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CERAMIUM INKYUII SP. NOV. (CERAMIACEAE, RHODOPHYTA) FROM KOREA: A NEW SPECIES BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE[1]

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***Ceramium inkyuii* sp. nov. is newly described based on samples collected from the east coast of Korea and compared with similar species such as *C. paniculatum* and *C. tenerrimum*. The new species is characterized by pseudo-dichotomously branched thalli with a twist in the upper part, a single row of spines on the abaxial side, strongly inrolled apices, and the presence of gland cells. In contrast, *C. paniculatum* has alternate branches and lacks gland cells, and *C. tenerrimum* is spineless and also lacks gland cells. *Ceramium inkyuii* was observed to be an annual species producing tetrasporangia in the spring to summer and cystocarps in the fall. Plastid-encoded *rbc*L and nuclear small subunit (SSU) rDNA sequences were determined in four samples of *C. inkyuii* from different locations and six samples of four putative relatives. All four *C. inkyuii* replicates from three different locations had identical sequences of each gene, and the interspecific sequence divergences were enough to warrant its natural entity. The phylogenies of the *rbc*L and SSU rDNA sequences also indicate the monophyly of *C. inkyuii*. The spinous *C. inkyuii* was more closely related to the spineless *C. tenerrimum* than to the spinous *C. paniculatum*.**

***Key index words: Ceramium*; *C. inkyuii*; Ceramiales; phylogeny; *rbc*L; Rhodophyta; SSU rDNA; taxonomy**

Ceramium is a cosmopolitan red algal genus that frequently occurs in the tropical to polar coasts of both hemispheres (Boo and Lee 1994). The genus is characterized by cylindrical axial cells that are incompletely to completely covered by cortical cells, alternate to pseudo-dichotomous branching, straight to inrolled apices, tetrasporangia produced from periaxial to cortical cells, spermatangia occurring on cortical nodes, and spherical cystocarps surrounded with involucral branches (Dixon 1960, Hommersand 1963, Womersley 1978, Cho et al. 2001). *Ceramium* is one of the largest genera in the rhodophytes, with approximately 191 species worldwide, and recent monographic studies have added new members to the genus (Meñeses 1995, South and Skelton 2000). However, the taxonomy of *Ceramium* at the species level is still in a state of chaos because of a lack of knowledge pertaining to morphological and anatomical variability (Boo and Lee 1994).

Taxonomic revisions of *Ceramium* in the North Pacific began with the first major treatment by Agardh (1894). Subsequent studies (Setchell and Gardner 1924, Dawson 1950, Itono 1977, Womersley 1978) reported numerous species but also synonymized additional taxa. Approximately 57 species are currently recognized for the North Pacific (Setchell and Gardner 1924, Dawson 1962, Nakamura 1965, Itono 1977, Abbott 1999). Of these species, *C. paniculatum* and *C. tenerrimum* are well-defined members in the Northwest Pacific (Nakamura 1965). *Ceramium paniculatum* is characterized by alternate branches, slightly incurved apices, and an abaxial row of spines on the upper branchlets (Okamura 1921, Nakamura 1965) and *C. tenerrimum* by pseudo-dichotomous branches, inrolled apices, and a thin cortex (Martens 1866, Nakamura 1965). The distributional ranges of these two species overlap in Japan (Nakamura 1965). However, distribution of *C. paniculatum* is reported to extend to Baja California, Mexico (Dawson 1950) and that of *C. tenerrimum* to the Mediterranean and Indian Ocean (Silva et al. 1996).

During continuing investigations of the marine benthic algae of Korea, a number of unidentified *Ceramium* specimens were collected along the east coast that superficially resembled both *C. tenerrimum* and *C. paniculatum*. Here we describe the unidentified species as *C. inkyuii* sp. nov. on the basis of its vegetative and reproductive morphology and provide further evidence of its natural entity using comparative analyses of plastid-encoded *rbc*L gene and nuclear small subunit (SSU) rDNA from selected members of *Ceramium*.

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MATERIALS AND METHODS

Morphology and phenology. Material of *C. inkyuii* was collected on the east coast of Korea (Fig. 1) and preserved in 4% formaldehyde/seawater for morphological observations. Microscopic observations were made from material stained with 1% aqueous aniline blue acidified with 1% HCl. Drawings were made with a camera lucida attached to a Vanox light microscope (Olympus Optical Co, Tokyo, Japan). Voucher specimens used in this study were deposited in the herbarium of Chungnam National University (CNUK), Daejon, Korea. A total of 25 individuals from 10 tufts were selected for measuring quantitative characters. *Ceramium paniculatum* and *C. tenerrimum* were collected on the south and east coasts of Korea for comparison (Fig. 1). The reproductive phenology of *C. inkyuii* was observed in Gampo on the east coast every 3 months from May 1999 to February 2000.

DNA extraction, amplification, and sequencing. Replicate samples of *C. inkyuii* used for DNA extraction were desiccated in silica gel or air dried in the field and isolated in the laboratory. Samples of *C. affine*, *C. paniculatum*, and *C. tenerrimum* were prepared for comparison, and a fully corticated spinous *C. horridum* was used as outgroup (Table 1).

Genomic DNA was prepared using a hexadecyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). Approximately 2 mg of ground algal tissue was incubated in 500 μL of 2% CTAB buffer (with the addition of 2% β-mercaptoethanol) at 60° C for 45 min. Extractions with chloroform-isoamyl alcohol (24:1 vol/vol) were performed repeatedly until complete removal of the interphase was achieved. Nucleic acids were precipitated with 1 mL of 100% cold ethanol (with 10 μL of 2M sodium acetate) at −70° C for 30 min, pelleted by centrifugation (13,000 rpm at 4° C for 10 min), air dried, and dissolved in 200 μL distilled water. The DNA was reprecipitated with the addition of 500 μL of 90% cold ethanol at −70° C for 30 min, washed with 70% ethanol twice, air dried, and then dissolved in 150 μL distilled water.

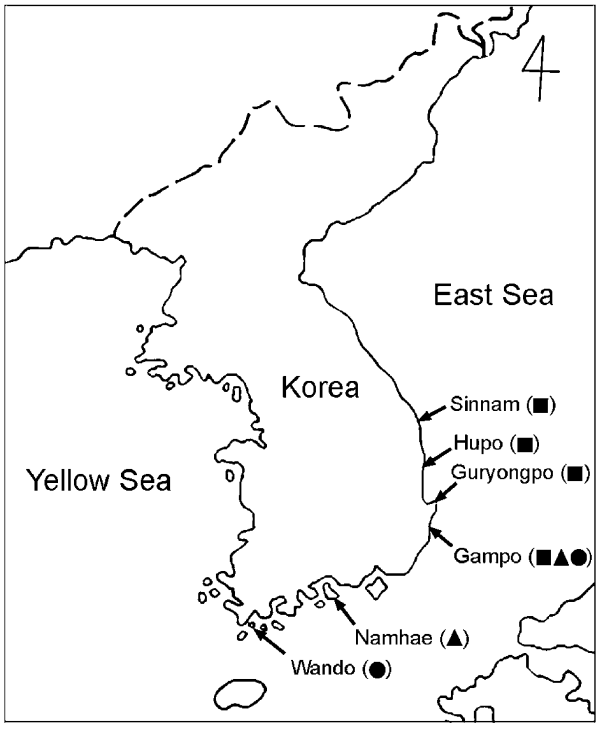

FIG. 1. Distribution of *Ceramium inkyuii* (■) in Korea, and collection sites of *C. paniculatum* (●) and *C. tenerrimum* (▲) for a molecular comparison. See Table 1 for details.

