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Cladosiphon takenoensis sp. nov. (Ectocarpales *s.l.*, Phaeophyceae) from Japan

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SUMMARY

The new brown algal species *Cladosiphon takenoensis* H. Kawai (Chordariaceae, Ectocarpales *s.l.*) is described from Takeno, Hyogo, Japan based on morphology and DNA sequences. The species is a spring annual, growing on subtidal rocks at more or less exposed sites. It resembles *C. umezakii* in its gross morphology, and the two often grow together, but is distinguishable from *C. umezakii* in having a more hairy appearance. *Cladosiphon takenoensis* has a slimy, cylindrical, multiaxial and sympodial erect thallus, branching once to twice, and is provided with long assimilatory filaments (up to 1.8 mm long, composed of up to 100 cells). Unilocular zoidangia are formed on the basal part of assimilatory filaments. The species is genetically most related to *C. umezakii* and has the same basic thallus structures, but differs from *C. umezakii* and other *Cladosiphon* species in lacking phaeophycean hairs and plurilocular zoidangia of the assimilatory filaments. DNA sequences of the mitochondrial *cox*1 and *cox*3, chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes and the nuclear rDNA ITS2 region support the distinctness of the species. The genus *Cladosiphon* was paraphyletic in our analyses because the clades of *C. okamuranus/C. zosterae* and *C. takenoensis/C. umezakii* were split by *Mesogloia vermiculata*. However, since the genus-level taxonomy of Chordariaceae needs considerable revision, we suspend the genus-level taxonomy of the new species, and tentatively describe it as *C. takenoensis*.

Key words: Chordariaceae, *Cladosiphon takenoensis* sp. nov., Ectocarpales *s.l.*, molecular phylogeny, morphology, taxonomy.

INTRODUCTION

Kützing (1843) established the genus *Cladosiphon* (type species: *C. mediterraneus* Kützing) in the Chordariaceae, Ectocarpales *sensu lato.* Based on the morphology of the central axis and the position of the meristematic region, Kylin (1940) recognized five genus groups in the Chordariaceae (*Mesogloia*-, *Myriogloea*-, *Cladosiphon*-, *Sphaerotrichia*- and *Chordaria*-groups). The *Cladosiphon*-group is characterized by a polysiphonous main axis and sympodial growth, and contains seven genera (*Cladosiphon*, *Eudesme*, *Mesogloiopsis*, *Polycerea*, *Sauvageaugloia*, *Suringariella* and *Tinocladia*; Kylin 1940; Womersley & Bailey 1987 in Womersley 1987). Kylin (1940) defined the genus *Cladosiphon* by the following characteristics: central axis of sympodial and polysiphonous growth; generally hollow medullary layer; relatively long medullary cells (4–8 times longer than wide); very thin (1–3 celled) subcortical layer; simple (or branched only at the base) assimilatory filaments; presence of hairs; and plurilocular zoidangia transformed from the terminal portion of assimilatory filaments. This definition of the genus has generally been followed by later researchers (Inagaki 1958; Lindauer, Chapman, & Aiken 1961; Womersley & Bailey 1987 in Womersley 1987; Ajisaka *et al.* 2007).

Currently, 13 species are described in the genus *Cladosiphon* (Guiry & Guiry 2016). Among them, only *C. okamuranus* Tokida (Tokida 1942) and the relatively recently described *C. umezakii* Ajisaka (Ajisaka *et al.* 2007) have been reported from Japan. In the present study, we propose the description of a third species of *Cladosiphon* from Japan, based on combined analyses of morphology and molecular phylogeny.

MATERIALS AND METHODS

Specimens used for morphological and moleclular studies

Specimens of the new *Cladosiphon* species and *C. umezakii* were collected by SCUBA diving at Takeno (35.6635N, 134.4780E), Hyogo, Japan and used for morphological observations and molecular studies (Fig. 1). Voucher specimen of *Cladosiphon zosterae* (J.Agardh) Kylin (BM633584) was used for DNA extraction. For reference, specimens of *C. mediterraneus* Kützing and *C. zosterae* were collected in the localities listed in Table S1 in Appendix S2. Photomicrographs were taken using a VB–7010 Digital Camera (Keyence, Tokyo, Japan) attached to a BX-51 microscope (Olympus, Tokyo, Japan). The type specimen of the new species (SAP115073) is housed in the Herbarium of the Graduate School of Hokkaido University (SAP), and additional silica gel-dried specimens used for the molecular analyses are deposited in the Kobe University Research Center for Inland Seas (KU-d899, KU-d903 and KU-d5852).

