New distributional records, taxonomy, morphology, and genetic variations of the endangered brackish-water species Lamprothamnium succinctum (Charales:…

Kato, Syou ; Tanaka, Jiro ; Tanaka, Norio ; Yokoyama, Jun ; Ito, Yu ; Fujiwara, Yoichiro ; Higa, Atsushi ; Kobayashi, Shingo ; Watanabe,…

(Citation)
Journal of Asia-Pacific Biodiversity,14(1):15-22

(Issue Date)
2021-03-01

(Resource Type)
journal article

(Version)
Version of Record

Journal of Asia-Pacific Biodiversity 14 (2021) 15–22

Contents lists available at ScienceDirect

Journal of Asia-Pacific Biodiversity

journal homepage: http://www.elsevier.com/locate/japb

Original Article

New distributional records, taxonomy, morphology, and genetic variations of the endangered brackish-water species *Lamprothamnium succinctum* (Charales: Charophyceae) in Japan

Syou Kato [a,j], Jiro Tanaka [b], Norio Tanaka [c], Jun Yokoyama [d], Yu Ito [e], Yoichiro Fujiwara [f], Atsushi Higa [g], Shingo Kobayashi [h], Makoto M. Watanabe [i], Hidetoshi Sakayama [a,*]

[a] Department of Biology, Graduate School of Science, Kobe University, Rokkodai 1-1, Nada-ku, Kobe, Hyogo 657-8501, Japan
[b] Department of Oceans Sciences, Faculty of Marine Science, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato-ku, Tokyo 108-8477, Japan
[c] Tsukuba Botanical Garden, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan
[d] Faculty of Science, Yamagata University, Kojirakawa 1-4-12, Yamagata 990-8560, Japan
[e] Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge 45-1, Hirakata, Osaka 573-0101, Japan
[f] Ehime Botanical Club (c/o Ehime Prefectural Science Museum), Oojouin 2133-2, Niihama, Ehime 792-0060, Japan
[g] Okinawa Environmental Analysis Center Co., Ltd., Maehara 3-7-24, Ginowan, Okinawa 901-2215, Japan
[h] Curatorial division, Ehime Prefectural Science Museum, Oojouin 2133-2, Niihama, Ehime 792-0060, Japan
[i] Algae Biomass and Energy System R&D Center, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

ARTICLE INFO

Article history:
Received 15 March 2020
Received in revised form
4 July 2020
Accepted 10 September 2020
Available online 28 September 2020

Keywords:
Charophyceae
Japanese endangered species
Lamprothamnium succinctum
Molecular phylogeny
Oospores

ABSTRACT

Members of the brackish-water species *Lamprothamnium succinctum* (Charales, Charophyceae) are widely distributed from tropical to temperate regions, including East Asia. In Japan, *L. succinctum* is listed as an endangered species and is protected by the government, because it was recorded only at two localities, Lake Hachiro-gata (Akita Prefecture) and Oo-ike pond (Deba-jima Island, Tokushima prefecture), and has become extinct in the former. In this study, we identified five new localities of this species in Japan. The morphological characteristics of their thalli agreed with those provided in the original description of this species, with distinctive reproductive characteristics. Moreover, the oospores of Japanese specimens of *L. succinctum* were examined for the first time using scanning electron microscopy. The oospores of Japanese specimens exhibited granulate fossa wall patterns, which were consistent with those described in previous studies. Our genetic analyses based on the DNA sequences of two chloroplast DNA markers, including both the coding and non-coding regions, revealed that the sample from Oo-ike pond is distinguishable from those from other Japanese specimens, although they are genetically very similar.

Introduction

The genus *Lamprothamnium* J. Groves (Charales, Charophyceae) is characterized by ecorticated thalli, unforked whorled branchlets, stipulodes occurring directly below the branchlets, and one tier of coronal cells (cronula) in the oogonia (Wood 1965; Imahori and Kasaki 1977; Bryant and Stewart 2011; Casanova 2013). In most species, the male organs (antheridia) are positioned above the female reproductive organs (oogonia). This genus usually develops spherical bulbils on the rhizoidal filaments, inhabits brackish to saline water environments, and has an almost cosmopolitan distribution (Wood 1965; García and Casanova 2003). Within the genus, 20 extant taxa have been described (Groves 1916; Ophel 1947; Corillion 1957; Wood 1965; de Donterberg 1984; García and Casanova 2003; Schubert and Blindow 2003; Casanova 2013).

Lamprothamnium succinctum (A. Braun) R. D. Wood is characterized by irregularly developed stipulodes and the occasional positioning of oogonia above antheridia (Wood 1965). Moreover, its members are distributed widely from tropical to temperate regions, including East Asia (Allen 1887; Groves 1919; Zaneveld 1940; Kasaki 1964; Wood 1965; Guerlesquin et al 1987; Choi and Kim

* Corresponding author. Tel.: +81 78 803 5723; fax: +81 78 803 5723.
E-mail address: hsak@port.kobe-u.ac.jp (H. Sakayama).
Peer review under responsibility of National Science Museum of Korea (NSMK) and Korea National Arboretum (KNA).
[j] Present address: Faculty of Education, Niigata University, Ninocho 8050, Ikarashi, Nishi-ku, Niigata 950-2181, Japan.

https://doi.org/10.1016/j.japb.2020.09.005
pISSN2287-884X eISSN2287-9544/

1997; Casanova 2013). In Japan, this species was recorded only in two localities, i.e. Lake Hachiro-gata (Akita Prefecture) and Oo-ike pond (Deba-jima Island, Tokushima prefecture) (Imahori 1963; Kasaki 1964). However, recent field surveys revealed that this species has become extinct in Lake Hachiro-gata because of the reclamation of the lake (Imahori and Kasaki 1977; Watanabe et al 2005). Consequently, *L. succinctum* was listed as a "critically endangered species (CR+EN)" in the Japanese Red List of Ministry of the Environment, Japan (MOEJ 2019), and the remaining viable populations of this species inhabiting in Oo-ike pond were protected as a natural monument designated by the government in 1972. In addition, individuals of this species collected at Oo-ike pond were cultured and are maintained in the Microbial Culture Collection at the National Institute for Environmental Studies (MCC-NIES, Tsukuba, Japan), for *ex situ* conservation.