Amplifying (F7-R753 and F645-RrbcS start) and sequencing (F7, F645, R753, and RrbcS start) primers of the *rbc*L are listed in Freshwater and Rueness (1994), Lin et al. (2001), and Gavio and Fredericq (2002). The amplification, purification of the PCR products, and cyclic sequencing were performed as described by Lin et al. (2001). Sequences were determined for both forward and reverse strands using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

Primers (G01–G10, G02–G14, G04–G13, and G06–G07) of the SSU rDNA, developed by Saunders and Kraft (1994), were used in the present study. Amplification conditions consist of 4 min at 94° C for denaturation, followed by 36 cycles of 30 s at 94° C, 30 s at 50° C, and 90 s at 72° C, with a final 6-min extension cycle at 72° C. Further processing of the SSU amplicons was the same as for the *rbc*L (see above).

The generated sequence data of the *rbc*L and SSU rDNA were compiled and the data sets for each gene were manually aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA). The *rbc*L data set was directly exported for phylogenetic analyses, whereas the alignment of the SSU rDNA sequences was edited in MacClade (v. 4.0, Maddison and Maddison 2000) and then exported for phylogenetic analyses. The final data matrix was restricted to 1434 bp for the *rbc*L except the first 33 bp because of priming sites and 1731 bp for the SSU rDNA. Both sequences are available at GenBank (Table 1). Phylogenetic analyses of the *rbc*L, SSU rDNA, and the concatenated *rbc*L + SSU rDNA data were performed using the maximum likelihood (ML) and maximum parsimony (MP) algorithms available in the PAUP* (v. 4.0b10, Swofford 2002). For the ML analyses, the aligned sequences were first analyzed with the software Modeltest (v. 3.0, Posada and Crandall 1998) which compared different models of DNA substitutions in a hierarchical hypothesis-testing framework to select a base substitution model that best fits our sequence data. The optimal model of the *rbc*L sequence was a GTR (general time reversible model, Rodriguez et al. 1990) + G (gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0.3235, C = 0.1591, G = 0.2077, T = 0.3097; substitution rate matrix with A-C substitutions = 0.4746, A-G = 3.6713, A-T = 4.5252, C-G = 0.5018, C-T = 45.5104, G-T = 1.0000; rates for variable sites assumed to follow a gamma distribution with shape parameter = 0.0742. The optimal model of SSU rDNA sequence was a TrN (Tamura-Nei model, Tamura and Nei 1993) + I (invariable sites). The parameters were as follows: assumed nucleotide frequencies A = 0.2451, C = 0.2072, G = 0.2852, T = 0.2625; substitution rate matrix with A-C substitutions = 1.0000, A-G = 2.3926, A-T = 1.0000, C-G = 1.0000, C-T = 5.4349, G-T = 1.0000; proportion of sites assumed to be invariable = 0.8545. The optimal model of *rbc*L + SSU rDNA sequence was a GTR + I. The parameters were as follows: assumed nucleotide frequencies A = 0.2807, C = 0.1860, G = 0.2501, T = 0.2832; substitution rate matrix with A-C substitutions = 0.9525, A-G = 2.6855, A-T = 2.3970, C-G = 1.0025, C-T = 15.9137, G-T = 1.0000; proportion of sites assumed to be invariable = 0.8016. The ML tree was generated by a heuristic search of 100 random additions holding one tree at each step under the invoked settings for the respective base substitution model.

MP trees of the *rbc*L, SSU rDNA, and *rbc*L + SSU rDNA were inferred from an exhaustive search option. For the MP analysis, the first 33 bp from the aligned sequence data were removed and the uninformative characters were also excluded. Support for nodes was determined by calculating bootstrapping proportion values (Felsenstein 1985) using 100 bootstrap replicates for the ML and 1000 replicates for MP analyses.

RESULTS

Ceramium inkyuii Cho, Fredericq et Boo, sp. nov.

Thalli epiphytici, affixi ad hospitem per curta ramosa rhizoidea, 2.0–5.5 cm alti, 80–20 μm diametro, partim prostrati, partim erecti, ramosi pseudodichotomi, leviter torti supra, corti-

TABLE 1. Samples of *Ceramium inkyuii* and relatives used for analyses of both *rbc*L and SSU rDNA sequences.

Species	Location, data, collector, and voucher	GenBank accession no.	
		*rbc*L	SSU
C. affine Setchell et Gardner	Pichilingue, Baja California, Mexico; 27.v.2000; T. O. Cho & R. R. Rodriguez; CNUK TC242a	AF521797	AF460859
C. inkyuii sp. nov.	Gampo, Kyungju, Korea; 19.v.1999; T. O. Cho & H. S. Yoon; CNUK 005023	AF521798	AF460860
	Guryongpo, Pohang, Korea; 8.iv.1999; S. M. Boo & T. O. Cho; CNUK TC190a	AF521799	AF460862
	Guryongpo, Pohang, Korea; 18.v.1999; T. O. Cho & H. S. Yoon; CNUK TC020a	AF521800	AF460861
	Sinnam, Gangwon, Korea; 7.vii.1999; T.O. Cho; CNUK TC122a	AF521801	AF460863
C. paniculatum Okamura	Gampo, Kyungbuk, Korea; 8.ix.1999; S. M. Boo & T. O. Cho; CNUK 002926	AF521802	AF460864
	Wando, Chunnam, Korea; 12.vi.1999; T. O. Cho & W. J. Lee; CNUK TC165a	AF521803	AF460865
C. tenerrimum (Martens) Okamura	Gampo, Kyungbuk, Korea; 8.ix.1999; S.M. Boo & T.O. Cho; CNUK TC187a	AF521804	AF460866
	Namhae, Kyungnam, Korea; 30.v.1999; T. O. Cho & H. S. Yoon; CNUK TC066a	AF521805	AF460867
C. horridum Setchell et Gardner	San Juan de La Costa, Baja California, Mexico; 15.vi.2000; T. O. Cho & R. R. Rodriguez; CNUK 004833	AF521796	AF460858

cati solum ad nodos; apices forcipulati, valde involuti; cellulae periaxiales 6–8; filamenta corticata 4, plerumque acropeta 3–5 cellulis; spinae 3–4 cellulis, spina unica ad nodum corticalem, disposita seriatim facie abaxiali; cystocarpia prope apices, circumcincta 5–6 ramis involucralibus; tetrasporangia 30–40 μm diametro, in verticilla usque, bracteata; antheridia ignota.

Thalli epiphytic, attached to host by short, branched rhizoids, 2.0 to 5.5 cm high, 80 to 120 μm in diameter, partly prostrate and partly erect, pseudo-dichotomously branched, twisted in the upper part, corticated only at the nodes; apices forcipulate, strongly inrolled; periaxial cells 6 to 8 in number; corticating filaments 4 in number, predominantly acropetal, 3 to 5 cells long; spines 3 to 4 celled, one spine per cortical node, arranged in a row on the abaxial side; cystocarps near the apices, surrounded by 5 to 6 involucral branches; tetrasporangia 30 to 40 μm in diameter, in whorls, bracteate; antheridia unknown.

Holotype: Tetrasporophyte, CNUK c000215 (Fig. 2). Collected by Boo & T. O. Cho on 8 September 1999. Deposited in the herbarium of CNUK.

Type locality: On cliff below the lighthouse in Gampo, Kyungju, Korea.

Etymology: The name *Ceramium inkyuii* is chosen to honor Dr. In Kyu Lee, Emeritus Professor at Seoul National University, for his significant contributions to the understanding of phycology and training of phycological students.

Representative specimens from Korea: Gampo, Kyungju (T. O. Cho & H. S. Yoon, 18.v.1999, CNUK 005023, 005025–005026, 005028, 005032–005033, 005039, 005041–005042, 005055–005056, vegetative; Boo & T. O. Cho, 17.viii.1999, CNUK 000218–000220, vegetative; Boo & T. O. Cho, 8.ix.1999, 000214–000216, tetrasporangial; T. O. Cho, 18.xi.1999, CNUK 000217, cystocarpic; Boo & T. O. Cho, 12.ii.2000, CNUK 002430, vegetative). Guryongpo, Pohang (Boo & T. O. Cho, 18.v.1999, CNUK TC020a; Boo & T. O. Cho, 19.iv.2001, CNUK TC190a). Hupo, Uljin (Boo & H. S. Yoon, 4.iii.2000, CNUK TC204a). Sinnam, Gangwon (T. O. Cho, 7.vii.1999, CNUK TC122a).

