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Revision of *Batrachospermum* section *Virescentia* (Batrachospermales, Rhodophyta) with the establishment of the new genus, *Virescentia* stat. nov.

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Abstract – In recent years, sections of the paraphyletic genus *Batrachospermum* have been investigated using DNA sequence data, as well as morphology and some have been raised to genera in order to resolve this paraphylly. The species of *Batrachospermum* section *Virescentia* form a well-supported clade and we propose the raising of this section to the genus *Virescentia* stat. nov. In addition, we re-evaluated the characters used to circumscribe species by reexamination of type specimens and new collections in historically important areas. Of the eleven previously accepted species, we recognize five species and a new species is proposed as follows: *V. crispata*, *V. gulbenkiana* (synomyms *V. azeredoi* and *V. ferreri*), *V. helminthosa* (type species), *V. viride-americana* sp. nov., *V. viride-brasiliensis* and *V. vogesiaca*. The species are circumscribed on the basis of morphological characteristics (shape of fascicles, occurrence of secondary fascicles, disposition of carpogonial branches and size of carpogonia), geographic distribution, and DNA sequence data (*rbc*L and COI-5P). Descriptions, identification key and photomicrographs are presented for each recognized species. Two potential species from Japan based on DNA sequence data remained undescribed due to lack of voucher specimens for morphological observation. Other species previously assigned to the section *Virescentia* are referred to other genera of Batrachospermales based on morphology (disposition of carpogonial branches, shape of trichogynes, types of gonimoblast filaments and arrangement of carposporophytes).

COI-5P / Batrachospermales / biogeography / morphology / phylogeny / rbcL / revision / Rhodophyta / taxonomy

INTRODUCTION

The genus *Batrachospermum* has been shown to be paraphyletic in previous phylogenetic studies of the freshwater red algal order Batrachospermales (Vis *et al.*, 1998; Entwisle *et al.*, 2009). The genus *Batrachospermum* included nine sections (*Acarposporophytum, Aristata*, *Batrachospermum*, *Gonimopropagulum*, *Helminthoidea*, *Macrospora*, *Setacea*, *Turfosa* and *Virescentia*) and was provisionally kept by Entwisle *et al.* (2009) until further detailed taxonomic research could be conducted; an informal group (named 'Australasica') was also recognized. The

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genus *Kumanoa* was established for species formerly classified in sections *Contorta* and *Hybrida* (Entwisle *et al.*, 2009). Subsequently, species of the monophyletic section *Helminthoidea* were investigated and the genus *Sheathia* was proposed with the transfer of previously described species and proposal of new species (Salomaki *et al.*, 2014). More recently, section *Setacea* was raised to the genus level as *Atrophycus* (Rossignolo & Necchi, 2016; Rossignolo *et al.*, 2016). However, Entwisle *et al.* (2016) adopted an alternative scheme combining section *Setacea,* all taxa of the 'Australasica' group and previously described species of *Nothocladus* into the genus *Nothocladus* "lato sensu" based on phylogenetic analyses of five genes.

Virescentia is one of the sections within the paraphyletic genus *Batrachospermum* that has yet to be revised. DNA sequence (plastid-encoded RuBisCO large-subunit gene, *rbc*L and nuclear small subunit ribosomal DNA, SSU) evidence has shown this section to be monophyletic (Vis *et al.*, 1998, 2001; Hanyuda *et al.*, 2004; Entwisle *et al.*, 2009; Agostinho & Necchi, 2014). Section *Virescentia* was proposed by Sirodot (1873) to include species of the genus with greenish thalli and carpogonia having cylindrical and stalked trichogynes. Additional characters have been added to the section including well-developed whorls, carpogonial branches composed of short cells that are well-differentiated from the fascicle cells, carposporophytes inserted centrally in the whorl, densely arranged and large (Necchi, 1990; Sheath *et al.*, 1994; Kumano, 2002; Agostinho & Necchi, 2014). The monograph of *Batrachospermum* by Sirodot (1884) represents the starting point for the studies on this section with seven new species described and illustrated. Subsequent taxonomic treatments for species of this section have been mostly in more general studies, in which species of the genus *Batrachospermum* for various regions of the world were described such as Israelson (1942) for Sweden, Mori (1975) for Japan, Necchi (1990) for Brazil and Eloranta *et al.* (2011) for Central Europe. Only two studies have focused exclusively on species of the section, Sheath *et al.* (1994) for North America and Agostinho & Necchi (2014) for Brazil.

Kumano (2002) recognized eleven species within the section: *B. azeredoi* Reis (1967), *B. bakarense* Kumano & Ratnas. (Ratnasabapathy & Kumano, 1984), *B. crispatum* Kumano & Ratnas. (Ratnasabapathy & Kumano, 1982a), *B. desikacharyi* Sankaran (1984), *B. elegans* Sirodot (1884), *B. ferreri* Reis (1967), *B. gombakense* Kumano & Ratnas. (Ratnasabapathy & Kumano, 1982b), *B. gulbenkianum* Reis (1965), *B. helminthosum* Bory (1808), *B. transtaganum* Reis (1970) and *B. vogesiacum* T.G. Schultz *ex* Skuja (1938). The following criteria have been applied to distinguish species within the section: sexuality (monoecious, dioecious or polyoecious), position and length of the carpogonial branch, size of carpogonia, number of cell storeys in primary fascicles, density of secondary fascicles and the presence of terminal hairs (Sirodot, 1884; Mori, 1975; Starmach, 1977; Necchi, 1990). The morphometric analyses by Sheath *et al.* (1994) of type specimens and North American populations resulted in the proposal of many synonymies and recognition of only two species, *B. helminthosum* Bory (synonyms *B. bruziense* Sirodot, *B. graibussoniense* Sirodot, *B. sirodotii* Reis – as *B. virgatum* (Kützing) Sirodot – *B. testale* Sirodot and *B. vogesiacum* Schultz – as *B. flagelliforme* Sirodot) and *B. elegans* Sirodot (synonym *B. coerulescens* Sirodot). Sheath *et al.* (1994) distinguished the two species based on the presence in *B. elegans* or absence in *B. helminthosum* of basal knobs or protuberances on the trichogynes. However, Vis *et al.* (2001) showed that most qualitative features are universally present in *B. helminthosum* and *B. elegans* and morphometric characteristics are highly variable, with significant overlap among specimens of the two species. Hanyuda *et*