Recent scanning electron microscopy (SEM) and molecular phylogenetic studies have shown that the morphology of the fully mature oospores is a reliable character for elucidating the species-level taxonomy of the charalean taxa (John and Moore 1987; John et al 1990; Leitch 1990; Ray et al 2001; Sakayama et al 2002; Sakayama et al 2004; Sakayama et al 2005; Casanova 2005, 2009; Sakayama 2008; Sakayama et al 2009; Urbaniak 2011a, 2011b; Perez et al 2014, 2015; Sakayama et al 2015; Perez et al 2017). However, the oospore morphology of Japanese *L. succinctum* has not been studied at the SEM level, and only two specimens of this species have been analyzed based on molecular phylogeny (Sakayama et al 2009; Perez et al 2017). More recently, Casanova (2013) pointed out that *L. succinctum* sensu R. D. Wood (1965) might include several species based on detailed morphological analyses. Therefore, the examination of the detailed morphology of fully mature specimens and genetic analyses are necessary to clarify the taxonomic status of Japanese specimens of *L. succinctum*.

During our recent field surveys, we identified five new localities of *L. succinctum* inhabitation in Japan. In this study, we determined the morphology of the vegetative thalli and the fine oospore structures of *L. succinctum* using newly collected fully matured specimens. In addition, we conducted genetic analyses of the chloroplast *rbc*L DNA sequences and of the intergenic spacer (IGS) region located between the *atp*B and *rbc*L genes (*atp*B-*rbc*L IGS) in Japanese specimens of *L. succinctum*, to elucidate their phylogenetic relationships and genetic differences.

Material and methods

The localities at which sample collections were performed are shown in Figure 1 and Table 1. The methods used for field collection, culture, light microscopy (LM), and SEM were all according to those reported by Sakayama et al (2002, 2004, 2009, 2015) and Kato et al (2008), with the following modifications: filtered seawater was diluted to a salinity of approximately 10‰ and used to prepare the brackish soil–water medium for the Charales (B-SWC). The pressed specimens were deposited at the Herbarium, Department of Botany, National Science Museum (TNS), Tsukuba, Japan.

The extraction of total DNA, amplification of DNA by polymerase chain reaction (PCR), direct sequencing of the PCR products, and phylogenetic analyses were conducted as described previously (Sakayama et al 2002; Sakayama et al 2006; Kato et al 2008), with

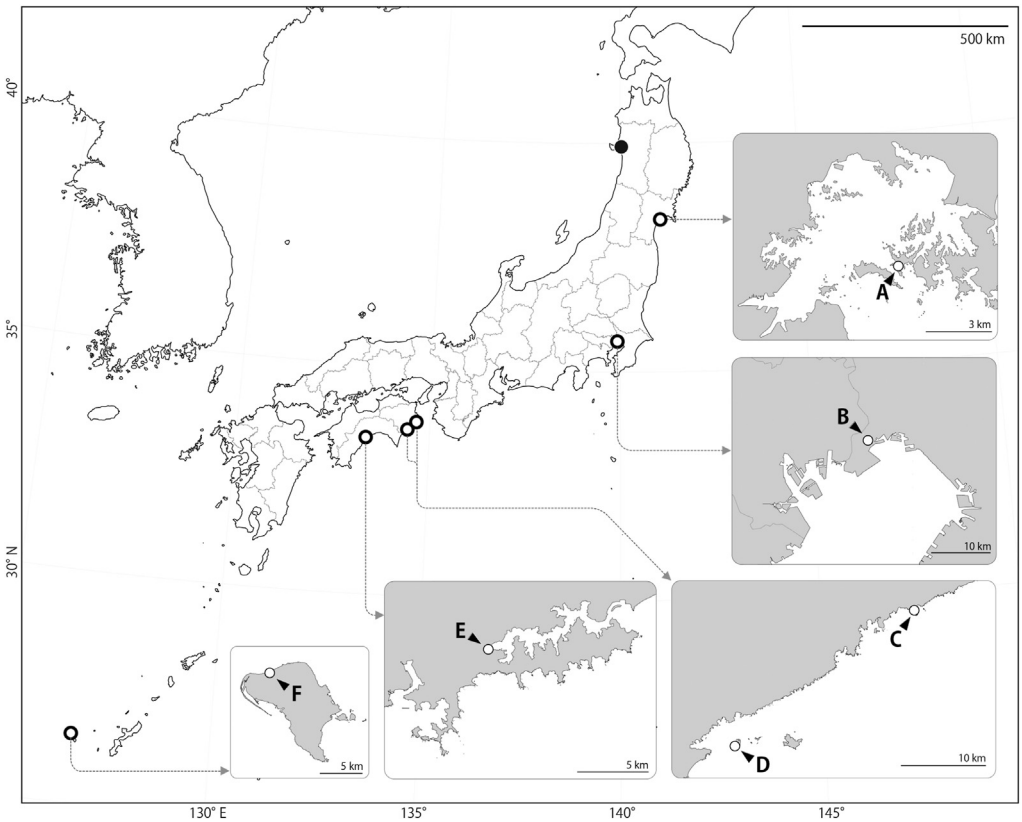

Figure 1. Map of the localities where the Japanese specimens of *Lamprothamnium succinctum* were collected for this study (open circles, A–F). The closed circle (Lake Hachiro-gata, Akita Prefecture) indicates the locality at which this species has become extinct. For detailed information on these localities, see Table 1.

Table 1. Sources of the specimens of *Lamprothamnium succinctum* used in this study.