Molecular phylogenetic analyses

Specimens used for molecular analyses are listed in Table S1 in Appendix S2. Genomic DNA was extracted from fresh specimens or specimens rapidly desiccated in silica gel

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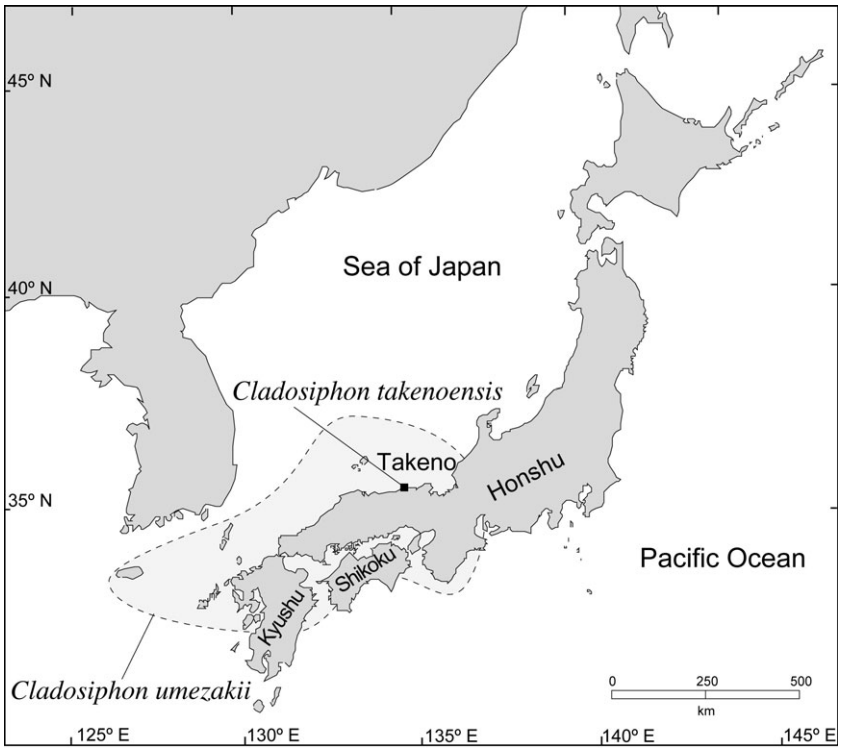

Fig. 1. Map showing collection site of *Cladosiphon takenoensis* sp. nov. and known distributional range of *C. umezakii*.

powder using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and QuickExtract Plant DNA Extraction Solution (Epicentre, Madison, WI, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplifications of the chloroplast *atp*B, *psa*A, *psb*A, and *rbc*L and mitochondrial *cox*1 and *cox*3 genes, and the nuclear rDNA ITS2 region were carried out using the KOD FX (ToYoBo, Osaka, Japan) PCR enzyme and the TaKaRa PCR Thermal Cycler Dice (Takara Bio, Kusatsu, Japan). Primers used for PCR and/or sequencing are listed in Table S2 in Appendix S2. After PEG purification (Lis 1980), PCR products were sequenced using a CE DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA) and a CEQ8000 DNA analysis system (Beckman Coulter) according to the manufacturer's instructions, or sequenced by a DNA sequencing service (FASMAC, Atsugi, Japan). Newly determined sequences were deposited in The DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) (Table 1). For the molecular phylogenetic analyses, published and newly determined sequence data of the Ectocarpales *s.l.* were used (Table 1). *Ectocarpus siliculosus* (Dillwyne) Lyngbye and *Pylaiella littoralis* (L.) Kjellman were used as the outgroup for dataset 1–3, and *Chordaria flagelliformis* (O.F. Müller) C. Agardh was used as the outgroup for dataset 4. Alignments were prepared using the program MAFFT v.6 (Katoh & Toh 2008) and then manually adjusted prior to phylogenetic analyses. The rate of nucleotide sequence divergence was calculated based on the alignments, and insertion and deletion were treated as a fifth base.

Table 1. Comparison of morphological data of C*ladosiphon takenoensis* with selected C*ladosiphon* species

	C. mediterraneus	*C. okamuranus*	*C. takenoensis* sp. nov.	*C. umezakii*	*C. zosterae*
Type locality	Mediterranean	Japan	Japan	Japan	Sweden
Thallus height (cm)	10–20 (–30)	0–25	–10	10–30	2–10 (–20)
Thallus thickness (mm)	1–3	1–1.5	2–3	1.5–2	1–2 (–3)
Subcortical layer	1–2	1–3	1–2	1–2	0–1
Assimilatory filaments					
Length (μm)	–400	150–250	–1800	400–840	150–270
Number of cells	9–20	5–20	60–80 (–100)	26–90	13–15
Diameter of cells (μm)	8–16	7–10	15–20	6–20	8–15
Phaeophycean hair	Present	Present	Absent	Present	Present
Unilocular zoidangia					
Length (μm)	Approximately 95	Approximately 60	110–145	60–110	65–90
Diameter (μm)	Approximately 65	Approximately 30	85–105	25–50	40–65
Plurilocular zoidangia	Present	Present	Absent	Present/absent	Present
References	Parke (1933), Hamel (1935) and Kylin (1940)	Inagaki (1958)	This study	Ajisaka *et al.* (2007)	Parke (1933), Hamel (1935) and Kylin (1940)

Molecular phylogenetic trees for each dataset (data set 1: 31 OTUs, six genes, total 7642 bp; data set 2: 31 OTUs, four chloroplast genes, total 5463 bp; data set 3: 31 OTUs, two mitochondrial genes, total 2179 bp; dataset 4: 10 OTUs, rDNA ITS2, 196 bp) were constructed by maximum likelihood (ML) and Bayesian inference (BI) analyses. The sites including insertion and deletion were removed from alignment. For ML analysis, we used RAxML GUI v.1.31 (Silvestro & Michalak 2012) run to conduct 10 000 Rapid Bootstrap searches followed by an ML search with the GTR + G model. BI analyses were run using MrBayes v.3.2.2 (Ronquist *et al.* 2012). With the aid of the Kakusan4 program (Tanabe 2011) the best-fit evolutionary model for each codon position of each gene was determined by comparing different evolutionary models via the corrected Bayesian Information Criterion (Schwarz 1978). The BI analyses were initiated with a random starting tree and four chains of Markov chain Monte Carlo iterations were run simultaneously for 10 000 000 generations, keeping one tree every 100 generations. The first 25 000 trees sampled were discarded as 'burn-in' based on the stationarity of ln L as assessed using Tracer v.1.5 (Rambaut & Drummond 2007). A consensus topology and posterior probability values were calculated from the remaining trees.