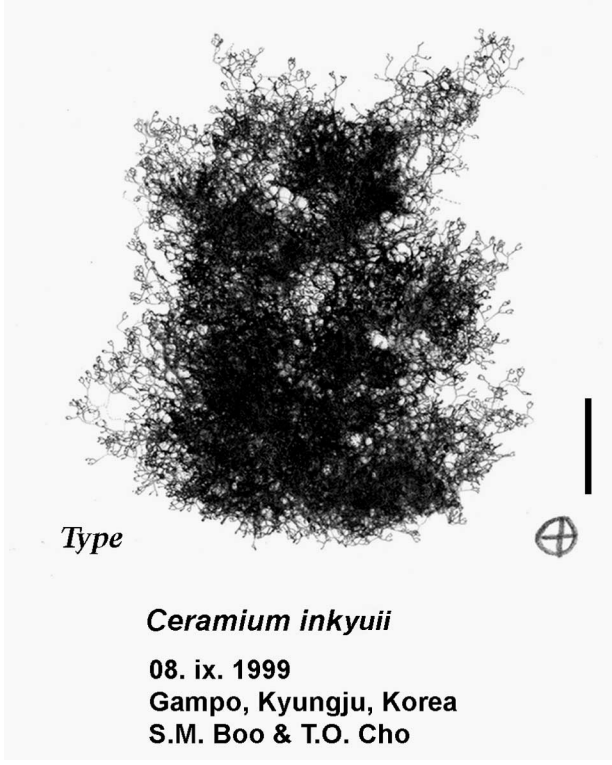

FIG. 2. Holotype (CNUK 000215) of *Ceramium inkyuii* sp. nov. Scale, 1 cm.

Morphology: Thalli are delicate, rose-pink in color, and 2.0 to 5.5 cm high. They form dense tufts and are twisted in the upper part (Fig. 3a). Thalli consist of interwoven prostrate axes giving rise to erect pseudo-dichotomous axes and more or less entangled mats bearing rhizoids at the base.

Erect axes have forcipulate strongly inrolled apical regions beset with spines (Fig. 3b) and are each composed of an axial cell and corticated nodes. Apical cells produce discoid segments by transverse division. Cortication develops first on the abaxial side of a discoid segment before the establishment of the periax-

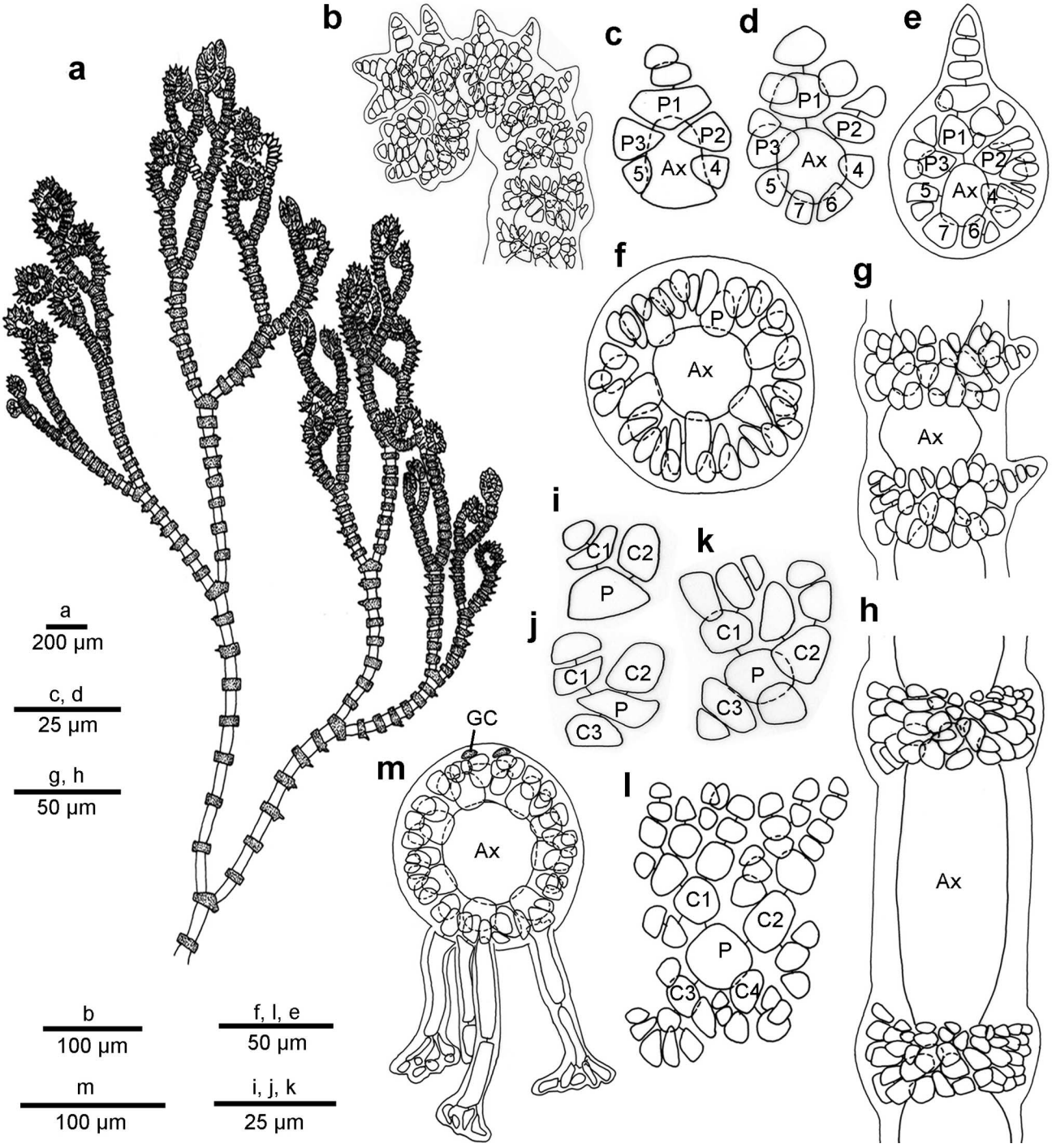

FIG. 3. *Ceramium inkyuii* sp. nov. (a) Thallus part showing pseudo-dichotomous twisted branching. (b) Apical region with spines on the abaxial side. (c–e) Alternate sequence of periaxial cell formation from an axial cell. (f) Cross-section showing axial, periaxial, and cortical cells. (g) Upper thallus part with narrow internode. (h) Lower thallus part with long internode. (i–l) Alternate sequence of cortical cell formation from periaxial cell. (m) Cross-section of prostrate part showing gland cell and rhizoid formation from periaxial and cortical cells. Ax, axial cell; C, cortical cell; C1–4, sequence of cortical cell formation; GC, gland cell; P, periaxial cell; P1–7, sequence of periaxial cell formation.

