Cladosiphon umezakii sp. nov. (Ectocarpales, Phaeophyceae) from Japan

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SUMMARY

The new species *Cladosiphon umezakii* Ajisaka (Ectocarpales, Phaeophyceae) is described from Japan based on morphology and DNA sequences. The species resembles *Cladosiphon okamuranus* Tokida in its gross morphology; somewhat slimy, cylindrical, multiaxial and sympodial erect thallus, arising from a small disc-shaped holdfast, and branching once to twice. However, *C. umezakii* has considerably longer assimilatory filaments (up to 840 μm long, composed of up to 90 cells) than any known taxa of the genus. The species is a winter to spring annual, growing on lower intertidal to subtidal rocks of more or less exposed sites on the north-eastern coast of Kyushu and on both the Pacific and the Sea of Japan coasts of Honshu. Specimens from the Sea of Japan coast had both unilocular and plurilocular zoidangia, whereas those from Kyushu and from the Pacific had only unilocular zoidangia. Unilocular zoidangia were formed on the basal part of assimilatory filaments, and plurilocular ones were transformed from the distal part of assimilatory filaments. DNA sequences of the Rubisco-spacer (*rbc*-spacer) region and the nuclear rDNA ITS region (ITS1, 5.8S and ITS2) supported the distinctness of the species.

Key words: Chordariaceae, *Cladosiphon*, *C. umezakii*, Ectocarpales s.*l*., molecular phylogeny, morphology, Phaeophyceae, *rbc*-spacer, rDNA ITS, taxonomy.

INTRODUCTION

Kützing (1843) established the genus *Cladosiphon* (type species *Cladosiphon mediterraneus* Kützing, described from Naples and Livorno, Mediterranean Sea) in the Chordariaceae, Ectocarpales *sensu lato.* Based on morphological studies of the central axis and the position of the meristematic region, Kylin (1940) recognized five species groups in the Chordariaceae (*Mesogloia*-, *Myriogloia*-, *Cladosiphon*-, *Sphaerotrichia*- and *Chordaria*-groups). The *Cladosiphon*-group was characterized by a polysiphonous main axis and sympodial growth, and included seven genera (*Cladosiphon, Eudesme, Mesogloiopsis, Polycerea, Sauvageaugloia, Suringariella* and *Tinocladia*; Kylin 1940; Womersley & Bailey 1987). Kylin (1940) defined the genus *Cladosiphon* by the following characteristics: sympodial and polysiphonous growth mode of the central axis; medullary layer often becoming hollow; medullary cells four to eight times longer than wide; border between medullary layer and assimilatory filaments very thin (a layer of about one to three cells in the subcortex); assimilatory filaments simple, or branched near the base, with hairs; and plurilocular zoidangia, when present, transformed from the terminal portion of assimilatory filaments. This definition of the genus has been followed by later researchers (Inagaki 1958; Lindauer *et al.* 1961; Womersley & Bailey 1987).

About 12 species have been described in the genus *Cladosiphon*. Among them, only *C. okamuranus* Tokida has been reported from the subtropical region (Ryukyu Islands) of Japan (Tokida 1942). This species had previously been identified as *Eudesme virescens* (Carmichael) J. Agardh by Okamura (1907, 1936), but Tokida (1942) recognized that the specimens from Ryukyu represent an independent taxon, and placed it in the genus *Cladosiphon*. Later, Shinmura (1977) reported the occurrence of both plurilocular zoidangia on the distal part of the assimilatory filaments, and phaeophycean hairs, in this species.

In the present paper we report *Cladosiphon umezakii* sp. nov. as a second species of the genus from Japan, which was first mentioned as *Cladosiphon* sp. in Ajisaka (1991), based on the morphology and DNA sequences of the Rubisco-spacer (*rbc*-spacer) and nrDNA ITS regions.

MATERIALS AND METHODS

Morphological observations

Morphological observations were made using Nomarski light microscopy (Nomarski light microscope DX-50; Olympus, Tokyo, Japan) on the following Japanese spe cimens (fresh or preserved in 5% formaldehyde-seawater): Sabiura, Kushimoto (type locality, 23 January 1977, 6 April 1981, 7 April 1982, 22 February 1983, 8 March 1986, 1 February 1990, collected by T. Ajisaka), Rinkai, Shirahama (27 March 1980, 10 March 1981, 8 April 1982, 24 March 1989, 6 June 1993, 22 May 1994, 18 March 2000, by T. Ajisaka), Kada, Wakayama (13 February 1986, by T. Ajisaka), Inami, Gobo (17 January 2002, by T. Ajisaka), Wakayama Prefecture; Maiko-jima and Nadachi, Tokushima (9 May 2000, by K. Yoshimi), Tokushima Pref.; Yura, Awaji I. (19 March 2000, by T. Ushirokawa), Hyogo Pref.; Kanmuri I., Wakasa Bay (19 May 1980, 10 June 1981, 24 April 1982, 29 June 2000, by T. Ajisaka), Kyoto Pref.; Nomozaki (1 May 1992 by T. Yotsui), Nagasaki Pref.; and Amakusa (1 April 1955, by S. Migita), Kumamoto Pref. (Fig. 1).

Molecular phylogenetic analyses

Specimens collected from the Pacific and the Sea of Japan coast of Honshu were used for molecular analyses (Table 1, Fig. 1). Genomic DNA was extracted from specimens rapidly desiccated in silica gel powder, or fresh specimens, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The *rbc*-spacer (non-coding region between *rbc*L and *rbc*S genes) and the nuclear rDNA

Fig. 1. Collection sites of *Cladosiphon umezakii* sp. nov. Specimens from black square sites were used in the molecular analyses.