From *Cladosiphon mediterranus* Kützing, we obtained only an ITS2 sequence. It was near-identical (see Results and Figs 5, S6 in Appendix S1) to ITS2 of *C. zosterae* from Brittany. We concluded that the two taxa are conspecific or very closely related and used the sequences of *C. zosterae* to represent the genus *Cladosiphon* in the other markers.

RESULTS

Morphology of *Cladosiphon takenoensis* sp. nov

At Takeno, specimens of the new species were solitary or sparsely gregarious on intertidal and subtidal rocks of more or less exposed coasts, often mixed with *Cladosiphon umezakii* and *Sphaerotrichia divaricata* (C. Agardh) Kylin (Fig. 2a). The new species resembled *C. umezakii* in gross morphology when emersed, but could be distinguished when submerged because of its more hairy appearance, and somewhat lighter color (Fig. 2a,b). The erect thalli were attached by a small discoid holdfast (Fig. 3a), moderately and irregularly branched once or twice, approximately 10 cm high and 2.0–3.5 mm thick, cylindrical, solid or hollow, embedded in a gelatinous matrix (Fig. 3b).

The erect thallus had a polysiphonous and sympodial structure, composed of a medullary layer corticated by assimilatory filaments (Fig. 3a–e,g). The medullary layer consisted of cylindrical or elongated cells, which were longitudinally, uniseriately and loosely arranged (Fig. 3d,e). Cells were 25–30 μm in diameter, and became narrower and smaller towards the periphery. The inconspicuous subcortical layer was only 1–2 cells thick (Fig. 3d). Assimilatory filaments were borne on the medullary/subcortical cells, up to 1800 μm long comprising 60–80 (–100) cells. Cells of the assimilatory filaments were cylindrical, barrel-shaped to globular, 15–20 μm in diameter (Fig. 3c–g and contained a few peripheral discoid chloroplasts with prominent pyrenoids. Phaeophycean hairs were absent. Unilocular zoidangia were ellipsoid or oviform, 110–145 μm × 80–105 μm in size, sessile, and produced solitary or gregariously on the cells near the base of assimilatory filaments (Fig. 3e,g,h). Released unizoids were biflagellated and had an eyespot (Fig. 3f).

In contrast, *Cladosiphon umezakii* had shorter assimilatory filaments comprising up to 90 cells, and the length of unilocular zoidangia rarely exceeded 200 μm (Fig. 3i), and often formed plurilocular zoidangia at the tip (Fig. 3j).

Molecular phylogenetic analyses

Four specimens of *Cladosiphon takenoensis* sp. nov. had the identical *cox*3 gene sequence. A molecular phylogenetic tree based on concatenated DNA sequences of mitochondrial *cox*1 and *cox*3, chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes (ML analysis) is shown in Fig. 4. *Cladosiphon takenoensis* sp. nov. was genetically closest to *C. umezakii*, supported by the maximum bootstrap value. *Cladosiphon okamuranus* formed a clade with the generitype *C. zosterae*, but the clade of *C. okamuranus*/*C. zosterae* formed a clade with *Mesogloia vermiculata* C.Agardh, rendering the genus *Cladosiphon* paraphyletic. The clade of *Corynophlaea crispa*

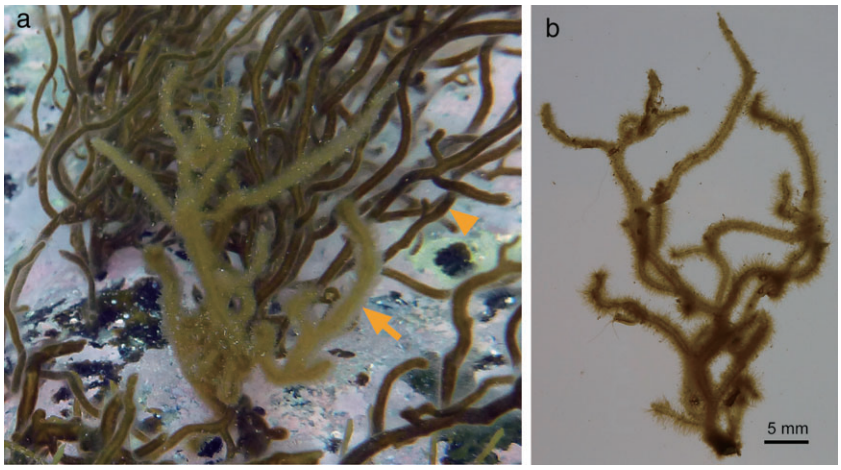

Fig. 2. (a) *C. takenoensis* sp. nov. Habit of epilithic specimen (arrow) growing mixed with *C. umezakii* (arrowhead). (b) Collected specimen (Type material, SAP115073).