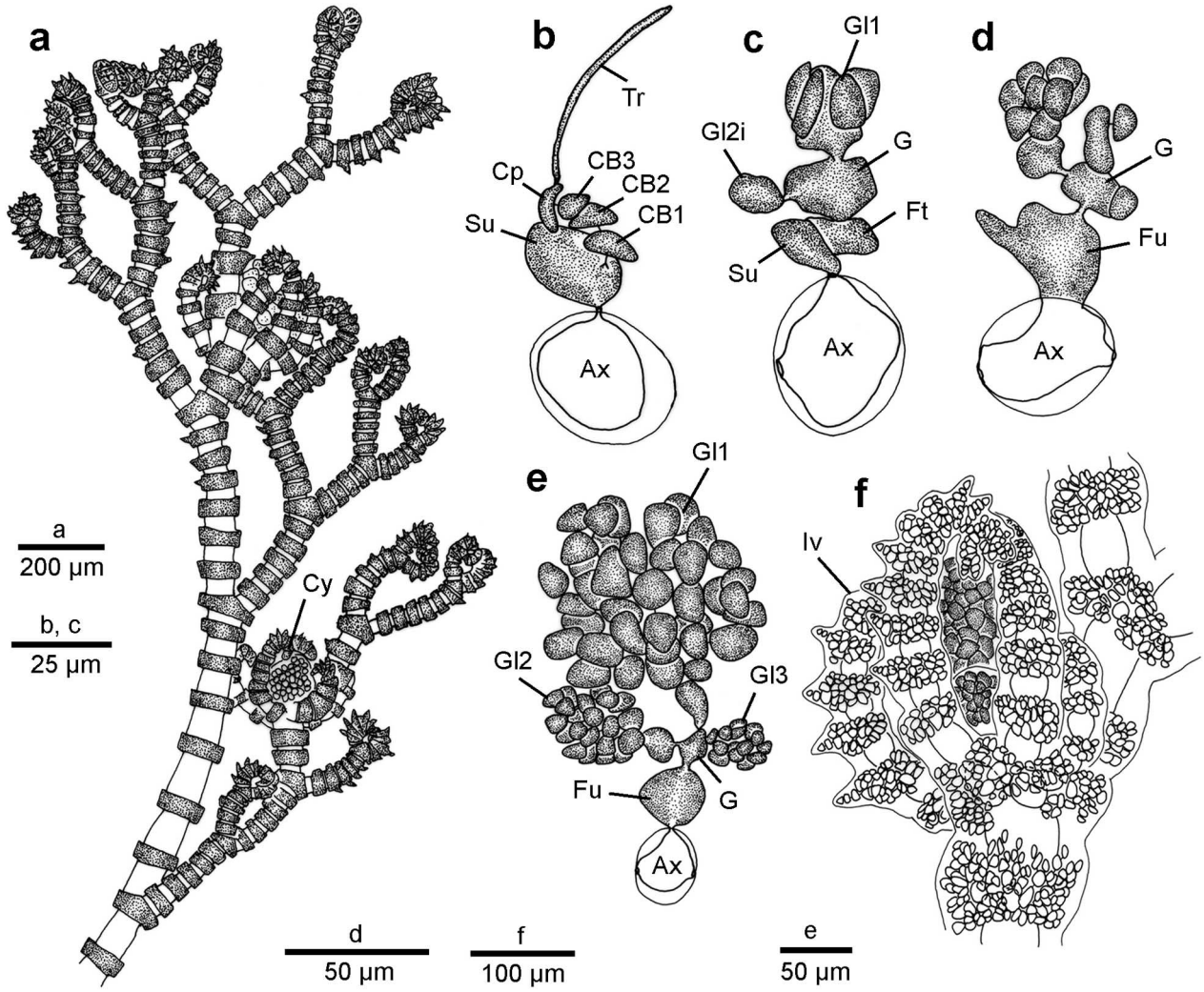

FIG. 4. *Ceramium inkyuii* sp. nov. (a) Female thallus bearing cystocarps. (b) Procarp composed of carpogonial branch, carpogonium, and supporting cell. (c) Formation of gonimolobe from gonimoblast initial. (d) Fusion cell by fusion of supporting cell, foot cell, and axial cell. (e) Three gonimolobes from gonimoblast initials. (f) Mature cystocarp surrounded by involucral branches. CB1–3, sequence formation of carpogonial branch cells; Cp, carpogonium; Cy, cystocarp; Ft, foot cell; Fu, fusion cell; G, gonimoblast initial; Gl1–3, sequence formation of gonimolobes; Gl2i, second gonimolobe initial; Iv, involucral branch; Su, supporting cell; Tr, trichogyne.

ial cells is completed on the adaxial side. Such uneven development of the cortication pattern that proceeds faster on the abaxial than on the adaxial side gives rise to the strongly inrolled apices (Fig. 3b). The axial cells are spherical to cylindrical and reach 220 ± 55 × 43 ± 5 μm at the level of the seventh dichotomy away from the apex. Six to eight periaxial cells are cut off obliquely from the upper part of each parent axial cell (Fig. 3, c–f) and remain at the nodes after axial cell elongation. The first periaxial cell is cut off on the abaxial side of the third to fourth axial cell below the apex. The remaining periaxial cells are then cut off in an alternating sequence, first to one and then to the other side of the first periaxial cell. All periaxial cells produce corticating filaments forming the cortex. The mature cortex is incomplete, covering the axial cells only at the nodes (Fig. 3, g and h). Four primary cortical cells are produced from each periaxial cell in an alternate sequence and develop into corticating filaments. The first two are cut off obliquely from the anterior end of periaxial cell and grow acropetally (Fig. 3i). The remaining two are produced obliquely from the posterior end and grow basipetally (Fig. 3, j and k). The acropetal corticating filaments predominate and are three to five cells long, and the basipetal corticating filaments occur in the lower thallus parts and are two to three cells long (Fig. 3l).

Branches are pseudo-dichotomous. When a new branch is produced, the terminal cell first divides obliquely, and the resulting adaxial apical initial functions as the leading apical cell of the main filament that elongates. Branching takes place at intervals of seven to nine (average, 6.8 ± 0.5) axial cells in the main axes and at the intervals of six to nine (average, 7.8 ± 0.5) cells in the lateral axes. In addition, adventitious branches develop from periaxial cells in the lower thallus parts.

A single spine occurs at each node on the abaxial side in the upper thallus parts (Fig. 3b) and is produced from the first-formed periaxial cell. Each spine is pigmented and three to four cells long with a multicellular base (Fig. 3e). Gland cells develop from cortical cells of acropetally and basipetally corticating filaments (Fig. 3m). They are ovoid to angular gland cells, averaging $7 \pm 1 \times 13 \pm 3$ μm. Rhizoids are multicellular, uniseriate, and branched near the tip. They are produced from periaxial and cortical cells (Fig. 3m).

Procarps are seriated in an abaxial row of the upper branches in female thalli. The carpogonial branch bearing the carpogonium with trichogyne is four-celled and borne laterally on the supporting cell, a transformed periaxial cell (Fig. 4b). After presumed fertilization, the supporting cell enlarges and cuts off an auxiliary cell. The auxiliary cell then divides into a foot cell and a gonimoblast initial. The gonimoblast initial cuts off the first gonimolobe terminally followed by one or two additional gonimolobe initials laterally (Fig. 4c). The foot cell fuses with the axial cell and the tiny supporting cell resulting in a large fusion cell (Fig. 4d). Nearly all gonimolobe cells become transformed into carposporangia at maturity (Fig. 4e). Mature cystocarps are spherical, 355 ± 30 μm long and 290 ± 35 μm in diameter. The involucral branches consist of five to