ITS regions were amplified using primers listed in Kim and Kawai (2002): 18F1 and 5.8R-1 for ITS1, 5.8F-1 and 26R-1 for ITS2, and rbcF3 and rbcR6 for the *rbc*-spacer region including 444 bp of 3′ region of the *rbc*L gene. Polymerase chain reactions (PCRs) were carried out using a GeneAmp PCR System 2400 or a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and a TaKaRa Ex Taq Reaction Kit (Takara Shuzo, Shiga, Japan). The profiles of PCRs were as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 7 min. PCR products were directly sequenced using the Cy5 Auto Cycle Sequencing Kit and ALF express DNA sequencer (Pharmacia Biotech AB, Uppsala, Sweden), or the ABI PRISM BigDye Terminator Cycle Sequencing Kit ver. 3.0 and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) DNA sequencer, all according to the manufacturers' protocols.

DNA sequences were aligned by Clustal W v. 1.8 (Thompson *et al.* 1994) and subsequently refined by eye. For the *rbc*-spacer region, 37 sequences of brown algae in the Ectocarpales *sensu lato* were retrieved from the international DNA database (Table 2) and the *rbc*-spacer regions of four chordariacean taxa with morphology similar to *C. umezakii* were newly determined (Table 1). The level of saturation of *rbc*L and its spacer region was inferred by graphing the transition/transversion ratio for pairs of sequences versus the number of transversions between those pairs of sequences. Molecular phylogenetic trees were constructed by neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods implemented in PAUP* v. 4.0b10 (Swofford 2002). For MP analyses, a heuristic search was carried out with random order sequence additions (10 replicates) and tree bisection-reconstruction (TBR) option. The Jukes-Cantor distance (Jukes & Cantor 1969) was used in the NJ analyses according to the guideline in Nei and Kumar (2000), and the estimated best-fit model selected by Modeltest v. 3.06 (Posada & Crandall 1998) was also used. The best-fit substitution models estimated for the *rbc*-spacer and ITS data using Modeltest v. 3.06 (Posada & Crandall 1998) were also used in ML analyses. Bootstrap analyses (1000 replicates) were carried out in MP and NJ analyses, in order to evaluate the robustness of each lineage. *Asterocladon rhodochortonoides* (Børgesen) Uwai, Nagasato, Motomura *et* Kogame and *Eudesme virescens* were used as outgroup taxa in the analysis of the *rbc*-spacer and the ITS regions, respectively. In addition, phylogenetic trees (NJ and MP) were reconstructed based on the unambiguously aligned sites of the *rbc*-spacer data set, for the Ectocarpales s*. l.* as well as for the Chordariaceae only (*sensu* Peters & Ramírez 2001); in order to
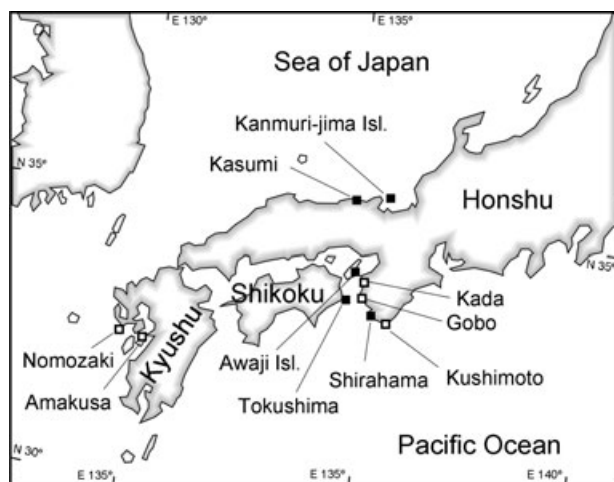
Table 1. Sources of DNA sequences determined in the present study. Collection sites in Japan, abbreviations of specimens used in Figures 12 and 13 for *Cladosiphon* spp. and accession numbers in DNA database are indicated

Species	Collection site	Abbreviation	Accession number 5.8S rDNA and ITS region	*rbc*L and spacer region
Cladosiphon umezakii sp. nov.	Kanmuri-jima I., Wakasa Bay, Kyoto Pref.	KAM	AB199621	AB199632
	Oura, Takeno, Hyogo Pref.	TAK	AB199620	AB199634
	Imagoura, Kasumi, Hyogo Pref.	IMA4	AB199619	AB199636
	Imagoura, Kasumi, Hyogo Pref.	IMA5	AB199618	–
	Yura, Awaji I., Hyogo Pref.	YUR1	AB199623	–
	Yura, Awaji I., Hyogo Pref.	YUR2	AB199625	AB199637
	Nadachi, Tokushima Pref.	YUR4	AB199627	–
	Yura, Awaji I., Hyogo Pref.	YUR3	AB199624	AB199633
	Maiko I., Anan, Tokushima Pref.	MAI1	AB199629	–
	Maiko I., Anan, Tokushima Pref.	MAI2	AB199626	–
	Hunase, Anan, Tokushima Pref.	HUN	AB199628	AB199635
	Rinkai, Shirahama, Wakayama Pref.	CLF1	AB199622	AB199638
Cladosiphon okamuranus Tokida	Kin, Okinawa Pref.	OKI1	AB199616	AB199630
	Kin, Okinawa Pref.	OKI2	AB199617	–
	Okinawa Pref.	–	–	AB199631
Eudesme virescens (Carmichael *ex* Berkeley) J. Agardh	Hokkaido	–	AB199615	AB199639
	Akkeshi, Hokkaido	–	–	AB199640
Heterosaundersella hattoriana Tokida	Akkeshi, Hokkaido	–	–	AB199641
Sauvageaugloia ikomae (Narita) Inagaki	Ehime Pref.	–	–	AB199642

remove the ambiguity in the alignment, about 60 sites on the 5′ region of the *rbc*-spacer region were removed from the alignment.