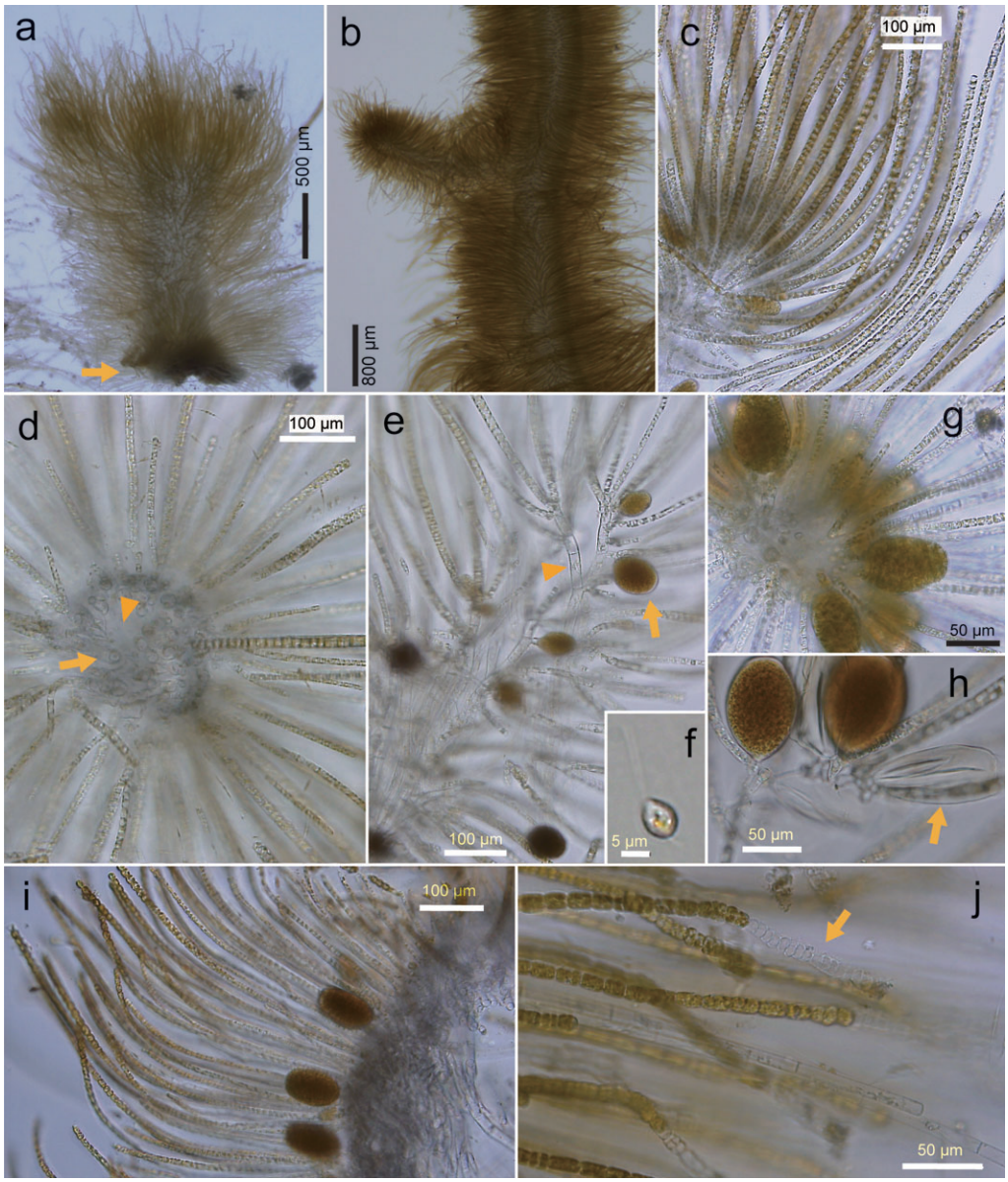

Fig. 3. Sporophyte morphology of *C. takenoensis* sp. nov. (a–h) and *C. umezakii* (i,j) collected at Takeno, Hyogo, Japan on 12 June 2012 by H. Kawai. (a) Habit of juvenile sporophyte showing discoidal holdfast (arrow). (b) Habit of young thallus showing branchlet. (c) Surface view of apex showing long assimilatory filaments. (d) Cross section showing somewhat hollow medulla (arrowhead) and fine medullary filament (arrow). (e) Surface view of upper portion of the thallus showing unilocular zoidangia (arrow) and cortical filament (arrowhead). (f) Released unizoid provided with eyespot. (g) Cross section near apical portion showing unilocular zoidangia. (h) Emptied unilocular zoidangium (arrow). (i) Cross section at mid of the thallus with unilocular zoidaingia. (j) Plurilocular zoidangia formed at the tip of assimilatory filament (arrow).

(Harvey) Kuckuck and *Leathesia difformis* Areschoug was sister to the *Cladosiphon*/*Mesogloia* clade. The phylogenetic relationships among *Cladosiphon*, *M. vermiculata* and *Cladosiphon*/*Mesogloia* were identical in the BI analysis (Fig. S1 in Appendix S1).