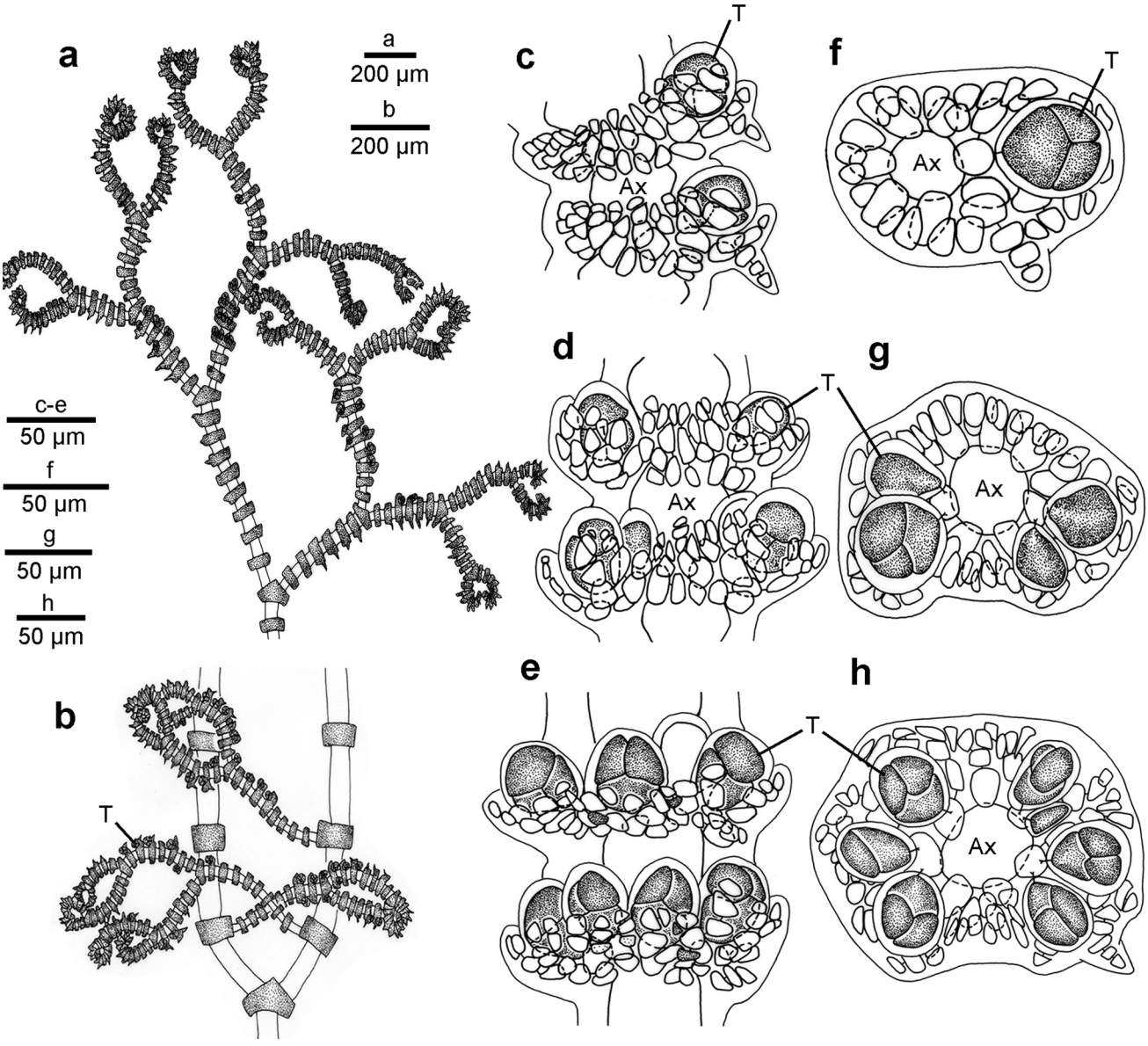

FIG. 5. *Ceramium inkyuii* sp. nov. (a) Tetrasporangial thallus. (b) Adventitious branch having tetrasporangia. (c) Detail of cortical node including tetrasporangia on the abaxial side. (d) Detail of cortical node including tetrasporangia on the both sides. (e) Detail of cortical node, including tetrasporangia in whorl. (f–h) Cross-section through cortical node having tetrahedral tetrasporangia, showing opposite sequence of their formation. T, tetrasporangia.

six finger-like branchlets (Fig. 4, a and f). Male thalli were not found in our collections.

Tetrasporangia are distributed in the upper part of the erect and adventitious branches (Fig. 5, a and b). They are produced initially from the first-formed periaxial cell on the abaxial side (Fig. 5, c–f) and then from the last-formed one on the adaxial side (Fig. 5, d, g, and h), resulting in a whorl around the axis (Fig. 5e) and indicating a pattern of opposite sequence. Two tetrasporangia develop from each periaxial cell (Fig. 5, g and h). Tetrasporangia are almost completely covered by cortical cells or partially project (Fig. 5, d and e). They are tetrahedrically divided, spherical to ellipsoidal, and average 41 ± 1 × 34 ± 3 μm excluding the sheath and 41 ± 4 × 39 ± 2 μm with the sheath. Photographs of vegetative and reproductive features of *C. inkyuii* are shown in Figure 6.

Habitat and phenology. *Ceramium inkyuii* was epiphytic on other algae such as *Corallina* spp. and occurred subtidally to a depth of 1 to 3 m in wave-exposed sites. All sites of its occurrence are on the east coast of Korea, where the warm Kuroshio Current coming from the south mixes with the cold Liman current flowing southward in the winter. Thalli were collected in February, May, September, and November in Gampo. Tetrasporangial thalli were collected in May and September and female thalli occurred in November. All thalli in February were vegetative.

Morphology and habitat of reference species. Specimens of *C. paniculatum* used for observation are deposited in CNUK as following: Gampo, Kyungju (T. O. Cho & Boo, 17.viii.1999, CNUK 000221–000222, 18.vii.2001, CNUK 006533). Thalli of *C. paniculatum* are 2 to 3 cm high and alternately branched (Fig. 7a). The erect axes have forcipulate and slightly incurved apices with spines (Fig. 7b). Periaxial cells are oblique and six to seven in number. The cortex is incomplete (Fig. 7c). Four primary cortical cells are produced from each of the periaxial cells. Although the acropetal corticating filaments predominate, measuring three to four cells long, basipetal corticating files are one to two celled in the lower thallus. Branching occurs at intervals of four to five (average, 4.2 ± 0.5) axial cells in the main axes and at intervals of five to six (average, 5.6 ± 0.6) cells in the lateral axes (Fig. 7a). Spines occur on the abaxial side of each node in the upper branches and develop from the first-formed periaxial cell. Each spine is pigmented and three to four cells long with a multicellular base (Fig. 7b). *Ceramium paniculatum* is epiphytic on other algae such as *Corallina* spp. in the subtidal zone of wave-exposed sites. It occurs mixed with *C. inkyuii* in Gampo and is commonly found on the south and east coast of Korea.

Specimens of *C. tenerrimum* used for observations were collected in Guryongpo on 18 May 1998 (CNUK 000107) and in Gampo on 17 August 1999 (CNUK 000223). Thalli of *C. tenerrimum* are 5 to 10 cm tall and pseudo-dichotomously branched (Fig. 7d). The erect axes have forcipulate and strongly inrolled apices lacking spines (Fig. 7e). Periaxial cells are oblique and six to seven in number. The cortex is incomplete (Fig. 7f). Acropetal corticating filaments predominate, whereas basipetal corticating filaments are rare. Branching occurs at intervals of 7 to 10 (average, 9.0 ± 0.8) axial cells in the main axes and at intervals of 7 to 11 (average, 9.5 ± 0.6) cells in the lateral axes (Fig. 7d). *Ceramium tenerrium* is epilithic or epiphytic on other algae and occurs in the intertidal zone and tide pools of the sheltered areas on the south and east coasts of Korea.

Table 2 summarizes tetrasporangial features and the dimension of cystocarp of *C. paniculatum* and *C. tenerrimum*, including other comparative morphology of both species to *C. inkyuii*. Spermatangial features of *C. paniculatum* and *C. tenerrimum* are of typical for the genus *Ceramium*.

Phylogenetic analyses. Both *rbc*L and SSU rDNA sequences were generated from a total of 10 samples: 4 from *C. inkyuii*, 2 from *C. paniculatum* and *C. tenerrimum*, and 1 from *C. affine* and *C. horridum*. A 1434-bp portion, with 79 parsimony informative sites, of the 1467-bp *rbc*L gene (= 97.7% sequenced) was determined. The *rbc*L sequences from the four samples of *C. inkyuii* were identical, as were each two samples of *C. paniculatum* and *C. tenerrimum*. However, the sequences of *C. inkyuii* and *C. paniculatum* differed by 36 nucleotides or 2.5% sequence divergence. Pair-wise comparison of *C. inkyuii* and *C. tenerrimum* revealed a difference of 39 positions or 2.7% sequence divergence. *Ceramium paniculatum* differed from *C. tenerrimum* by 54 nucleotides or 3.8% sequence divergence.