RESULTS

Specimens referable to the present species were collected from several localities on the Pacific and the Sea of Japan coasts of Honshu, and on the north-eastern coast of Kyushu, Japan (Fig. 1). They grew solitary or sparsely gregarious on intertidal and subtidal rocks of moderately exposed coasts. The main axis of the erect thallus, developing from a small discoid holdfast, was 10–30 cm high and 1.5–2.0 mm thick, cylindrical, solid or somewhat hollow, embedded in gelatinous substance, and moderately and irregularly branched. The erect thallus branched once to twice, and often bore sparse branchlets (Figs 2,3).

The erect thallus had a polysiphonous and sympodial structure, composed of a medullary layer and assimilatory filaments (Figs 4,9). The medullary layer consisted of cylindrical or elongated cells that were longitudinally, uniseriately and loosely arranged. Cells were 50–120 μm long, 15–40 μm thick, and became narrower and smaller towards the periphery. The subcortical layer was thin, only composed of one to two cells (Fig. 9). Cells in the subcortical layer were 7–20 μm × 20–70 μm, shorter than cells in the medullary layer, and they produced assimilatory filaments and phaeophycean hairs. Assimilatory filaments in the middle or upper portions of the thalli were 540–640 μm long and were composed of 40–61 cells, and those in the lower portion were 690–840 μm long, and composed of 65–90 cells in the specimens collected from the Pacific coast. In contrast, they were 400–500 μm long and 26–28 cells in the specimens from the Sea of Japan coast. Assimilatory filaments were uniseriate, constricted at cross walls, and slightly curved in the distal portion (Figs 4,9). Cells of the assimilatory filaments contained peripheral chloroplasts with some prominent pyrenoids (Figs 6,10,11). The lower cells of assimilatory filaments were cylindrical, 6–15 μm in diameter and 6–17 μm in length, and the upper cells were swollen, barrel-shaped to globular, about 12–20 μm in diameter (Figs 6,10,11). Phaeophycean hairs were unsheathed, hyaline, about 2.0–2.5 mm long, 6–12 μm in diameter, and arose from the growing points of the central axis or from subcortical cells (Fig. 9). Unilocular zoidangia were ellipsoid or oviform, 60–110 μm × 25–50 μm in size, sessile, and produced solitary or gregariously on the cells near the base of assimilatory filaments (Figs 5,7). Plurilocular zoidangia were formed on the distal portion of assimilatory filaments, uniseriate, partly bi- or tri-seriate (Figs 8,10,11). The erect thalli collected from the Sea of Japan coast had both unilocular and plurilocular zoidangia, whereas those from the Kyushu and the Pacific coasts had only unilocular zoidangia.

Table 2. Taxa included in the molecular analyses based on *rbc*L and its spacer region, and their accession numbers in the DNA database

Species	*Rbc*L and spacer
Acrothrix pacifica Okamura *et* Yamada	AB066060
Adenocystis utricularis (Bory) Skottsberg	AJ295823
Ascoseirophila violodora A. F. Peters	AJ439835
Asperococcus fistulosus (Hudson) Hooker	AY095321
Asterocladon rhodochortonoides (Børgesen) Uwai, Nagasato, Motomura *et* Kogame	AJ295825
Austrofilum incommodum (Skottsberg) Peters	AJ439838
Caepidium antarcticum J. Agardh	AJ295826
Chordaria chordaeformis (Kjellman) Kawai *et* S. H. Kim	AB066069
Coelocladia arctica Rosenvinge	AF055395
Delamarea attenuata (Kjellman) Rosenvinge	AF055396
Dictyosiphon foeniculaceus (Hudson) Greville	AF055397
Ectocarpus siliculosus (Dillwyn) Lyngbye	X52503
Elachista antarctica Skottsberg	AJ439841
Feldmannia irregularis (Kützing) Hamel	AF207800
Giraudia sphacelarioides Derbès *et* Solier	AF055399
Halothrix lumbricalis (Kützing) Reinke	AF207801
Hincksia hincksiae (Harvey) Silva	AF207803
Hummia onusta (Kützing) Fiore	AF055402
Laminariocolax tomentosoides (Farlow) Kylin	AF055404
Litosiphon pusillus (Lyngbye) Harvey	AF055406
Microsporangium globosum Reinke	AF207805
Mikrosyphar porphyrae Kuckuck	AF207806
Myelophycus cavum J. Tanaka *et* Chihara	AY095319
Myriogloea simplex (Segawa *et* Ohta) Inagaki	AB079015
Myrionema balticum (Reinke) Foslie	AJ439855
Papenfussiella kuromo (Yendo) Inagaki	AB079004
Pogotrichum filiforme Reinke	AF055409
Polytretus reinboldii (Reinke) Sauvageau	AF207809
Protectocarpus speciosus (Børgesen) Kuckuck *ex* Kornmann	AF207810
Pylaiella littoralis (Linnaeus) Kjellman	X55372
Scytosiphon lomentaria (Lyngbye) Link	AF207811
Sorocarpus micromorus (Bory) Silva	AF055411
Sphaerotrichia divaricata (C.Agardh) Kylin	AB066061-2
Stictyosiphon soriferus (Reinke) Rosenvinge	AF055413
Streblonema tenuissimum Hauck	AF055414
Tinocladia crassa (Suringar) Kylin	AB079014

