangia. The spermatangia are cut off from outer cortical cells. The tetrasporangia are zonately or cruciately or irregularly divided. Life history, where known, is triphasic with isomorphic or heteromorphic gametophytes and tetrasporophytes.

Type genus: *Dumontia* J.V.Lamouroux.

Dudresnaya ryukyuensis *M.Hoshino, Wakeman, Kitayama & Kogame sp. nov.*

For the description, see the Results section.

Himehibirhodia *M.Hoshino, Kitayama & Kogame gen. nov.*

Description: The gametophytes are erect, gregarious, attached to substrata by a small discoid holdfast, uniaxial, cylindrical, irregularly subdichotomously branched. Color is crimson to light purple. Texture very soft, gelatinous and lubricous. The branches are annulated. The cortical fascicles are branched di- or tri-chotomously, with a colorless hair at the terminus. The outer cortical cells are cylindrical. The gametophytes dioicous. The spermatangia are produced at the distal end of the terminal cells of the cortical fascicles. The carpogonial branches are usually 6–7 celled including the basal cell and strongly crooked near the terminus. The auxiliary cell branches are typically 6–9 celled, all cells are modified and rounded, including the terminal cells; the auxiliary cell is intercalary, usually the third cell from the base, the same in size and shape as neighboring cells of the branch, but more transparent and showing a large nucleus. After fertilization, the carpogonium directly fuses with the fourth cell (counting from the terminal carpogonium) of the carpogonium branch that is in contact with it, and then cuts off a primary connecting filament fusing with the fifth cell. The resulting two fusion complexes cut off secondary connecting filaments to the auxiliary cells. The trichogyne degenerates and disappears by the time the primary connecting filament fuses with the fifth cell. Carposporophytes up to 150 μm in diameter. The released carpospores are 28–35 μm in diameter. The tetrasporophytes are crustose, and the tetrasporangia are irregularly cruciate. The released tetraspores are 20–25 μm in diameter.

Diagnosis: This genus resembles *Dudresnaya*, *Thuretellopsis* and *Nudresdaya* in being uniaxial, gelatinous and lubricous. However, it differs from *Dudresnaya* with respect to the disappearance of the trichogyne during the post-fertilization development of the carpogonial branch; from *Thuretellopsis* with respect to lacking production of monospores; and from *Nudresdaya* with respect to having a heteromorphic life cycle.

Generitype: *Himehibirhodia minima* (Okamura) M.Hoshino, Kitayama & Kogame comb. nov.

Etymology: The genus is named after the Japanese name of the generitype *H. minima*, Hime-hibirodo (Okamura 1932). The genus name is feminine in gender.

Japanese name: Hime-hibirodo.

Remark: The description and the diagnosis of this genus are based on the descriptions of *D. minima* (Okamura 1932; Hasegawa 1949; Segawa & Ichiki 1958, as *Thuretellopsis japonica*; Kawashima 1959; Notoya 1988).

Himehibirhodia minima *(Okamura) M.Hoshino, Kitayama & Kogame comb. nov.*

Basionym: *Dudresnaya minima* Okamura, in Okamura, K., 1932, Icones of Japanese algae. Vol. VI: 81, pl. CCXCII: figs 6–12.

Heterotypic synonym: *Thuretellopsis japonica* Segawa & Ichiki.

Lectotype: A specimen (Ondo, Hiroshima Pref., Japan; 3 June 1931) in the SAP Okamura collection (Yoshida 1998).

Distribution: Japan.

Remark: Considering the relatively large intraspecies variation in *cox*1 sequences (3.5% in *p*-distance), this species may include at least two cryptic species.

Japanese name: Hime-hibirodo (Okamura 1932).