Collection and strain/specimen information			*rbc*L		*atp*B-*rbc*L IGS	
Collection site[a]	Collection date	Strain/specimen designation	Accession number	Source	Accession number	Source
Pond at Nonoshima Island, Shiogama, Miyagi (A)	2012/10/5	MYG006/TNS-AL 213317	LC536055	This study	LC536062	This study
Pond at Niihama, Ichikawa, Chiba (B)	2007/6/29	SK166/TNS-AL 213318	LC536056	This study	LC536063	This study
	2008/5/24	-/TNS-AL 213319	LC536057	This study	LC536064	This study
Jaga-ike Pond at Minami, Kaifu, Tokushima (C)	2003/12/14	-/TNS-AL 213320	LC536058	This study	LC536065	This study
	2004/6/14	KGK1947/NY01089138	KX431014	Perez et al (2017)	-	-
Oo-ike pond, Deba-jima Island, Muki, Tokushima (D)	-	S038/TNS-AL 213321	AB440262	Sakayama et al (2009)	LC536066	This study
Pond at Uranouchi	2006/9/20	-/TNS-AL 213322	-	-	-	-
Nishibun, Susaki, Kochi (E)	2012/8/28	EHM117/TNS-AL 213323	LC536059	This study	LC536067	This study
Pond at Kume-jima Island, Kume-jima, Okinawa (F)	2006/5/13	SK164/TNS-AL 213324	LC536060	This study	LC536068	This study
	2012/9/3	KMJM002/TNS-AL 213325	LC536061	This study	LC536069	This study
	2018/5/26	S399/TNS-AL 213326	-	-	-	-

[a] The characters placed in parentheses after the collection site indicate the localities shown in Figure 1.

the exception of the use of the four primers designed by us for the *atp*B-*rbc*L IGS (Table 2).

For molecular phylogenetic analyses, we used a data matrix containing 1,194 base pairs (bp) of unambiguously aligned *rbc*L gene sequences [corresponding to nucleotides 31 to 1224 of the *rbc*L gene of *Chara vulgaris* L.; Accession No. NC_008097 (Turmel et al 2006)] from nine specimens of *L. succinctum*, as well as 19 species from the genera *Chara* L., *Lamprothamnium*, *Nitellopsis* Hy, and *Lychnothamnus* (Rupr.) Leonh. (Table 1, Appendix A; TreeBASE accession number S26033). Two species of the genera *Nitellopsis* and *Lychnothamnus* were selected as an outgroup because recent phylogenetic studies demonstrated that the tribe Chareae (*Chara*, *Lamprothamnium*, *Lychnothamnus*, and *Nitellopsis*) is monophyletic, where a clade comprising of species of genera *Nitellopsis* and *Lychnothamnus* is sister to a clade comprising of species of genera *Chara* and *Lamprothamnium* (McCourt et al 1996; McCourt et al 1999; Sakayama et al 2002; Sakayama et al 2004; Sakayama 2008; Sakayama et al 2009; Perez et al 2014; Perez et al 2017). The aligned dataset was subjected to the Bayesian inference (BI) method using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). Kakusan4 (Tanabe 2011) was used to identify the sequence evolution model that fitted the dataset. The criteria used for model selection were Bayesian Information Criterion (BIC4) for BI analyses and corrected Akaike's Information Criterion (AICc4) for maximum likelihood (ML) analyses. The substitution models used for each codon position of the *rbc*L gene in the BI analyses were HKY85 + I (first codon position), JC69 (second codon position), and GTR + I (third codon position), which were selected by Kakusan4. BI analyses were performed using MrBayes 3.2.6, as previously described (Nakada and Nozaki 2009). The parameters of the substitution models for each codon position were unlinked. The Markov chain Monte Carlo iteration process was stopped at 1,000,000 generations. The first 25% of generations were discarded as burn-in, whereas the remaining trees were used to calculate a 50% majority-rule tree and to determine the posterior probabilities of individual branches. The average standard deviations of the split frequencies were below 0.01, indicating convergence of the iterations. ML analyses were performed with 1,000 bootstrap replications (Felsenstein 1985) using RAxML v. 8.2.10 (Stamatakis 2014), and the GTR + G model (selected by Kakusan4) was used for each codon position. Maximum-parsimony analyses were performed with 1,000 bootstrap replications (using a heuristic search with the stepwise addition of 10 random replications using the tree-bisection–reconnection branch-swapping algorithm) using PAUP* 4.0b10 (Swofford 2002).

Results

Morphological observations

The thalli of the present specimens of *L. succinctum* were monoecious, bright to dark green, and up to 40 cm high. The axes were up to 440 μm in diameter, the internodes were up to ca. 4.5 cm long, and cortical cells were absent (Figure 2A). The stipulodes were developed, grew downward, were placed opposite the branchlets in one tier, and were up to ca. 900 μm long and ca. 110 μm in diameter (their length varied) (Figure 2B). Whorls comprised 6–8 branchlets that were up to ca. 4 cm long, lacked cortical cells, and were composed of three or four (or five) segments (Figure 2A). The terminal segments of branchlets were one- or two-celled, were elongated, and were up to ca. 2 cm long (Figure 2A). The branchlets exhibited one or two bracteoles that were up to ca. 810 μm long and included two or three bract cells that were weakly or well developed and up to ca. 650 μm long (Figure 2A and 2C). The bulbils were spherical and up to ca. 900 μm in diameter and were arranged in clusters of 1 to 4 units attached to the rhizoidal filaments (Figure 2D). The oogonia and antheridia were usually sejoined (rarely conjoined) and formed at the basal two-branchlet nodes and at the base of the whorl (Figure 2C). The oogonia were bright yellow, up to ca. 890 μm long (excluding coronula), up to ca. 550 μm wide, and had 9–11 convolutions; the coronula were ca. 80–110 μm long and 130–160 μm wide (Figure 2C). Finally, the antheridia were orange and 360–460 μm in diameter (Figure 2C). These vegetative and reproductive characters were essentially consistent with those included in previous descriptions of Japanese specimens (Kasaki 1964; Imahori and Kasaki 1977).