Molecular phylogenetic analyses
rbc-spacer

Sequences of the *rbc*-spacer region were determined for the seven specimens of *Cladosiphon umezakii* and two specimens of *C. okamuranus* (Table 1). The *rbc*-spacer of all specimens was 175 bp in length. A short horizontal portion of the graph of the transition/transversion ratio versus number of transversions indicated saturation, but only weakly, therefore we included all sites in the analyses. There were 35–40 base substitutions between *Cladosiphon umezakii* and *C. okamuranus*, of which 23–26 bp were present in the spacer region. Zero to three bp substitutions (zero to two bp in the spacer) were observed within *C. umezakii*. There was a single consistent distinction between the Sea of Japan and the Pacific specimens of *C. umezakii*; at position 1416 in

the *rbc*L, specimens from the Pacific possessed a T, whereas those from the Sea of Japan had a C.

Because the NJ, MP and ML trees were similar to each other, at least regarding the position of *Cladosiphon* spp., only the ML tree, including bootstrap supports of the MP and NJ trees, is shown in Figure 12. Two NJ trees estimated from JC distance and estimated best-fit model (GTR model) also resulted in very similar topologies and bootstrap supports. *Cladosiphon umezakii* and *C. okamuranus* were included within a single clade in all analyses; however, bootstrap support of the clade was very weak (<50%). Furthermore, the clade including *Cladosiphon* spp. also harbored *Sauvageaugloia ikomae* (Narita) Inagaki, which was more closely related to *C. umezakii* than *C. okamuranus* in the NJ tree, although the bootstrap values were low (55%). On the other hand, *S. ikomae* was monophyletic with

Figs 2,3. *Cladosiphon umezakii* sp. nov. Habit of specimens from Kushimoto, the Pacific coast (Fig. 2) and Kanmuri I., the Sea of Japan coast (Fig. 3).

C. okamuranus in most of the MP trees (179 of 232 equally most parsimonious trees [MPT]).

Specimens from the Pacific and the Sea of Japan were separated in the NJ tree with weak (60% and 62%, respectively) bootstrap supports. In the ML and MP (207 of 232 equally most parsimonious trees) trees, the Pacific specimens were monophyletic, however, the relationships between the specimens from both coasts as well as among the specimens from the Sea of Japan were not resolved.

The analyses based on the unambiguously aligned sites of the Ectocarpales s. *l.* also resulted in topologies similar to that of Figure 12; however, significant bootstrap support could not be obtained for the *Cladosiphon* clade including *S. ikomae*, both in the MP and NJ analyses. On the other hand, for the data set of the Chordariaceae the relationships between *Cladosiphon* species and *S. ikomae* were not resolved due to a polytomy among most species included. Comparison of the MP analyses for the Ectocarpales s. *l.* including all site(s), excluding ambiguously aligned sites and the Chordariaceae, is shown in Table 3; the exclusion of operational taxonomic units (OTUs) other than Chordariaceae slightly improved the consistency index (CI), retention index (RI) and rescaled consistency index (RC) values, but largely increased the number of MPTs.

rDNA ITS regions

ITS sequences of 12 specimens of *C. umezakii* and two of *C. okamuranus*, as well as of an individual of *Eudesme virescens*, were determined (Table 1). The ITS1 region of *C. umezakii* was 667–670 bp in length, and ITS2 was 388–392 bp long. Both ITS regions were entirely alignable within each species; 3–21 bp differences among specimens from the Sea of Japan, and 9–40 bp among the Pacific specimens. Differences of 17–45 bp were observed between the Pacific and the Sea of Japan.

Molecular phylogenetic trees inferred by MP, ML and NJ methods resulted in similar topologies (Fig. 13, MP analysis); *C. umezakii* formed a monophyletic clade. Monophyly of the specimens from the Sea of Japan was supported by moderate or weak bootstrap support (85% in NJ and 72% in MP trees); the Pacific specimens were paraphyletic to the Sea of Japan specimens in all trees.

Cladosiphon umezakii Ajisaka sp. nov.