In the analyses of dataset 2 using four chloroplast gene sequences (Figs S2, S3 in Appendix S1) as well as dataset 3 using two mitochondrial gene sequences (Figs S4, S5 in Appendix S1), the tree topology was identical as to the relationships among the *Cladosiphon* spp. and *Mesogloia*. Again, close phylogenetic relationships between *C. okamuranus*/*C. zosterae* and *C. umezakii*/*C. takenoensis* were indicated, and *Cladosiphon* was paraphyletic.

rDNA ITS2

A high degree of similarity (99.6%, ambiguous 4 bp were removed from the calculation) was found between the ITS2 sequences of *C. zosterae* and the generitype *C. mediterraneus*, comparable to intraspecific variation in ITS2 sequences in *C. okamuranus* (Figs 5, S6 in Appendix S1). *C. umezakii*

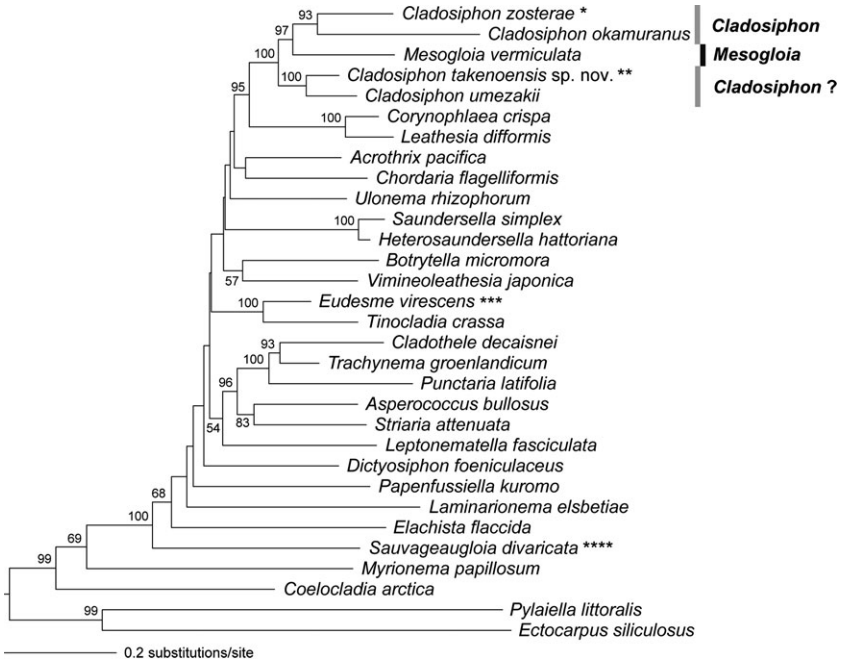

Fig. 4. Maximum likelihood tree based on the concatenated DNA sequences of mitochondrial *cox*1 and *cox*3, and chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes (Total 7642 bp). Numbers at nodes indicate bootstrap values. Only values >50% are shown. *The partial sequences of *cox*3 gene of KU-d13873 (259 bp) was identical to that of KU-3197. **The *cox*3 gene sequences of KU-d899, –d903 and –d5852 were identical to that of SAP115073. ***The partial sequences of *cox*3 gene of KU-d13871 (449 bp) was identical to that of KU-d4645. ****The partial sequences of *cox*3 gene of *Sauvageaugloia griffithsiana* (436 bp) was identical to that of *S. divaricata* (KU-3324).

specimens from the Sea of Japan (Kasumi and Wakasa) and the Pacific (Anan and Awaji) clustered together with 92.2–99.5% similarity. The new species was most closely related to *C. umezakii*, however, *Cladosiphon* was again paraphyletic because *Mesogloia vermiculata* was nested in the clade. In the ML analysis (Fig. 5), *M. vermiculata* was sister to the *C. umezakii*/*C. takenoensis* clade, but it was a member of the *C. umezakii*/*C. takenoensis* clade in the BI analysis (Fig. S6 in Appendix S1).

Cladosiphon takenoensis H. Kawai sp. nov.

Thalli attached to rocks by a small disc, cylindrical, tubular, lubricous, up to 10 cm high, 2.0–3.5 mm thick, moderately and irregularly branched once or twice; medullary cells 25–30 μm in diameter; assimilatory filaments up to 1.8 mm long, 60–80 (–100) cells long, lower cells cylindrical, upper cells swollen, 15–20 μm in diameter, nearly as long as wide; phaeophycean hairs absent; unilocular zoidangia formed at the base of the assimilatory filaments, elliptical to obovate, 110–145 μm long, 80–105 μm broad. Plurilocular zoidangia on the erect thallus absent. The species resembles *Cladosiphon umezakii* in overall morphology but is distinctive in having a more hairy appearance due to longer assimilatory filaments, and larger unilocular zoidangia.

DISCUSSION

The distinctiveness of the new *Cladosiphon* species is revealed by combined morphological and genetic investigations. The species resembles *C. umezakii* in gross morphology and anatomy, but is distinguishable when submerged by its more hairy appearance, due to the considerably longer assimilatory filaments. The new species was genetically most closely related to *C. umezakii* in all DNA sequences examined in the present study, but was genetically different from *C. umezakii* with sequence divergences of 6.6% (*cox*1), 7.8% (*cox*3), 1.9% (*atp*B), 1.0% (*psa*A), 0.4% (*psb*A), 0.6% (*rbc*L), and 14.8% (rDNA ITS2, including insertions and deletions).