In the SSU rDNA sequences, a 1731-bp portion was sequenced that included 27 informative nucleotide positions. Sequences from the infraspecific populations of each species were identical. However, *C. inkyuii* differed from *C. paniculatum* by 12 nucleotides or 0.7% sequence divergence and from *C. tenerrimum* by 9 nucleotides or 0.5% sequence divergence. There was a difference of 17 nucleotides or 1.0% sequence divergence between *C. paniculatum* and *C. tenerrimum*.

All analyses of the *rbc*L, the SSU rDNA, and the *rbc*L + SSU rDNA sequence data showed the monophyly of *C. inkyuii*, clearly separated from both *C. tenerrimum* and *C. paniculatu*m (Fig. 8). The monophyly of *C. inkyuii* was supported by maximum bootstrap values in the *rbc*L (Fig. 8a) and the *rbc*L + SSU trees (Fig. 8c), despite their declines (59% for ML and 79% for MP) in the SSU tree (Fig. 8b). *Ceramium tenerrimum* was a sister to *C. inkyuii* in the SSU rDNA (Fig. 8b) and the concatenated trees (Fig. 8c). The sister relationship was supported by low bootstrap values (<50% for ML and 58% for MP) in the SSU tree (Fig. 8b) from low (54% for ML) to high (99% for MP) in the concatenated data analysis (Fig. 8c). In the *rbc*L analysis, *C. paniculatum* and *C. inkyuii* are sister groups (Fig. 8a), but the support for this topology is very low (<50% for both the ML and MP analysis). In a recently completed global *rbc*L + SSU rDNA analysis of 40+ *Ceramium* species worldwide, *C. tenerrimum* was consistently identified as the sister species to *C. inkyuii* (data not shown).

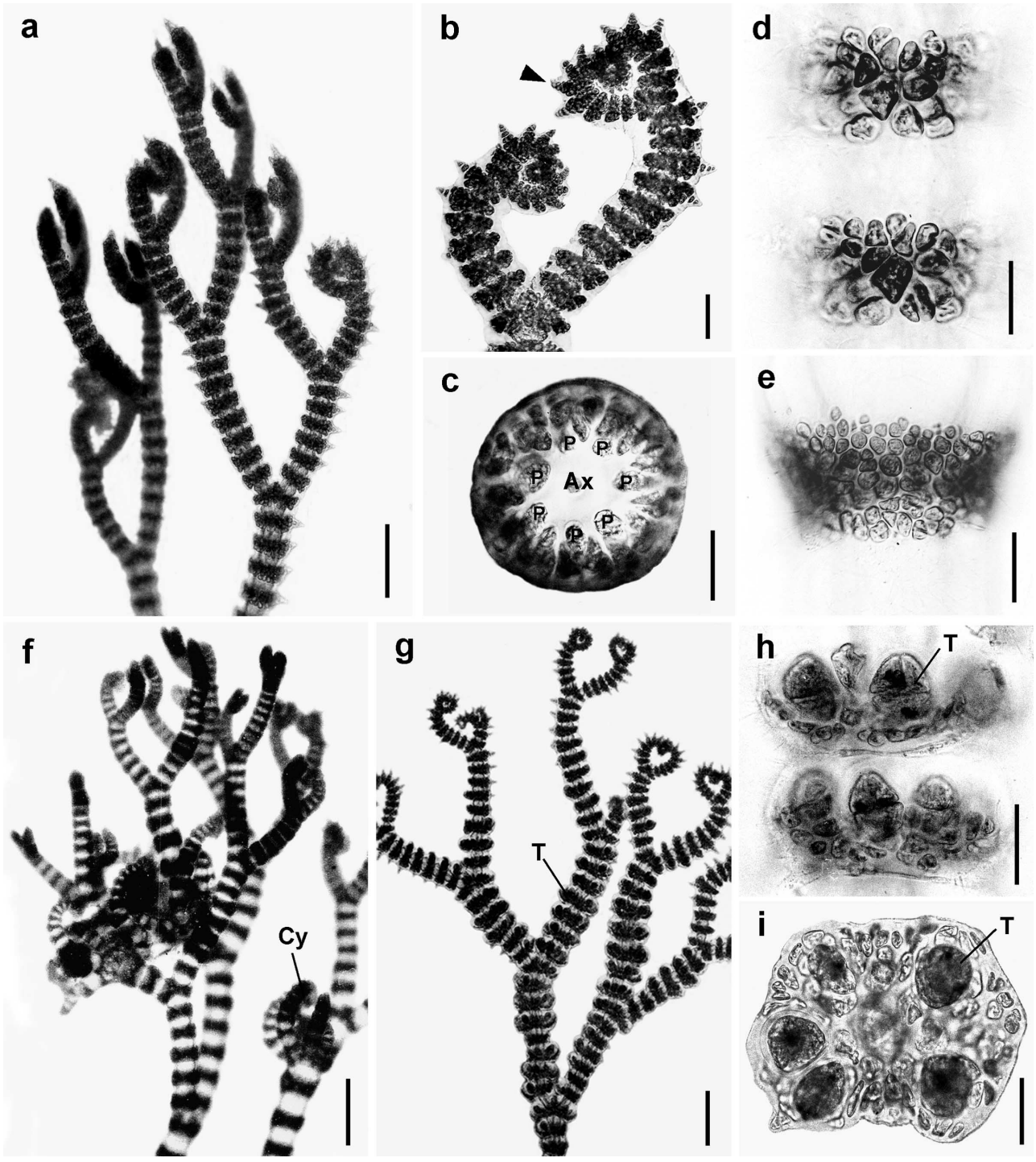

FIG. 6. *Ceramium inkyuii* sp. nov. (a–e, CNUK 005025; f, CNUK 000217; g–i, CNUK 000214). (a) Thallus part with pseudo-dichotomous branches. Scale, 200 μm. (b) Apical region bearing spines (arrowhead). Scale, 50 μm. (c) Cross-section through cortical node. Scale, 25 μm. (d) Cortical nodes in the upper thallus part. Scale, 25 μm. (e) Cortical node in the lower thallus part. Scale, 25 μm. (f) Female thallus bearing cystocarps. Scale, 200 μm. (g) Tetrasporangial thallus. Scale, 200 μm. (h) Cortical nodes showing tetrasporangia in whorl. Scale, 50 μm. (i) Cross-section through cortical node showing the opposite sequence of tetrasporangial formation. Scale, 50 μm. Abbreviations are as in Figures 4 and 5.

DISCUSSION

Ceramium inkyuii is newly described from the east coast of Korea based on morphological and molecular evidence. The new species is recognized by the following characteristics: a single row of spines on the abaxial side, strongly inrolled apices, pseudo-dichotomous branches with a twist in the upper part, and gland cells. Although the species resembles *C. panicu-*