Description

Fronde in rupibus disco radicali parvo adfixa, cylindrica, tubulosa, lubrica, exssiccatione chartae arcte adhaerenti, usque ad 30 cm alta, 1.5–2.0 mm crassa,
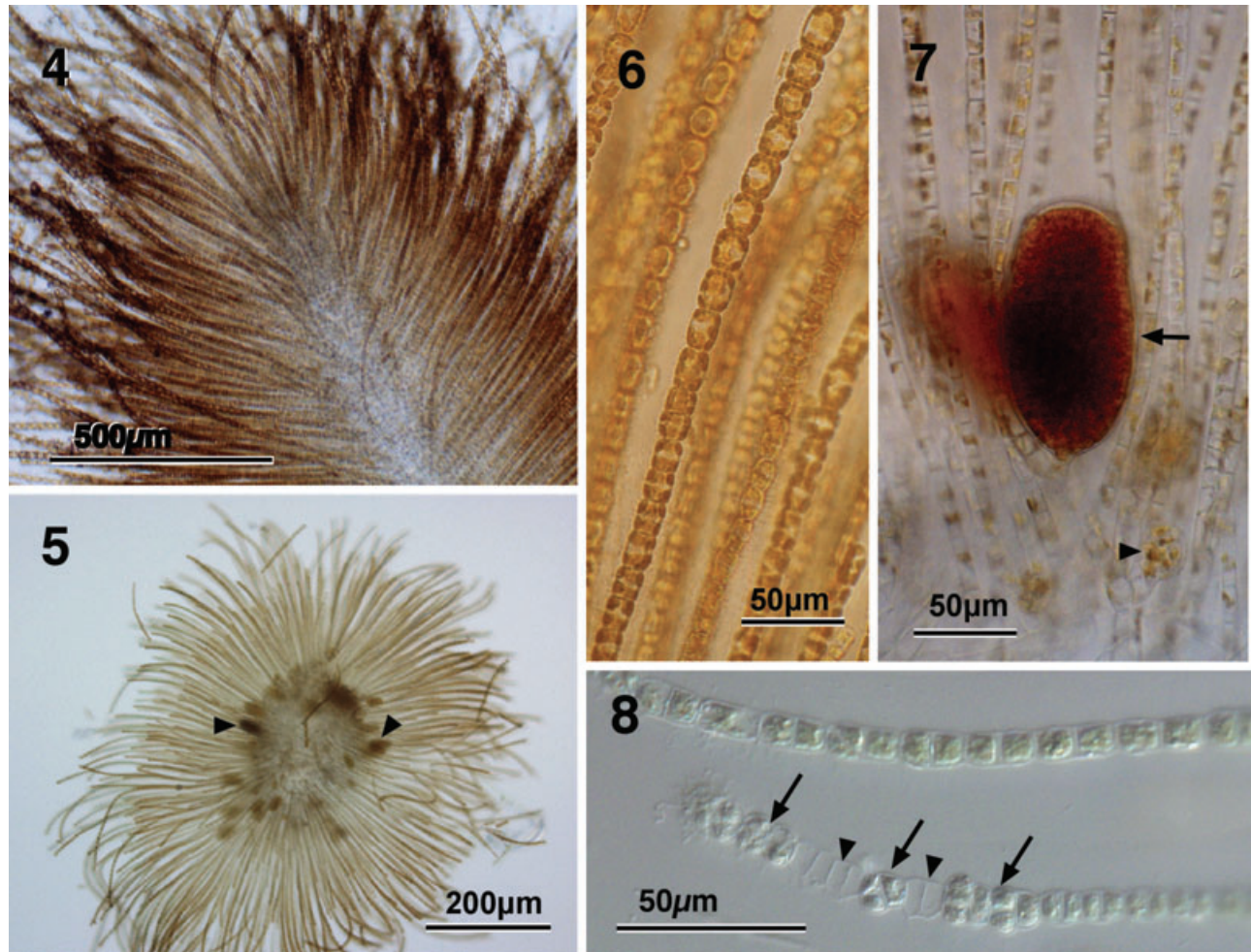
Figs 4–8. Sporophyte anatomy of *Cladosiphon umezakii* sp. nov. 4. Surface view of the tip portion, showing long assimilatory filaments. 5. Cross section of middle portion of fertile erect thallus with unilocular zoidangia (arrowheads) among long assimilatory filaments. 6. Assimilatory filaments. 7. Unilocular zoidangium (arrow). 8. Plurilocular zoidangia (arrows) transformed from the upper part of the assimilatory filaments, and their emptied cells (arrowheads).

moderate *et al*. terne pinnata; cellulis medullaribus 15–40 μm crassis, 50–120 μm longis; filis assimilantibus 400–840 μm altis, superiore parte saepe curvatis, 26–90 cellularibus, cellulis inferioribus cylindricis, 6–15 μm crassis, 6–17 μm longis, superioribus inflatis, 12–20 μm crasis, diametro prope aequalibus; pilis hyalinus, 6–12 μm crassis; sporangiis utriusque formae in eodem individuo; sporangiis unilocularibus ad basinn filorum assimilantium evolutis, elliptico-obovatis, 60–110 μm longis, 25–50 μm latis; sporangiis plurilocularibus e transformatione articulorum superiorum filorum asimilantium ortis, seriatis, maturis cum aperturis unilatiralibus.

Frond attached to rocks by a small disc, cylindrical, tubular, lubricous, tightly adhering to paper when dried, up to 30 cm high, 1.5–2.0 mm thick, moderately and alternately pinnate; medullary cells 15–40 μm in diameter, 50–120 μm long; assimilatory filaments 400–800 μm high, often curved in the upper portions, 26–90 cells long, lower cells cylindrical, 6–15 μm in diameter, 6–17 μm long, upper cells swollen, 12–20 μm in diameter, nearly as long as wide; hairs hyaline, 6–12 μm in diameter; unilocular and plurilocular zoidangia borne on the same individual; unilocular zoidangia formed at the base of the assimilatory filaments, elliptical to obovate, 60–100 μm long, 25–50 μm broad; plurilocular zoidangia transformed from the upper segments of assimilatory filaments, seriate, at maturity with unilateral opening.

Japanese name: Kishu-Mozuku

Habitat: Growing on lower intertidal to upper subtidal rocks and stones down to one to two meters below the low tide mark.