In the present study, the oospore morphology of Japanese specimens of *L. succinctum* was examined using SEM for the first time. The oospores were dark-brown to black colored and ellipsoid in shape, and had 9–11 spiral ridges (Figure 3A). Moreover, they were ca. 550–680 μm long, ca. 280–360 μm wide, and up to 74 μm across the fossa (Figure 3A). The spiral ridges were weakly developed and lacked ribbon-like structures (Figures 3A and 3B). The

Table 2. Primers used for the amplification and sequencing of the full-length *atp*B-*rbc*L IGS performed in the present study.

Designations	Sequence (5' to 3')	Position	Source
CH-*atp*B-R1	AGACCATCAGTAGCACTCATAG	248–227[b]	Kato et al (2008)
CHAR-RR-5[a]	GCTAAAATGTCAGTATCTTTAG	113–92[c]	Kato et al (2008)
Cb_*atp*B_R84-63	AACAGGTCCAATAATTTGAGTA	84–63[b]	This study
Ls_IGS-F23	ATCAATGGTATATATGAATTTGAA	436–459[d]	This study
Ls_IGS-R14[a]	AGTTTCTTTATATTGAAAAGCATGA	619–595[d]	This study
CH_*rbc*L_R65-44[a]	AATCTGTAATCTTTTACCCCTG	65–44[c]	This study

IGS, intergenic spacer.
[a] Reverse primer.
[b] Coordinate number from the *atp*B gene of *Chara vulgaris* (Turmel et al 2006).
[c] Coordinate number from the *rbc*L gene of *C. vulgaris* (Turmel et al 2006).
[d] Coordinate number from the *atp*B-*rbc*L IGS of *Lamprothamnium succinctum* (strain S038).

oospore wall ornamentations were granulate, as observed using LM (Figure 3D). Under SEM, it was observed that the fossa wall had an irregular granulate pattern (Figure 3B); the granules rarely had a pore at the apex and were ca. 0.3–2.7 μm in diameter (Figure 3C).

Molecular phylogeny and genetic variations

The *rbc*L DNA sequences (1,194 bp) of our seven Japanese specimens of *L. succinctum* were identical to the previously published sequences of the specimens from Oo-ike pond (strain S038) and Jaga-ike pond (KGK1947) (Figure 4, Table 1). The Japanese specimens of *L. succinctum* formed a robust clade with three other species of *Lamprothamnium* [*L. papulosum* (Wallroth) J. Groves, *L. macropogon* (A. Braun) Ophel, and *L. heraldii* Adr. García & Casanova]. In our *rbc*L phylogeny, the phylogenetic position of the genus

Figure 2. Thalli of *Lamprothamnium succinctum* collected from Ichikawa, Chiba, Japan: A, apical part of a thallus consisting of a main axis and whorled branchlets; B, node of the main axis, from which whorled branchlets (above) and developed stipulodes (below) occur; C, node of the main axis, showing whorled branchlets with antheridia and bracteoles/bract cells, as well as oogonia that formed on the inside of the base of the whorl (OO: oogonia, AN: antheridia); D, white spherical bulbils attached to the rhizoidal filaments. <scale bar: 1 mm (A–D)>.

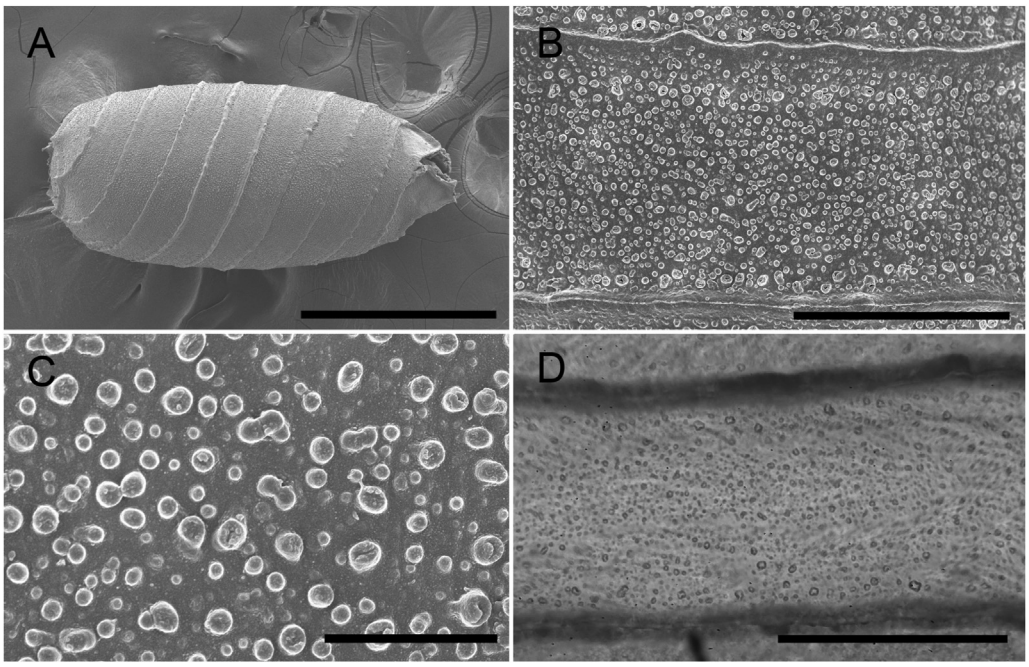

Figure 3. Oospores of *Lamprothamnium succinctum* collected from Ichikawa, Chiba, Japan: A, overall appearance of an oospore, as imaged using scanning electron microscopy (SEM), showing 9 or 10 flanged spiral ridges on the surface, and fossa walls between the spiral ridges; B, part of the fossa wall, as imaged using SEM, showing an irregular granulate ornamentation; C, detail of the fossa wall, as imaged using SEM, showing large and minute granules on its surface; D, part of the fossa wall, as imaged using light microscopy, showing an irregular granulate pattern. <scale bar: 300 μm (A); 50 μm (B and D); 10 μm (C)>.