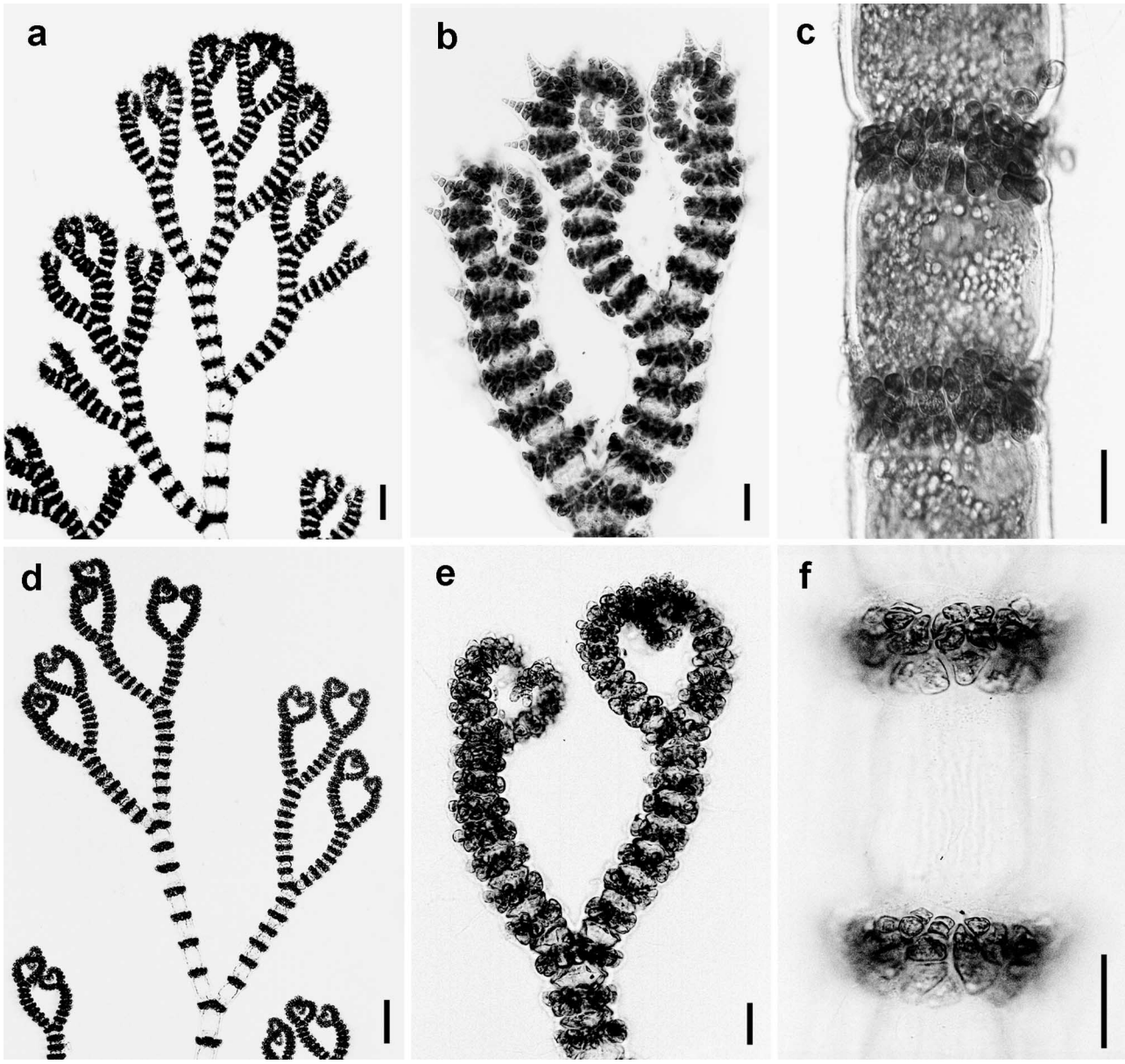

FIG. 7. *Ceramium paniculatum* (a–c, CNUK 000221) and *C. tenerrimum* (d–e, CNUK 000107). (a) Thallus part showing alternate branching. Scale, 200 μm. (b) Incurved apex with spines on the abaxial side. Scale, 50 μm. (c) Cortical node in the upper thallus. Scale, 50 μm. (d) Thallus part showing pseudo-dichotomous branching. Scale, 200 μm. (e) Inrolled apex lacking spines. Scale, 50 μm. (f) Cortical node in the lower thallus. Scale, 50 μm.

latum and *C. tenerrimum* in appearance, *C. inkyuii* is distinguishable from *C. paniculatum* by the strongly inrolled apices and pseudo-dichotomous branches and from *C. tenerrimum* by the acute spines with a multicellular base and gland cells. The distinctness of *C. inkyuii* is strongly supported by phylogenetic analyses inferred from both *rbc*L and SSU rDNA sequences.

In recognizing the *Ceramium* species, vegetative characters such as cortical spines, branching pattern, and degree of incurvature of the apices are viewed to be important diagnostic characters (Dixon 1960, Womersley 1978, Boo and Yoon 1993, Maggs and Hommersand 1993, Cho et al. 2001). Tetrasporangial arrangement and development is also a valuable character at the species level (Womersley 1978, South and Skelton 2000, Cho et al. 2001), although gametangial reproductive structures are of less taxonomic value (Womersley 1978, Maggs and Hommersand 1993, Boo and Lee 1994).

Spines are useful for identifying species because shape and number of spine cells differs among species (Dixon 1960, Womersley 1978). A diversity of spines has been reported in *C. ciliatum* (Ellis) Ducluzeau, *C. dumosertum* R. E. Norris et Abbott, *C. hamatispinum* Dawson, *C. horridum* Setchell et Gardner, *C. monacanthum* J. Agardh, *C. paniculatum* Okamura, *C.*

TABLE 2. Morphological character comparison among *Ceramium inkyuii*, *C. paniculatum*, and *C. tenerrimum*.

Character	*C. inkyuii*	*C. paniculatum*	*C. tenerrimum*
Thallus length (cm)	2.0–5.0	1.5–3.0	5–10
Apices	Strongly inrolled	Slightly incurved	Strongly inrolled
Branching pattern	Pseudo-dichotomous, twisted in the upper thallus	Alternate, complanate	Pseudo-dichotomous, complanate
Branch interval	7–9 segments	4–6 segments	6–11 segments
Axial cell			
Length (μm)	220 ± 55	184 ± 28	480 ± 60
Diameter (μm)	43 ± 5	91 ± 8	48 ± 8
Periaxial cells	6–8	6–7	6–7
Cortical node			
Length (μm)	45 ± 9	55 ± 8	53 ± 7
Diameter (μm)	91 ± 13	125 ± 17	98 ± 15
Gland cells	Present	Absent	Absent
Spines	Present	Present	Absent
Tetrasporangia			
Arrangement	Opposite to whorled	Alternate to whorled	Opposite to whorled
Length (μm)	41 ± 6	40 ± 2	39 ± 5
Diameter (μm)	34 ± 3	35 ± 2	38 ± 3
Cystocarp			
Length (μm)	355 ± 30	144 ± 15	348 ± 22
Diameter (μm)	290 ± 35	130 ± 35	270 ± 45

puberulum Sonder, and *C. shepherdii* Womersley in the Pacific Ocean (Dawson 1962, Nakamura 1965, Womersley 1978, Abbott 1999). Spines of *C. ciliatum*, *C. dumosertum*, *C. hamatispinum*, and *C. horridum* are nonpigmented, and the spine of *C. shepherdii* is pigmented and uniseriate. All the above spines are one to several per node of the upper branches and in a whorl with the exception of *C. horridum*, in which the spine occurs irregularly on the abaxial side of the upper branches. On the contrary, spines of *C. inkyuii, C. monacanthum*, *C. paniculatum*, and *C. puberulum* are pigmented, acute with a multicellular base, and arranged in a single row on the abaxial side at each node of the upper branches. *Ceramium puberulum* is distinct in having an intermediate cortication (Womersley 1978, Boo 1993), whereas *C. inkyuii, C. monacanthum*, and *C. paniculatum* have an incomplete cortex. *Ceramium monacanthum* has four- to six-celled spines and is exclusively distributed in Tasmania and Australia (Womersley 1978). *Ceramium paniculatum* and *C. inkyuii* have three- to four-celled spines, a feature that appears to distinguish these two species from the other spinous taxa in the Pacific Ocean.

Nakamura (1965) described small spines at the outer edge of the apex of only tetrasporangial branches in *C. tenerrimum* from Japan. The spines were two cells long and never found on other parts of the frond of *C. tenerrimum* (Nakamura 1965, p. 135). However, these kinds of spines are not found in our collections from Korea. Because of the superficial similarity between *C. tenerrimum* and *C. inkyuii*, *C. inkyuii* thalli have likely been misidentified as *C. tenerrimum*.