Distribution: Pacific and the Sea of Japan coast of south-central Honshu, Shikoku and Kyushu, Japan.
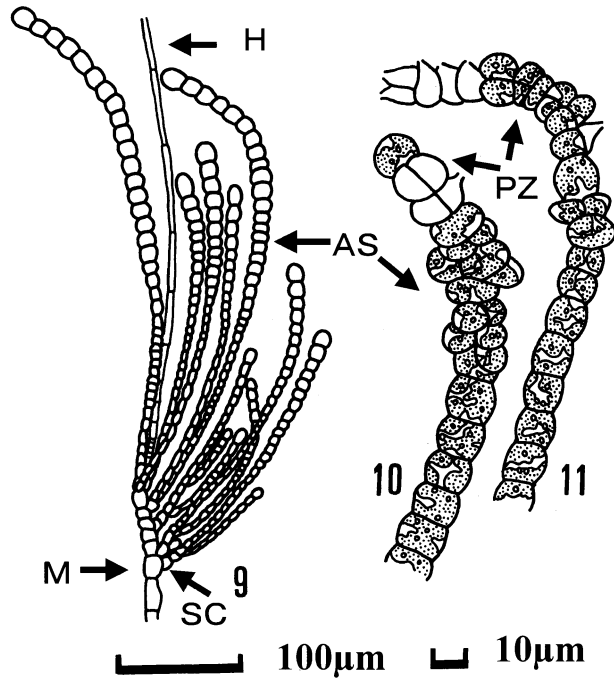
Figs 9–11. Sporophyte anatomy of *Cladosiphon umezakii* sp. nov. 9. Sympodial growth point of the thallus. 10,11. Plurilocular zoidangia transformed from the upper part of the assimilatory filaments. AS, assimilatory filament; H, hair; M, medullary cell; PZ, plurilocular zoidangia; SC, subcortical cell.

Type locality: Sabiura, Kushimoto, Wakayama Prefecture, Japan.
Etymology: The specific epithet is named in memory of the late Professor Isamu Umezaki (1925–1995).
Holotype: SAP 100676. Collected by Tetsuro Ajisaka. 23 January 1977.
Isotype: SAP 100677, 100678.

DISCUSSION

The new species is considered to belong to the genus *Cladosiphon* based on the following basic morphological features: (i) polysiphonous central axis with sympodial growth; (ii) thin subcortical layer only one to two cells thick; (iii) plurilocular zoidangia transformed from upper segments of the assimilatory filaments; (iv) unilocular zoidangia formed on the basal portion of assimilatory filaments. *Cladosiphon umezakii* is morphologically compared with other previously described species of the genus in Table 4. Most morphological data were referenced from Sansón *et al*. (2006), and we added the data of *C. umizakii* in this study, *C. novae-caledoniae* Kylin from New Caledonia (Ajisaka 1991), and *Sauvageaugloia ikomae* from the Sea of Japan (Inagaki 1954, 1958; Kajimura 1986). Kylin (1940) had reported two other species, *C. sibogae* Kylin and *C. lubricus* (Sauvageau) Kylin;
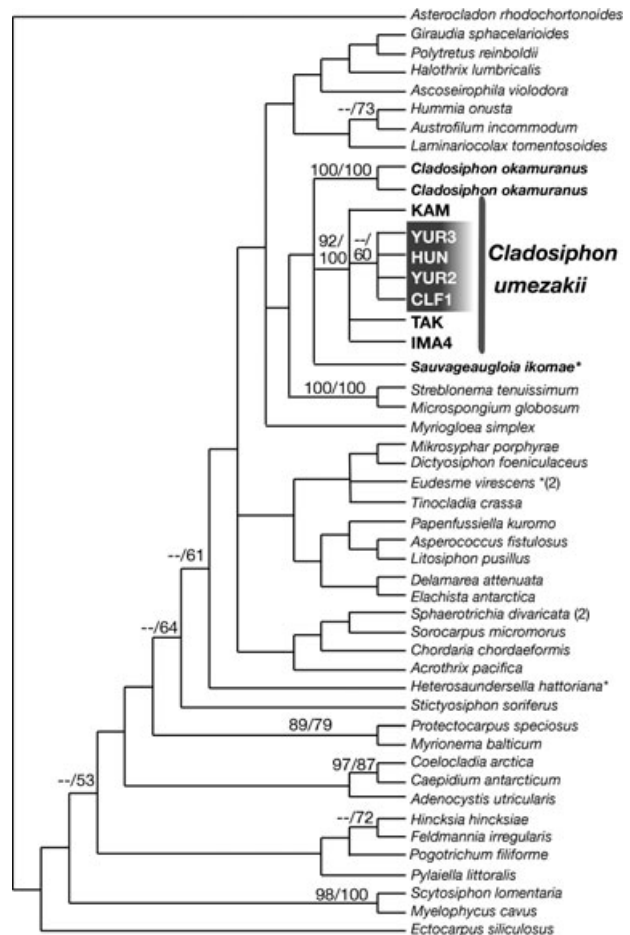
Fig. 12. Maximum likelihood (ML) tree based on *rbcL* and its spacer region sequences. Maximum parsimony (MP) (left) and neighbor joining (NJ) (right) bootstrap values (%) are shown on the branches; indicated as – when below 50%. Specimens of *Cladosiphon umezakii* from the Pacific coast represented by white letters. Sequences newly determined in the present study, other than *Cladosiphon* species, are represented by an asterisk.

however, we could not find comparable morphological data for these two species. Because the assimilatory filaments of *C. umezakii* are remarkably longer than those of the other related taxa (up to 90 cells and 840 μm long), it can be distinguished easily from the other taxa in the genus *Cladosiphon* and *S. ikomae* (Table 4). Assimilatory filaments of *C. filum* (Harv.) Kylin from Australia are also long (400–500 μm, rarely to 800 μm long) (Womersley & Bailey 1987), however, the latter species has only 20–30 (rarely up to 45) cells compared to 90 cells in *C. umezakii*. Only *C. umezakii* varies in the occurrence of plurilocular sporangia according to locality (Kyushu and the Pacific, or the Sea of Japan).