Lamprothamnium was not clearly supported. This finding was similar to that reported previously (McCourt et al 1999; Sakayama et al 2009; Perez et al 2014; Perez et al 2017). Based on the full-length sequences of the *atp*B-*rbc*L IGS (1,065 bp), two haplotypes were detected within the eight specimens of *L. succinctum* examined in the present study, in which a single-nucleotide variant was found in the *atp*B-*rbc*L IGS sequences of the S038 strain (from Oo-ike pond, Deba-jima Island) (Appendix B).

Systematic accounts

Lamprothamnium succinctum (A. Braun) R. D. Wood

(Figures 2, 3)

Lamprothamnium succinctum (A. Braun) R. D. Wood, Taxon 11: 15, 1962 (Wood 1962).—*Chara succincta* A. Braun in Asch., Oesterr. Bot. Zeitschr. 28: 257, 1878 (Ascherson 1878).

Diagnosis. Lamprothamnium succinctum is similar to *L. hansenii* (C. Sond.) Corill. and *L. tasmanicum* (A. Braun) Casanova in having monoecious thalli without foxtail-like apices, in which whorled branchlets are not inflated (Wood 1965; Casanova 2013). However, *L. succinctum* can be distinguished clearly from *L. hansenii* by the size of stipulodes (irregular in size and up to 1,000 μm long in *L. succinctum*, and uniform in size and well developed in *L. hansenii*) and the position and arrangement of reproductive organs [usually sejoined (when conjoined, oogonium are positioned above or beside antheridium) and formed at the base of the whorl in *L. succinctum*, and conjoined (oogonium are positioned below antheridium) and not formed at the base of the whorl in *L. hansenii*], and also distinguished clearly from *L. tasmanicum* by the oospore wall ornamentation (irregular glanulate in *L. succinctum* and smooth to roughened in *L. tasmanicum*) and the position and arrangement of reproductive organs [usually sejoined (or rarely conjoined) in *L. succinctum*, and uniformly sejoined in *L. tasmanicum*].

Distribution. Japan (Imahori 1963; Kasaki 1964), Egypt (Ascherson 1878), Libya (Compère 1986), Spain (Canary Islands) (Henríquez and Villalba 1995), Morocco (Muller et al 2017), Oman (Hussain et al 2003), Pakistan (Langangen and Leghari 2001), India (Dixit 1931; Khan 1980), China (Groves 1919; Ling et al 2000), Korea (Choi and Kim 1997), New Caledonia (Wood 1966), Mauritius (Allen 1887), and Bolivia (Guerlesquin 1981).

Japanese specimens examined in this study. TNS-AL 213317 (S. Kato, strain MYG006; collected by S. Kato and J. Yokoyama from Nonoshima Island, Miyagi); TNS-AL 213318 (S. Kato, strain SK166; collected by S. Kato, Y. Ito and N. Tanaka from Ichikawa, Chiba); TNS-AL 213319 (collected by H. Morishima from Ichikawa, Chiba); TNS-AL 213320 (collected by S. Kinoshita, A. Narita and M. Saji from Jaga-ike Pond, Tokushima); TNS-AL 213321 (H. Sakayama, strain S038; collected by E. Yoshida from Oo-ike pond, Deba-jima Island, Tokushima); TNS-AL 213322 (collected by Y. Ito and N. Tanaka from Susaki, Kochi); TNS-AL 213323 (S. Kato, strain EHM117; collected by S. Kato, S. Kobayashi and Y. Fujiwara from Susaki, Kochi); TNS-AL 213324 (S. Kato, strain SK164; collected by J. Tanaka from Kume-jima Island, Okinawa); TNS-AL 213325 (S. Kato, strain KMJM002; collected by S. Kato and A. Higa from Kume-jima Island, Okinawa); TNS-AL 213326 (H. Sakayama, strain S399; collected by H. Sakayama and A. Higa from Kume-jima Island); MAK A12442, MAK A12443, MAK A12444, MAK A12445 and MAK A12446 (collected by K. Kato from Lake Hachiro-gata).

Other specimen examined in this study. MAK A20679 (collected by A. S. Hitchkock from Mauritius).

Discussion

Although the vegetative morphology is similar among the species of *Lamprothamnium*, they exhibit very marked differences in the arrangement of their reproductive organs and oospore characteristics (Wood 1965; García and Casanova 2003; García and Chivas 2004; Casanova 2013). In a monograph on Charales (Wood