Morphological diversity within the genus *Cladosiphon* including *C. mediterraneus*, *C. zosterae*, *C. okamuranus*, *C. umezakii* and *C. takenoensis* is rather high (Table 1). Species may have small adults, up to 1.5 cm high, whereas others reach 50 cm, and the length of assimilatory filaments ranges from 200 to 1800 μm (Ajisaka *et al.* 2007). The genus is also genetically rather diverse, with 0.6–3.2% and 7.8–14.5% interspecific sequence divergences in *rbc*L and *cox*3, respectively, suggesting that a taxonomic reconsideration at the genus level might be necessary. However, the *Cladosiphon* taxa studied in this work share many of the basic morphological features that Kylin (1940) used for defining

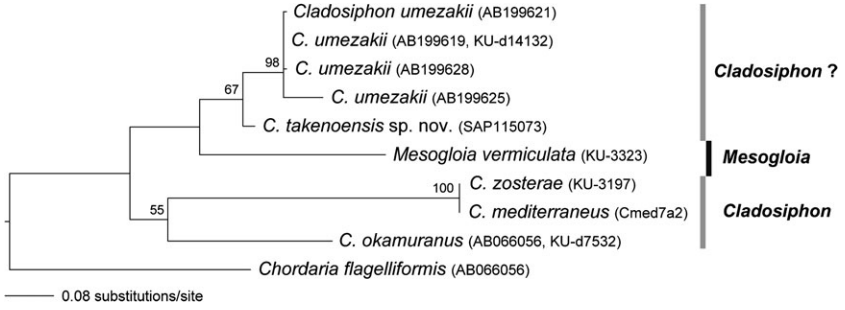

Fig. 5. Maximum-likelihood phylogenetic tree based on the nuclear rDNA ITS2 region sequence (10 OTUs, 196 bp). Numbers at nodes indicate bootstrap values. Only values >50% are shown on the branches.

the genus (*i.e.*, sympodial and polysiphonous central axis, thin subcortex and long assimilatory filaments). Consequently, we suspend taxonomic conclusions about the generic assignment of the species of *Cladosiphon*.

Chordariacean species basically show a heteromorphic life history alternating between macroscopic sporophyte and microscopic gametophytes. The sporophyte generally forms unilocular zoidangia, where meiosis occurs, and the gametophytes form plurilocular gametangia. However, some chordariacean taxa, such as *Cladosiphon*, form asexual plurilocular zoidangia on the sporophyte as an accessory reproductive cycle (Peters 1987 for review). They are transformed from the upper cells of assimilatory filaments, synchronous with unilocular zoidangia or prior to the formation of unilocular zoidangia. Although plurilocular zoidangia in *C. takenoensis* sp. nov. were not observed even in fertile sporophytes bearing unilocular zoidangia, it is possible that this species forms them in later stages, or in different populations and habitats. In *C. umezakii*, plurilocular zoidangia have not been found in Pacific coast populations, although they are common in the Sea of Japan populations (Ajisaka *et al.* 2007). Although the new species differs morphologically from known taxa of *Cladosiphon* in lacking phaeophycean hairs and plurilocular zoidangia, it formed a statistically well-supported clade with *C. umezakii* in the genetic analyses.

There remains doubt about the inclusion of *C. umezakii* and *C. takenoensis* in the genus *Cladosiphon*. The branch of *C. mediterraneus*/*C. zosterae*/*C. okamuranus* and *C. umezakii*/*C. takenoensis* was not monophyletic: *C. mediterraneus*/*C. zosterae*/*C. okamuranus* formed a clade with *Mesogloia vermiculata*. However, the phylogenetic resolution of commonly used DNA sequence regions such as mitochondrial *cox*1, *cox*3, chloroplast *psa*A, *psb*A, *rbc*L and rDNA ITS2 as used in the present study do not appear to have sufficient resolution for elucidating the phylogeny of taxa within families of the Ectocarpales *s.l.* In addition, the numbers of genera in the orders of brown algae are greatest in Ectocarpales: more than 100 genera have been described and are currently accepted taxonomically in Chordariaceae, many of which are monotypic (Reviers, Rousseau, & Silberfeld 2015; Guiry & Guiry 2016). In contrast, the genetic divergences within the order are not greater than in other orders (Silberfeld *et al.* 2011, Kawai *et al.* 2015). Therefore, it is possible that chordariacean (and ectocarpalean) taxa are overdivided in genus level taxonomy, perhaps due to the taxonomic difficulty of interpreting morphological characters. We consider that considerable taxonomic revision of the family is necessary. In order to avoid further confusion by describing a new genus, we suspend the discussion of generic assignment in the present paper and provisionally describe the new species in the genus *Cladosiphon*.

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REFERENCES

Ajisaka, T., Kim, S.-H., Uwai, S. and Kawai, H. 2007. *Cladosiphon umezakii* sp. nov. (Ectocarpales, Phaeophyceae) from Japan. *Phycol. Res.* **55**: 203–13.

Assali, N. E., Mache, R. and Loiseaux-de Goer, S. 1990. Evidence for a composite phylogenetic origin of the plastid genome of the brown alga *Pylaiella littoralis* (L.) Kjellm. *Plant Mol. Biol.* **15**: 307–15.