The monophyly of *S. ikomae* with the Japanese *Cladosiphon* species and a polytomy among these species suggested that *C. umezakii* could also be included in

Table 3. Comparisons of maximum parsimony (MP) scores of each dataset. For details of each dataset, see Materials and methods, and Discussion

	Ectocarpales s. *l.* (all site)	Ectocarpales s. *l.* (excluding ambiguous site)	Chordariaceae	Chordariaceae + Atlantic *Cladosiphon*
Number of OTUs	50	50	39	41
Length	720	661	665	665
Number of variable sites	331	278	219	219
Number of informative sites	244	200	120	123
Number of MPTs	232	1048	833	401
Tree length	1316	1021	543	553
Consistency Index	0.447	0.457	0.567	0.561
Retention Index	0.432	0.458	0.511	0.516
Rescaled Consistency Index	0.193	0.209	0.290	0.286

MPTs, most parsimonious trees; OUTs, operational taxonomic units.

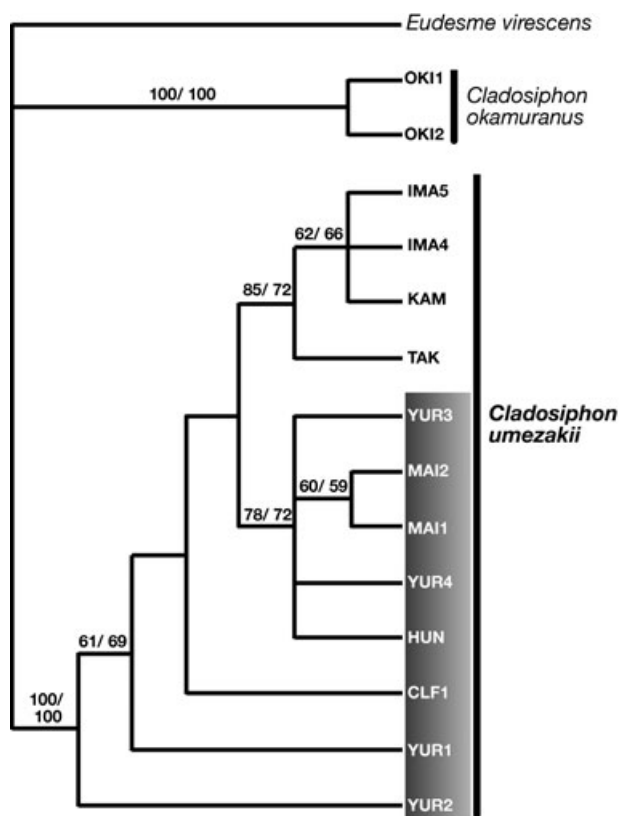**Fig. 13.** Maximum parsimony (MP) tree based on the ITS regions. MP (left) and neighbor joining (NJ) (right) bootstrap values (%) are shown on the branches. Specimens of *Cladosiphon umezakii* from the Pacific coast represented by white letters.

Chordariaceae (Table 3). This preliminary analysis including these two European species showed that the two Japanese *Cladosiphon* species were monophyletic with the two European species, and that these two European *Cladosiphon* species were close to *S. ikomae* rather than the two Japanese species. The data set including the two European species showed MP scores similar to that of the Chordariaceae data set (Table 3); however, significant bootstrap support for the *Cladosiphon* clade was not obtained in either NJ or MP analyses. Nonetheless, these analyses support the close relationship of *Cladosiphon umezakii* with other *Cladosiphon* taxa, and suggested a close relationship between *Cladosiphon* species and *Sauvageaugloia ikomae*. For the moment we consider it most appropriate to place the new species in the genus *Cladosiphon*.

Regarding the relationship between the Pacific and the Sea of Japan coast populations, they differed in the length of assimilatory filaments (the Pacific specimens had remarkably longer assimilatory filaments than the Sea of Japan ones) and presence or absence of plurilocular zoidangia (plurilocular zoidangia were only found in the Sea of Japan specimens). However, the two populations are genetically very close, and we decided to treat them as the same species.

Regarding the relationship between *Cladosiphon* and *Sauvageaugloia*, we could find few good characters to distinguish the two genera. From the description of the genus *Sauvageaugloia* in Kylin (1940), the most important characteristic was the length of the medullary cells, 20 times the diameter in *Sauvageaugloia*, and four to eight times that in *Cladosiphon.* The type species *Sauvageaugloia griffthiana* had only unilocular zoidangia, whereas *S. chordariaeformis* rarely had unilocular zoidangia but usually had plurilocular zoidangia on the terminal part of the assimilatory filaments. Both species have been reported from Europe, and Inagaki (1954) described *S. ikomae* from the Sea of Japan. He described somewhat longer medullary filaments (200–300 µm = four to 10 times the diam-

the genus *Sauvageaugloia*; however, conversely, it is also possible that *S. ikomae* should be included in *Cladosiphon* rather than *Sauvageaugloia*. Unfortunately, the present study included neither generitype, *Cladosiphon mediterraneus* Kützing nor *Sauvageaugloia griffithsiana* (Greville) Hamel *ex* Kylin. However, the partial *rbc*-spacer sequences of *C. mediterraneus* and *C. zosterae* (J. Ag.) Kylin (A. F. Peters, unpubl. data) were available for inclusion in the data set of the