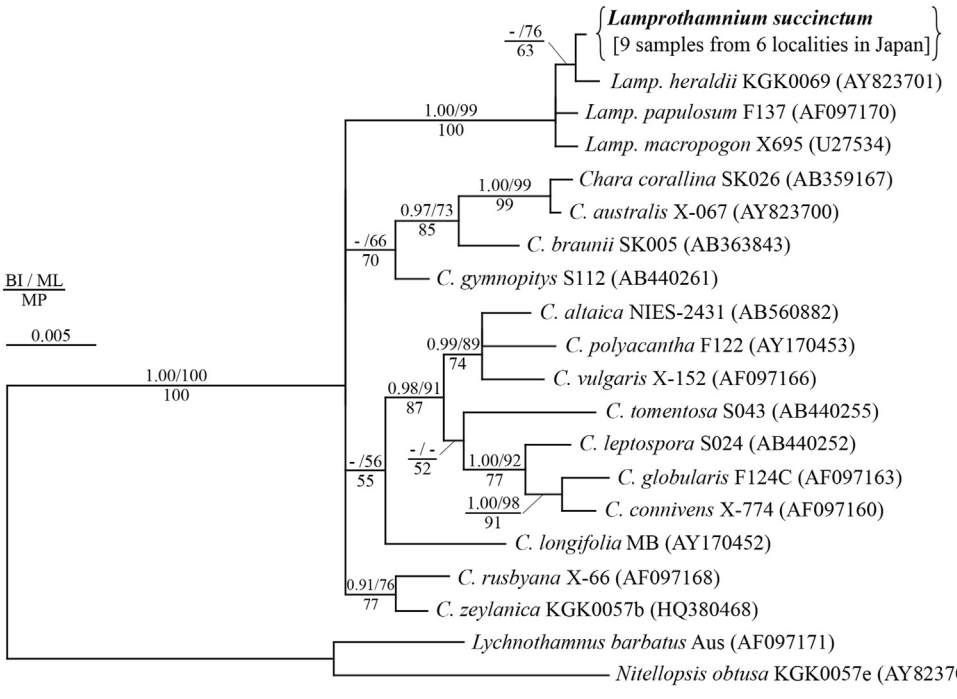

Figure 4. The Bayesian phylogenetic tree based on the 1,194 bp of the *rbc*L DNA sequences of 20 operational taxonomic units (OTUs). Specimens with identical sequences were treated as a single OTU (Table 1, Appendix A). The characters annotated after the species name and the subsequent numbers in parentheses represent the strain/specimen names and the accession numbers on the GenBank database, respectively. The numbers placed above the branches indicate the posterior probabilities of Bayesian inference (BI, left) and the bootstrap values obtained from maximum likelihood (ML, right) analyses. The numbers placed below the branches indicate the bootstrap values obtained from a maximum-parsimony (MP) analysis. Only posterior probabilities ≥ 0.90 and bootstrap values ≥ 50% are shown. Branch lengths and the scale bar represent the expected number of nucleotide substitutions per site.

1965), the taxa of *Lamprothamnium* were classified into three species with eight infraspecific taxa, mainly based on vegetative and reproductive characteristics. Recently, Casanova (2013) analyzed a large number of Australian specimens of the genus and revised their taxonomic status, mainly based on reproductive and oospore characteristics. That author reported that the variation in the arrangement of reproductive organs is useful for delineating the monoecious species of the genus.

The thalli of the present specimens were identified as *L. succinctum* based on their vegetative and reproductive characteristics (Figure 2). Moreover, our SEM observations showed that the overall appearance and fossa wall patterns of oospores of Japanese *L. succinctum* are consistent with those of the specimens analyzed in previous studies (Frame 1977; García et al 2002; García and Chivas 2004) (Figure 3). In the thalli of Japanese specimens, the oogonia and antheridia were usually sejoined; the antheridia were solitary and formed at the basal two-branchlet nodes, whereas the oogonia were usually formed on the inside of the base of the whorl (Figure 2C). However, the present specimens were slightly different from the type material of *L. succinctum* collected in North Africa, because the type material has one or two oogonia both on the outside and the inside of the base of the whorl, and antheridia conjoined with oogonia on the branchlets (Casanova 2013). Wood (1962, 1965) selected the specimen collected in Mauritius as the neotype specimen, because the holotype specimen collected in North Africa was not found. Casanova (2013) pointed out that the North-African specimens are different from the specimens from Mauritius (Allen 1887; Wood and Imahori 1964) as well as the specimens from New Caledonia (Zaneveld 1940; Wood 1966). Therefore, for future taxonomic revisions, morphological and molecular analyses are needed to clarify the taxonomic status of *L. succinctum* using fully matured specimens from these localities as well as large numbers of specimens from various localities located worldwide.

We performed genetic analyses based on the *rbc*L and *atp*B-*rbc*L IGS sequences (Figure 4, Appendix B). Our *rbc*L phylogeny demonstrated that all of the Japanese specimens of *L. succinctum* had an identical *rbc*L DNA sequence and that this species formed a robust clade with three other species of *Lamprothamnium* (*L. papulosum*, *L. macropogon*, and *L. heraldii*) (Figure 4). Moreover, based on the *atp*B-*rbc*L IGS sequences of the eight Japanese specimens (Table 1), only two haplotypes (IGS-1 and IGS-2) differing by a single nucleotide were identified, with the *L. succinctum* sample from Oo-ike pond (strain S038) having a distinct haplotype compared with those from other Japanese specimens (Appendix B). Our results indicate that the degree of genetic divergence of Japanese *L. succinctum* is very low and that they are genetically very close to each other.

Our morphological and molecular analyses of Japanese specimens of *L. succinctum* revealed the detailed morphological characteristics and genetic differences among Japanese populations of this species. We only analyzed specimens collected in Japan because this study focused on the relationship among Japanese populations, although this species has been reported in many countries and morphological variations have been recognized within this species. Moreover, 20 extant taxa have been described for *Lamprothamnium*. Therefore, in future taxonomic studies, further morphological and molecular analyses of these taxa in a large number of specimens collected globally are needed to resolve their natural relationships.

Finally, in Japan, *L. succinctum* is listed as an endangered species and is protected by the government, because this species has been recorded only at two localities (Lake Hachiro-gata and Oo-ike pond) and has become extinct in one of them (Lake Hachiro-gata)

(Watanabe et al 2005). In this study, however, we identified five new localities of this species in Japan. Therefore, further studies are needed to improve our understanding of the distribution and conservation status of this species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Mr Satoru Kinoshita, Mr Eiji Yoshida, Mr Satomi Sano, and Mr Hideharu Morishima, for their kind assistance with sample collection; Dr Yoshiji Okazaki for providing culture strains; and Dr Fumie Kasai, Dr Hisayoshi Nozaki, Dr Hiroshi Kawai, and Dr Masanobu Kawachi, for their kind suggestions and the kind use of their facilities. This work was supported in part by a Grant-in-Aid for Scientific Research (Nos. 24570100, 15K07185, 16H05764 and 18K06382 to H.S.) and a Grant-in-Aid for Scientific Research Fellows (No. 24·1993 to S.K.) from the Japan Society for the Promotion of Science and the 28th Botanical Research Grant of ICHIMURA Foundation for New Technology (to H.S.). The study was conducted as a part of the Environment Research and Technology Development Fund of the Ministry of the Environment, Japan (No. RFd-1102 to H.S.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.japb.2020.09.005.