Cho, T. O., Cho, G. Y., Yoon, H. S., Boo, S. M. and Lee, W. J. 2003. New records of *Myelophycus cavus* (Scytosiphonaceae, Phaeophyceae) in Korea and the taxonomic position of the genus on the basis of a plastid DNA phylogeny. *Nova Hedw.* **76**: 381–97.

Cho, G. Y., Lee, S. H. and Boo, S. M. 2004. A new brown algal order, Ishigeales (Phaeophyceae), established on the basis of plastid protein-coding *rbc*L, *psa*A, and *psb*A region comparisons. *J. Phycol.* **40**: 921–36.

Cock, J. M., Sterck, L., Rouze, P. *et al.* 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* **465**: 617–21.

Guiry, M. D. and Guiry, G. M. 2016. *AlgaeBase*. World-wide Electronic Publication. National University of Ireland, Galway. [Cited on 26 March 2016]. Available from: http://www.algaebase.org

Hamel, G. 1935. Phéophycées de France. Fasc. 2, pp. 81-176, Paris.

Hanyuda, T., Suzawa, Y., Suzawa, T. *et al.* 2004. Biogeography and taxonomy of *Batrachospermum helminthosum* Bory (Batrachospermales, Rhodophyta) in Japan inferred from *rbcL* gene sequences. *J. Phycol.* **40**: 581–8.

Inagaki, K. 1958. A systematic study of the order Chordariales from Japan and its vicinity. *Sci. Pap. Inst. Algol. Res., Fac. Sci. Hokkaido Univ.* **4**: 87–197.

Katoh, K. and Toh, H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* **9**: 286–98.

Kawai, H., Hanyuda, T., Bolton, J. and Anderson, R. 2016. Molecular phylogeny of *Zeacarpa* (Ralfsiales, Phaeophyceae) proposing a new family Zeacarpaceae and its transfer to Nemodermatales. *J. Phycol.* DOI: 10.1111/jpy.12419

Kawai, H., Hanyuda, T., Draisma, S. G. A. and Müller, D. G. 2007. Molecular phylogeny of *Discosporangium mesarthrocarpum* (Phaeophyceae) with a reassessment of the Discosporangiales. *J. Phycol.* **43**: 186–94.

Kawai, H., Hanyuda, T., Lindeberg, M. and Lindstrom, S. C. 2008. Morphology and molecular phylogeny of *Aureophycus aleuticus* gen. et sp. nov. (Laminariales, Phaeophyceae) from the Aleutian Islands. *J. Phycol.* **44**: 1013–21.

Kawai, H., Hanyuda, T., Mumford, T. and Waaland, J. R. 2015. An introduced population of *Chorda asiatica* (Chordaceae, Laminariales) in Puget Sound, Pacific coast of North America. *Phycol. Res.* **63**: 154–8.

Kawai, H., Hanyuda, T., Ridgway, L. M. and Holser, K. 2013. Ancestral reproductive structure in basal kelp *Aureophycus aleuticus*. *Sci. Rep.* **3**: 2491.

Kawai, H., Kogishi, K., Hanyuda, T. and Kitayama, T. 2012. Taxonomic revision of the genus *Cutleria* proposing a new genus *Mutimo* to accommodate *M. cylindrica* (Cutleriaceae, Phaeophyceae). *Phycol. Res.* **60**: 241–8.

Kawai, H., Muto, H., Fujii, T. and Kato, A. 1995. A linked 5S rRNA gene in *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycol.* **31**: 306–11.

Kawai, H., Sasaki, H., Maeda, Y. and Arai, S. 2001. Morphology, life history and molecular phylogeny of *Chorda rigida* sp. nov. (Laminariales, Phaeophyceae) from the Sea of Japan and the genetic diversity of *Chorda filum*. *J. Phycol.* **37**: 130–42.

Kim, S.-H. and Kawai, H. 2002. Taxonomic revision of *Chordaria flagelliformis* (Chordariales, Phaeophyceae) including novel use of the intragenic spacer region of rDNA for phylogenetic analysis. *Phycologia* **41**: 328–9.

Kützing, F. T. 1843. *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange*. F.A. Brockhaus, Leipzig, 80 pls.

Kylin, H. 1940. Die Phaeophyceenordnung Chordariales. *Lunds Univ. Årsskr. N. F. Avd. 2* **36**: 1–67.

Lane, C. E., Lindstrom, S. C. and Saunders, G. W. 2007. A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Mol. Phylogenet. Evol.* **44**: 634–48.

Le Corguillé, G., Pearson, G., Valente, M. *et al.* 2009. Plastid genomes of two brown algae, *Ectocarpus siliculosus* and *Fucus vesiculosus*: further insights on the evolution of red-algal derived plastids. *BMC Evol. Biol.* **9**: 253–66.

Lindauer, V. W., Chapman, V. J. and Aiken, M. 1961. The marine algae of New Zealand. II: Phaeophyceae. *Nova Hedw.* **3**: 129–350.

Lis, J. T. 1980. Fractionation of DNA fragments by polyethylene glycol induced precipitation. *Methods Enzymol.* **65**: 347–53.

Ni-Ni-Win, Hanyuda, T., Arai, S., Uchimura, M., Abbott, I. A. and Kawai, H. 2008. New records of Padina species from the western coast of the Pacific Ocean. *Phycol. Res.* **56**: 288–300.