Nudresdaya *M. Hoshino, A. R.Sherwood & Kogame gen. nov.*

Description: The gametophytes are erect, pale red in color uniaxial, cylindrical, narrow and irregularly branched. Texture is very soft, gelatinous and lubricous. The cortical fascicles are branched dichotomously. The distal cells of the cortical fascicles are oval to obpyriform and the proximal cells are cylindrical. The gametophytes are dioicous. The spermatangia are produced at the distal end of the terminal cells of the cortical fascicles, or form corncob-like spermatangial structures. The carpogonial branches are 6–10 celled including the basal cell, and strongly crooked near its terminal end. The auxiliary cell branches are 10–16 celled of which the 4–6 cells are enlarged and rounded, and the auxiliary cell is the second of the enlarged cells from the proximal end. After fertilization, the carpogonium fuses with the fourth cell (counting from the terminal carpogonium) of the carpogonial branch by an extremely short primary connecting filament, and resulting fusion complex cuts off a second primary connecting filament fusing with the fifth cell. The resulting two fusion complexes cut off secondary connecting filaments to the auxiliary cells. The trichogyne degenerates and disappears during the post-fertilization development. The carposporophytes are up to 430 μm in diameter. The released carpospores are 24 μm in diameter. The tetrasporophytes are isomorphic to the gametophytes. Tetrasporangia are zonately divided.

Diagnosis: This genus resembles *Dudresnaya*, *Thuretellopsis* and *Himehibirhodia* in that plants are uniaxial, gelatinous and lubricous. However, it differs from *Dudresnaya* in the disappearance of the trichogyne during the post-fertilization of the carpogonial branch, and differs from *Thuretellopsis* and *Himehibirhodia* in having an isomorphic life cycle.

Generitype: *Nudresdaya littleri* (I.A.Abbott) M.Hoshino, A.R.Sherwood & Kogame comb. nov.

Etymology: The name is an anagram of *Dudresnaya*. The genus name is feminine in gender.

Remark: The description and the diagnosis of this genus are based on the descriptions of *Dudresnaya littleri* (Littler 1974, as *Dudresnaya lubrica*; Abbott 1999; Abbott and McDermid 2001).

Nudresdaya littleri *(I.A.Abbott) M. Hoshino, A. R. Sherwood & Kogame comb. nov.*

Basionym: *Dudresnaya littleri* I.A.Abbott, in Abbott, I. A., Abbott 1996, Pacific Science 50: 151.

Heterotypic synonym: *Dudresnaya lubrica* Littler, *nom. illeg.*

Holotype: BISH 518022 collected offshore of Mākua, O'ahu, Hawai'i, deposited in BISH (Abbott 1999).

Distribution: Hawai'i.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Maximum likelihood tree based on the second dataset showing the phylogeny of Gigartinales families: 58 OTUs, concatenated DNA sequences of mitochondrial *cox*1 and chloroplast *rbc*L and nuclear 28S ribosomal RNA genes (total 4804 bp). Only bootstrap values >70 and posterior probabilities >0.95 are shown. For details, see Fig. 9.

Fig. S2. Maximum likelihood tree based on DNA sequences of mitochondrial *cox*1 (664 bp). Only bootstrap values >80 are shown. For details, see Fig. 9.

Fig. S3. Maximum likelihood tree based on DNA sequences of chloroplast *rbc*L (1363 bp). Only bootstrap values >70 are shown. For details, see Fig. 9.

Fig. S4. Maximum likelihood tree based on DNA sequences of 28S (2774 bp). Only bootstrap values >75 are shown. For details, see Fig. 9.

Table S1. *Dudresnaya* specimens examined in the present study.

Table S2. Primers used in the present study.

Table S3. Specimen codes and DNA sequences of the first dataset of the phylogenetic analyses: the dataset to infer the phylogeny of dumontiacean species, consisting of 63 OTUs (operational taxonomic units) from the Dumontiaceae, five phylogenetically close families (22 OTUs from the Gainiaceae, Rhizophyllidaceae, Kallymeniaceae, Etheliaceae, Ptilocladiopsidaceae) and the *Polyides rotunda* (Polyidaceae) as outgroup.

Table S4. Specimen codes and DNA sequences of the second dataset of the phylogenetic analyses: the dataset to infer the phylogeny of Gigartinales, consisting of 56 OTUs from the 32 Gigartinales families and two OTUs from the Acrosymphytales as outgroup.