References

Allen TF. 1887. Some notes on Characeae. *Bulletin of the Torrey Botanical Club* 14: 211–215.
Ascherson P. 1878. Noch einige Bemerkungen über die orientalischen Schismus-Formen und über Pflanzen der kleinen Oase. *Österreichische Botanische Zeitschrift* 28:254–257 [in German].
Bryant JA, Stewart NF. 2011. Order Charales. In: John DM, Whitton BA, Brook AJ, editors. *The Freshwater Algal Flora of the British Isles, 2nd edition*. Cambridge: Cambridge University Press. pp. 742–765.
Casanova MT. 2005. An overview of *Chara* L. in Australia (Characeae, Charophyta). *Australian Systematic Botany* 18:25–39.
Casanova MT. 2009. An overview of *Nitella* (Characeae, Charophyceae) in Australia. *Australian Systematic Botany* 22:193–218.
Casanova MT. 2013. *Lamprothamnium* in Australia (Characeae, Charophyceae). *Australian Systematic Botany* 26:268–290.
Choi KC, Kim YH. 1997. Taxonomic study on the charophytes in Korea I. *Chara and Lamprothamnium*. *Algae* 12:177–206 [in Korean with English abstract].
Compère P. 1986. Algues récoltées par J. Léonard dans le désert de Libye. *Bulletin du Jardin botanique national de Belgique* 56:9–50 [in French with English abstract].
Corillion R. 1957. Les charophycées de France et d'Europe occidentale. *Bulletin de la Société scientifique de Bretagne* 32:1–471 [in French].
Dixit S. 1931. Some Charophyta from Salsette. *Journal of the Indian Botanical Society* 10:205–208.
de Donterberg CCC. 1984. *Lamprothamnium haesseliae* C. C. Dont. nov. sp., una nueva Characeae para la Argentina. *Comunicaciones Museo argentino de Ciencias naturales "Benardino Rivadavia"* 2:93–101 [in Spanish with German abstract].
Felsenstein J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 38:16–24.
Frame P. 1977. *Fine structural studies of oospore ornamentation and bulbil development in charophytes*. PhD Thesis. University of Toronto. p. 182.
García A, Casanova MT. 2003. *Lamprothamnium heraldii* sp. nov. (Charales, Charophyta) from Australia: the first dioecious representative of the genus. *Phycologia* 42:622–628.
García A, Chivas AR. 2004. Quaternary and extant euryhaline *Lamprothamnium* Groves (Charales) from Australia: Gyrogonite morphology and paleolimnological significance. *Journal of Paleolimnology* 31:321–341.
García A, Jones BG, Chenhalland BE, et al. 2002. The charophyte *Lamprothamnium succinctum* as an environmental indicator: a Holocene example from Tom Thumbs Lagoon, eastern Australia. *Alcheringa* 26:507–518.
Groves J. 1916. On the name *Lamprothamnus* Braun. *Journal of Botany* 54:336–337.
Groves J. 1919. Notes on Lychnothamnus. *Journal of Botany* 57:125–129.
Guerlesquin M. 1981. Contribution à la connaissance des characées d'Amérique du Sud (Bolivie, Equateur, Guyane française). *Revue d'Hydrobiologie Tropicale* 14: 381–404 [in French with English abstract].
Guerlesquin M, Elkhiati N, Ramdani M, et al. 1987. *Lamprothamnium succinctum* A.Braun espece de Characee nouvelle pour le Maroc. *Bulletin de l'Institut Scientifique, Rabat.* 11:129–133 [in French with English abstract].
Henríquez NG, Villalba MB. 1995. Notas sobre la Characeae de "El Charco de Maspalomas" (Gran Canaria, islas Canarias). *Botánica Macaronésica* 21:37–42 [in Spanish with English abstract].
Hussain MI, Victor R, Khoja TM. 2003. Charophytes of the Sultanate of Oman, Southern Arabia. *Nova Hedwigia* 77:429–444.
Imahori K. 1963. Contributions to the East Asiatic Charophytes (1) Japan. *The Science Reports of the Tohoku University. Fourth Series, (Biology)* 29:153–164.
Imahori K, Kasaki H. 1977. Class Charophyceae. In: Hirose H, Yamagishi T, editors. *Illustrations of the Japanese fresh-water algae*. Tokyo: Uchida Rokakuho. pp. 761–829 [in Japanese].
John DM, Moore JA. 1987. An SEM study of the oospore of some *Nitella* species (Charales, Chlorophyta) with descriptions of wall ornamentation and an assessment of its taxonomic importance. *Phycologia* 26:334–355.
John DM, Moore JA, Green DR. 1990. Preliminary-observations on the structure and ornamentation of the oosporangial wall in *Chara* (Charales, Chlorophyta). *British Phycological Journal* 25:1–24.
Kasaki H. 1964. The Charophyta from the lakes of Japan. *Journal of the Hattori Botanical Laboratory* 27:215–314.
Kato S, Sakayama H, Sano S, et al. 2008. Morphological variation and intraspecific phylogeny of the ubiquitous species *Chara braunii* (Charales, Charophyceae) in Japan. *Phycologia* 47:191–202.
Khan M. 1980. Occurrence of *Lamprothamnium* J. Gr. (Charophyta) in India. *Acta Botanica Indica* 8:97–98.
Langangen A, Leghari S. 2001. Some charophytes (Charales) from Pakistan. *Studiea Botanica Hungarica* 32:63–85.
Leitch A. 1990. The oosporangium of the Characeae (Chlorophyta, Charales). *Progress in Phycological Research* 7:213–268.
Ling YJ, Xie SL, Langangen A. 2000. Charales of China. *Nova Hedwigia* 71:69–94.
McCourt RM, Karol KG, Casanova MT, et al. 1999. Monophyly of genera and species of characeae based on *rbc*L sequences, with special reference to Australian and European *Lychnothamnus barbatus* (Characeae, Charophyceae). *Australian Journal of Botany* 47:361–369.
McCourt RM, Karol KG, Guerlesquin M, et al. 1996. Phylogeny of extant genera in the family Characeae (Charales, Charophyceae) based on *rbc*L sequences and morphology. *American Journal of Botany* 83:125–131.
MOEJ. 2019. *The Japanese Red List of Ministry of the Environment, Japan, Version 2019*. Available at: https://www.env.go.jp/nature/kisho/hozen/redlist/index.html. (Accessed 1 March 2020).
Muller SD, Rhazi L, Soulie-Marsche I, et al. 2017. Diversity and distribution of Characeae in the Maghreb (Algeria, Morocco, Tunisia). *Cryptogamie Algologie* 38:201–251.
Nakada T, Nozaki H. 2009. Taxonomic study of two new genera of fusiform green flagellates, *Tabris* gen. nov. and *Hamakko* gen. nov. (Volvocales, Chlorophyceae). *Journal of Phycology* 45:482–492.
Ophel I. 1947. Notes on the genera *Lychnothamnus* and Lamprothamnium (Characeae). *Transactions of the Royal Society of South Australia* 71:318–323.
Perez W, Casanova MT, Hall JD, et al. 2017. Phylogenetic congruence of ribosomal operon and plastid gene sequences for the Characeae with an emphasis on *Tolypella* (Characeae, Charophyceae). *Phycologia* 56:230–237.
Perez W, Hall JD, McCourt RM, et al. 2014. Phylogeny of North American *Tolypella* (Charophyceae, Charophyta) based on plastid DNA sequences with a description of *Tolypella ramosissima* sp nov. *Journal of Phycology* 50:776–789.
Perez W, Hall JD, McCourt RM, et al. 2015. Oospore dimensions and morphology in North American *Tolypella* (Charophyceae, Charophyta). *Journal of Phycology* 51:310–320.
Ray S, Pekkari S, Snoeijs P. 2001. Oospore dimensions and wall ornamentation patterns in Swedish charophytes. *Nordic Journal of Botany* 21:207–224.
Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
Sakayama H. 2008. Taxonomy of *Nitella* (Charales, Charophyceae) based on comparative morphology of oospores and multiple DNA marker phylogeny using cultured material. *Phycological Research* 56:202–215.
Sakayama H, Arai S, Nozaki H, et al. 2006. Morphology, molecular phylogeny and taxonomy of *Nitella comptonii* (Charales, Characeae). *Phycologia* 45:417–421.
Sakayama H, Hara Y, Nozaki H. 2004. Taxonomic re-examination of six species of *Nitella* (Charales, Charophyceae) from Asia, and phylogenetic relationships within the genus based on *rbc*L and *atp*B gene sequences. *Phycologia* 43:91–104.
Sakayama H, Kai A, Nishiyama M, et al. 2015. Taxonomy, morphology, and genetic variation of *Nitella flexilis* var. *bifurcata* (Charales, Characeae) from Japan. *Phycological Research* 63:159–166.
Sakayama H, Kasai F, Nozaki H, et al. 2009. Taxonomic reexamination of *Chara globularis* (Charales, Charophyceae) from Japan based on oospore morphology