Oudot-Le Secq, M. P., Fontaine, J. M., Rousvoal, S., Kloareg, B. and Loiseaux-De Goer, S. 2001. The complete sequence of a brown algal mitochondrial genome, the ectocarpale *Pylaiella littoralis* (L.) Kjellm. *J. Mol. Evol.* **53**: 80–8.

Parke, M. 1933. A contribution to knowledge of the Mesogloiaceae and associated families. *Publ. Hartley Bot. Lab. Liverpool* **9**: 1–43.

Peters, A. F. 1987. Reproduction and sexuality in the Chordariales (Phaeophyceae). A review of culture studies. *Prog. Phycol. Res.* **5**: 224–63.

Peters, A. F. 2003. Molecular identification, distribution and taxonomy of brown algal endophytes, with emphasis on species from Antarctica. *Proc. Int. Seaweed Symp.* **17**: 293–302.

Rambaut, A. and Drummond, A. 2007. Tracer v1.5. [Cited on 27 March 2016]. http://tree.bio.ed.ac.uk/software/tracer/

Reviers, B.de, Rousseau, F. and Silberfeld, T. 2015. Phaeophyceae. *In* Frey, W. (Ed.) *Syllabus of Plant Families A. Engler's Syllabus der Pflanzenfamilien*. Forntraeger, Stuttgart, pp. 139–76.

Ronquist, F., Teslenko, M., van der Mark, P. *et al.* 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**: 539–42.

Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* **6**: 461–4.

Siemer, B. L., Stam, W. T., Olsen, J. L. and Pedersen, P. M. 1998. Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and Spacer sequences. *J. Phycol.* **34**: 1038–48.

Silberfeld, T., Leigh, J. W., Verbruggen, H., Cruaud, C., de Reviers, B. and Rousseau, F. 2010. A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". *Mol. Phylogenet. Evol.* **56**: 659–74.

Silberfeld, T., Racault, M.-F. L. P., Fletcher, R. L., Couloux, A., Rousseau, F. and de Reviers, B. 2011. Systematics and evolutionary history of pyrenoid-bearing taxa in brown algae (Phaeophyceae). *Eur. J. Phycol.* **46**: 361–77.

Silberfeld, T., Rousseau, F. and de Reviers, B. 2014. An updated classification of brown algae (Ochrophyta, Phaeophyceae). *Cryptog. Algol.* **35**: 117–56.

Silvestro, D. and Michalak, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* **12**: 335–7.

Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Mol. Ecol. Resour.* **11**: 914–21.

Tanaka, A., Uwai, S., Nelson, W. and Kawai, H. 2010. *Phaeophysema* gen. nov. and *Vimineoleathesia* gen. nov., new brown algal genera for the minute Japanese members of the genus *Leathesia*. *Eur. J. Phycol.* **45**: 109–17.

Tokida, J. 1942. Phycological observations V Trans Sapporo. *Nat. Hist. Soc.* **17**: 82–95.

Womersley, H. B. S. and Bailey, A. 1987. Family Chordariaceae Greville. *In* Womersley, H. B. S. (Ed.) *The Marine Benthic Flora of Southern Australia*. South Australian Government Printing Division, Adelaide, pp. 103–27.

Yoon, H. S., Hackett, J. D. and Bhattacharya, D. 2002. A single origin of the peridinin–and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci. U. S. A.* **99**: 11724–9.

SUPPORTING INFORMATION

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

Appendix S1

Fig. S1. Bayesian consensus tree based on the concatenated DNA sequences of mitochondrial *cox*1 and *cox*3, and chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes (total 7,642 bp). Numbers on branches indicate posterior probabilities. Only posterior probabilities >0.90 are shown.

Fig. S2. Maximum likelihood tree based on the concatenated DNA sequences of chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes (total 5,463 bp). Numbers at nodes indicate bootstrap values. Only values >50% are shown.

Fig. S3. Bayesian consensus tree based on the concatenated DNA sequences of chloroplast *atp*B, *psa*A, *psb*A and *rbc*L genes (total 5,463 bp). Numbers on branches indicate posterior probabilities. Only posterior probabilities >0.90 are shown.

Fig. S4. Maximum likelihood tree based on the concatenated DNA sequences of mitochondrial *cox*1 and *cox*3 genes (total 2,179 bp). Numbers at nodes indicate bootstrap values. Only values >50% are shown.

Fig. S5. Bayesian consensus tree based on the concatenated DNA sequences of mitochondrial *cox*1 and *cox*3 genes (total 2,179 bp). Numbers on branches indicate posterior probabilities. Only posterior probabilities >0.90 are shown.

Fig. S6. Bayesian consensus tree based on the nuclear rDNA ITS2 region sequence (10 OTUs, 196 bp). Numbers on branches indicate posterior probabilities. Only posterior probabilities >0.90 are shown.

Appendix S2

Table S1. Origin of samples and sequence data used for molecular analyses, including their database accession numbers. Sample codes in [KU-###] correspond to KU-MACC (Kobe University Macroalgal Culture Collection) strain code, and [KU-d###] corresponds to silica gel-dried specimens housed at Kobe University Research Center for Inland Seas.

Table S2. List of primers used for PCR and sequencing.

[Correction added on 11 November 2016, after first online publication: The supporting information descriptors have been corrected and the missing supporting information Table S1 and S2 have been added online].