Type of branches has been shown to be a valid character for delimiting some species of *Ceramium* (Boo and Yoon 1993), as is the number of axial cells between branches (Maggs and Hommersand 1993). *Ceramium paniculatum* has alternate branches, but most species of *Ceramium* have pseudo-dichotomous branches with relatively consistent intervals, as is in *C. inkyuii* and *C. tenerrimum*. However, in *C. inkyuii* the branch intervals average 6.8 segments in the main branches and 7.8 segments in the lateral branches, whereas in *C. tenerrimum* the average of branch intervals is 9.0 segments in the main branches and 9.5 segments in the lateral branches. In addition, *C. inkyuii* has twisted apical branches, whereas *C. paniculatum* and *C. tenerrimum* have more or less complanate branches.

Because tetrasporangia are usually produced from periaxial cells in most of completely corticated species such as *C. codicola* J. Agardh, *C. kondoi* Yendo, and *C. pacificum* (Collins) Kylin (Cho et al. 2001), their development follows the pattern of periaxial cell development. *Ceramium californicum* J. Agardh has been known as the only species in which tetrasporangia are produced in an opposite sequence, even though periaxial cells are produced in an alternate pattern (Cho et al. 2001). The opposite pattern of tetrasporangial formation appears more common, considering its occurrence in *C. inkyuii* and *C. tenerrimum* in our study. Tetrasporangial development may be important for the taxonomy of *Ceramium*, but detailed observations of additional species are necessary.

Our molecular phylogenetic analyses using *rbc*L and SSU rDNA gene sequences revealed sufficient sequence divergence between *C. inkyuii* and similarly looking species such as *C. paniculatum* and *C. tenerrimum* to classify our new species as a natural entity. The sequence divergence of the *rbc*L gene is in a range of 2.5% to 2.7% and the SSU rDNA sequences diverged at a level of 0.5% to 0.7%. Higher interspecific sequence variation values for the *rbc*L than for SSU rDNA have similarly been reported in other red algae, for instance between species (1.8%–6.8% vs. 0.2%–3.5%) of the Gracilariales and between mem-

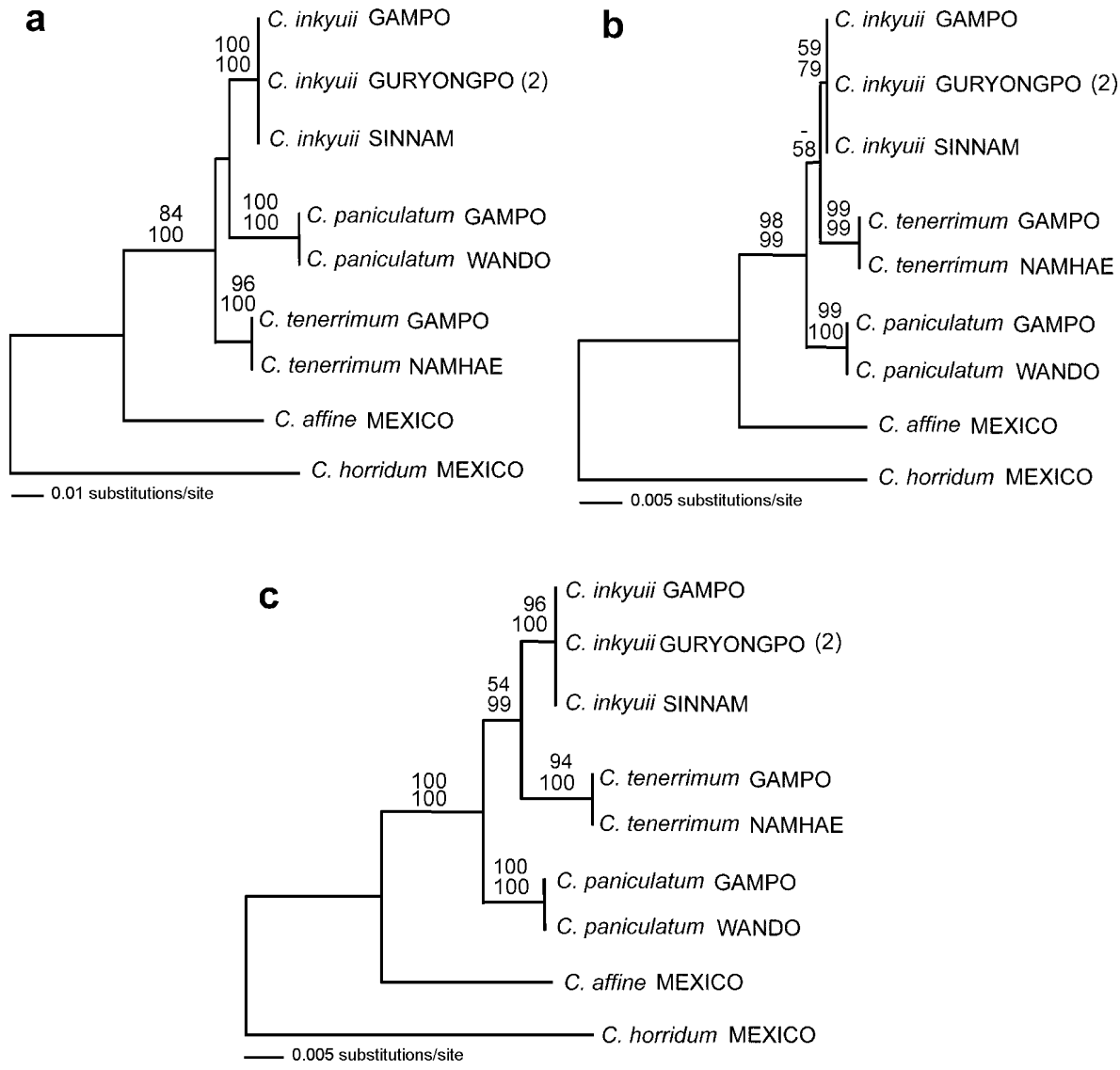

FIG. 8. Phylogeny of *Ceramium inkyuii* sp. nov. Number in parenthesis indicates the number of samples, and bootstrap proportion values (>50%) for all ML (top) and MP (bottom) trees are shown at each branch. (a) ML tree of the *rbc*L sequences using a GTR+G evolutionary model (Rodriguez et al. 1990). (b) ML tree of the SSU rDNA sequences using a TrN+I substitution model (Tamura and Nei 1993). (c) ML tree of the concatenated data of the *rbc*L + SSU rDNA sequences using a GTR+I model (Rodriguez et al. 1990).

bers (3.1%–11.5% vs. 0%–0.4%) of the Gelidiales (Bailey and Freshwater 1997). The fact that geographically distant samples of each of these three species had identical sequences of the *rbc*L and SSU rDNA suggests that each species is genetically isolated.

Although *C. inkyuii* is superficially similar to *C. paniculatum* in having pigmented acute spines with a multicellular base and inhabiting exposed sites, the *rbc*L and SSU rDNA sequence data show a sister relationship of *C. inkyuii* to *C. tenerrimum*. The shared characters between the latter two species are strongly inrolled apices and opposite sequence of tetrasporangial development, which may be synapomorphic. These results suggest that the spinous *C. inkyuii* might have a more recent common ancestor with the spineless *C. tenerrimum* and thus the presence of the acute spines with a multicellular base is phylogenetically uninformative. *Ceramium monocanthum*, endemic to Australia and similar to *C. paniculatum* (Womersley 1978, Boo 1993), is therefore considered more divergent from *C. inkyuii*, although it was unavailable in this study.

In conclusion, detailed observations of unidentified specimens collected on the east coast of Korea throughout the year caused us to add *C. inkyuii* to the world flora of the genus *Ceramium*. Comparative analyses of plastid *rbc*L and nuclear SSU rDNA gene sequences provide additional evidence for describing the new species. Because marine red algae such as *Ceramium* are morphologically very variable, the combined study of morphology and gene sequences is

necessary for concrete identification and description of new members.

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