Table 4. Comparison of morphological data of *Cladosiphon umezakii* with those of previously described species in the genus (Sansón *et al.* 2006) and *Sauvageaugloia ikomae*

	C. contortus	*C. cylindricus*	*C. cymodoceae*	*C. filum*	*C. irregularis*	*C. mauritianus*	*C. mediterraneus*
Thallus height (cm)	10–40	to 1.5	to 15	10–50	0.5–1.5	30 or more	10–20 (–30)
Thallus thickness (mm)	1–4	No data	to 2.5	(1–) 2–4 (–6)	No data	to 2	1–3
Medullary cell							
Length (µm)	50–200 (–750)	No data	107–300	(25–) 50–200 (–480)	No data	No data	60–80
Diameter (µm)	20–50 (–210)	No data	32–100	25–50 (–80)	No data	No data	20–25
Subcortical layer	3–4 (–7)	0–1	1 (–2)	1–3	0–1	1–2	1–2
Assimilatory filaments							
Length (µm)	150–200 (–475)	200–250	(182–) 210–425	400–500 (–800)	400–600	to 200	to 400
Number of cells	12–15 (–21)	15–20	12–30	20–30 (–45)	18–20	13–15	9–20
Diameter of lower cells (µm)	8–10	5–7	5–8	6–10	5–8	8	8–12
Diameter of upper cells (µm)	10–12	8–12	(5–) 10–13	8–12	8–10	12	12–16
Hair							
Sheath	Present	Absent	Present	Absent	Absent	Present	Present
Diameter (µm)	10–12	10–12	10–15	(16–) 20–25 (–30)	12	7	6–9 (–10)
Unilocular zoidangia							
Length (µm)	30–100	c. 65	53–80	80–100	54–62	60–65	c. 95
Diameter (µm)	22–65	c. 40	32–48	60–80	30–40	35–40	c. 65
Plurilocular zoidangia	Present	Present	Present	Present	Present	Present	Present
References	Kuckuck (1929); Hamel (1935); Kylin (1940); Sansón *et al.* (2006)	Sauvageau (1924); Hamel (1935); Kylin (1940); Coppejans (1983)	Sansón *et al.* (2006)	Womersley and Bailey (1987)	Sauvageau (1924); Hamel (1935); Kylin (1940); Coppejans (1983)	Børgesen (1941)	Parke (1933); Hamel (1935); Kylin (1940)

	C. occidentalis	*C. novae-caledoniae*	*C. okamuranus*	*C. umezakii*	*C. vermicularis*	*C. zosterae*	*S. ikomae*
Thallus height (cm)	10–30	10–30	20–25	10–30	10–50	2–10 (–20)	40–50 (–30*)
Thallus thickness (mm)	1–3	1–1.5	1–1.5	1.5–2	1.5–4 (–7)	1–2 (–3)	2–4 (1.5–2*)
Medullary cell							
Length (µm)	(112–) 200–400 (–625)	80–400	20–150	50–120	(25–) 35–270	20–240	200–300 (3–30 times diam.*)
Diameter (µm)	(40–) 50–130	40–150	20–50	12–40	25–45	20–60	25–50 (20–30*)
Subcortical layer	1–3	1–2	1–3	1–2	1–3	0–1	1–2**
Assimilatory filaments							
Length (µm)	100–300	160–300	150–250	400–840	200–400 (–500)	150–270	No data (120–210*)
Number of cells	(9–) 10–13 (–24)	10–30	5–20	26–90	15–30	13–15	7–13 (–19) (15–20*)
Diameter of lower cells (µm)	5–10	7–8	7–8	6–17	6–8	8–10	No data (3–10*)
Diameter of upper cells (µm)	10–15	8–10	10	12–20	10–14	12–15	10–15 (6–10*)
Hair							
Sheath	Present	Present	Present	Absent	Absent	Present	Absent
Diameter (µm)	7–10 (–15)	8–10	8–10	6–12	8–12	10–15	No data (10*)
Unilocular zoidangia				Present/absent			Absent (present/absent*)
Length (µm)	40–55 (–80)	50–55	c. 60	60–110	(50–) 60–80	65–90	No data (45–70*)
Diameter (µm)	25–40	25–30	c. 30	25–50	20–45	40–65	No data (25–70*)
Plurilocular zoidangia	Present	Present	Present	Present/absent	Present	Present	Present
References	Børgesen (1914); Taylor (1928); Kylin (1940); Schneider and Searles (1991); Sansón *et al.* (2006)	Kylin (1940); Ajisaka (1991)	Inagaki (1958)	This study	Womersley and Bailey (1987)	Sauvageau (1927); Parke (1933); Hamel (1935); Kylin (1940)	Inagaki (1954, 1958); Kajimura (1986)(*, (**: fig. of upper references))

eter, Table 4), and plurilocular zoidangia were transformed from the terminal cells of the assimilatory filaments. However, Kajimura (1986) noted that the length of the medullary cells of *S. ikomae* were three to 30 times the diameter (Table 4). Based on the molecular data, we consider that *S. ikomae* may be included in the genus *Cladosiphon*, but reserve taxonomic treatment until additional molecular data of the Mediterranean materials become available.

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