and *rbc*L gene sequences, and the description of *C. leptospora* sp. nov. *Journal of Phycology* 45:917–927.
Sakayama H, Miyaji K, Nagumo T, et al. 2005. Taxonomic reexamination of 17 species of *Nitella* subgenus *Tieffallenia* (Charales, Charophyceae) based on internal morphology of the oospore wall and multiple DNA marker sequences. *Journal of Phycology* 41:195–211.
Sakayama H, Nozaki H, Kasaki H, et al. 2002. Taxonomic re-examination of *Nitella* (Charales, Charophyceae) from Japan, based on microscopical studies of oospore wall ornamentation and *rbc*L gene sequences. *Phycologia* 41:397–408.
Schubert H, Blindow I. 2003. *Charophytes of the Baltic sea*. Ruggell: A. R. G. Gantner Verlag. p. 326.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.
Swofford DL. 2002. *PAUP* – Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0b10*. Sunderland, MA, USA: Sinauer Associates.
Tanabe AS. 2011. Kakusan4 and Aminosan: two programs for comparing non-partitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources* 11: 914–921.
Turmel M, Otis C, Lemieux C. 2006. The chloroplast genome sequence of *Chara vulgaris* sheds new light into the closest green algal relatives of land plants. *Molecular Biology and Evolution* 23:1324–1338.
Urbaniak J. 2011a. A SEM and light microscopy study of the oospore wall ornamentation in Polish charophytes (Charales, Charophyceae) - genus. *Chara. Nova Hedwigia* 93:1–28.
Urbaniak J. 2011b. A SEM study of the oospore wall ornamentation in Polish charophytes (Charales, Charophyceae) - genera *Lychnothamnus, Nitella* and. *Nitellopsis. Nova Hedwigia* 93:537–549.
Watanabe MM, Nozaki H, Kasaki H, et al. 2005. Threatened states of the Charales in the lakes of Japan. In: Kasai F, Kaya K, Watanabe MM, editors. *Algal culture collections and the environment*. Kanagawa: Tokai University Press. pp. 217–236.
Wood RD. 1962. New combinations and taxa in the revision of Characeae. *Taxon* 11:7–25.
Wood RD. 1965. Monograph of the Characeae. In: Wood RD, Imahori K, editors. *A revision of the Characeae*, vol. 1. Weinheim: J. Cramer. pp. 1–904.
Wood RD. 1966. Characeae of New Caledonia. *Cryptogamie Algologie* 8:10–42.
Wood RD, Imahori K. 1964. Iconograph of the Characeae. In: Wood RD, Imahori K, editors. *A revision of the Characeae*, vol. 2. Weinheim: J. Cramer. pp. 1–395.
Zaneveld JS. 1940. The Charophyta of Malaysia and adjacent countries. *Blumea* 4:1–224.