al. (2004) also concluded that characters generally used to distinguish species (sexuality, origin of the carpogonium bearing branch and trichogynes with or without basal knobs or branching) are not fixed genetically, but are affected by environmental conditions (seasonally or environmentally induced changes in the gametophyte) and proposed that *B. elegans* should be placed in synonymy with *B. helminthosum*. Agostinho & Necchi (2014) noted considerable divergence in morphometric characters (size of carpogonia, carposporophytes and carposporangia) among samples from Brazil and North America and type specimens from Europe indicating the existence of at least three distinct species with non-overlapping geographic distributions: Brazil, USA and Europe. The recognition of these groups was supported by the sequence analyses of *rbc*L and barcode region of the mitochondrial encoded cytochrome c oxidase subunit 1, COI-5P (Hanyuda *et al.*, 2004; Rueness, 2010; Agostinho & Necchi, 2014). Sequence data of the *rbc*L also indicated the occurrence of more than one species within the section *Virescentia* from Japan (Hanyuda *et al.*, 2004). Chiasson *et al.* (2014) revisited historically important areas in France, where several species were originally collected by Bory (1808) and Sirodot (1884). They presented *rbc*L sequenced data clearly showing *B. vogesiacum* being a species distinct from *B. helminthosum*.

While the morphological study of Sheath *et al.* (1994) reduced the number of species in the section, DNA sequence data have suggested that there is considerable genetic variation among populations and this variation is partitioned geographically (Hanyuda *et al.*, 2004; Agostinho & Necchi, 2014). We re-analyzed the DNA sequence data to evaluate the taxonomic status of the section. In addition, we have examined the morphology of type specimens, new collections from historically important and specimens for which there is DNA sequence data to provide updated species information.

MATERIALS AND METHODS

Sixteen type specimens of species attributed to section *Virescentia* were examined (Table 1). Type specimens were borrowed from the following herbaria (acronyms according to Thiers *et al.*, 2018): COI, PC and TNS-AL. In addition, taxonomically relevant specimens from which sequence data were reported were examined as follows: two specimens from France (Chiasson *et al.*, 2014) and ten specimens from USA (Vis *et al.*, 2001; House *et al.*, 2008; Sherwood *et al.*, 2008) in BHO. The synonyms listed for each species are all designated in this study.

All morphological and morphometric characters referred to in relevant taxonomic literature of section *Virescentia* (Sirodot, 1884; Israelson, 1942; Mori, 1975; Necchi, 1990; Sheath *et al.*, 1994; Kumano, 2002; Agostinho & Necchi, 2014) were evaluated. For observations and measurements, we used an Olympus SZH10 stereoscope and an Olympus BH-2 light microscope (Olympus Corp., Tokyo, Japan). Photomicrographs were taken with a Leica DFC 320 digital camera using LAS capture and image analysis software coupled to a Leica DM5000 microscope (Leica Microsystems, Wetzlar, Germany).

Tree reconstruction analyses were conducted using DNA sequence data from previously published studies (Tables S1-S2) available in GenBank (Benson *et al.*, 2013). For COI-5P data, distance analysis using Neighbor Joining (NJ) and Kimura two-parameter distances was performed using a plugin of Geneious version

Table 1. Type and historically important specimens of *Batrachospermum* section *Virescentia* examined in this study.

Species	*Type and herbarium number*[1]	*Locality, collector and date*
B. azeredoi Reis	Holotype, COI	Portugal, Vila da Feira, River Caster; M.P. Reis & A. Santos, no. 482, 17.vi.1966
B. bakarense Kumano & Ratnas.	Isotype, TNS-AL 169085	Malaysia, Kelantan, Sungai Bakar; M. Ratnasabapathy no. 3, 03.vi.1982
B. bruziense Sirodot	Lectotype, PC0452411	France, town of Bruz near Rennes, Fontaine de Chatillon; S. Sirodot, 27.iii.1882
B. coerulescens Sirodot	Not type, PC0452372	France, Le Meu at the top of Montfort near Tréguil; S. Sirodot, 29.vii.1874
B. crispatum Kumano & Ratnas.	Isotype, TNS-AL 169089	Malaysia, Pulau Tioman, Sungai Ayer Besar; M. Ratnasabapathy no. 21, 24.v.1974
B. elegans Sirodot	Lectotype, PC0452233	France, Morbihan near Compénéac, Fontaine de Pont-Garnier; S. Sirodot, 02.v.1869
B. ferreri Reis	Holotype, COI	Portugal, Vale de Cambra, Mina do Pintor; M.P. Reis & A. Santos, no. 485, 17.vi.1966
B. gibbosum Reis	Holotype, COI	Portugal, São Pedro do Sul, River Arões; M.P. Reis & A. Santos, no. 449, 07.vii.1965
B. gombakense Kumano & Ratnas.	Isotype, TNS-AL 169097	Malaysia, Selangor, Sungai Gombak; M. Ratnasabapathy no. 1221, 31.v.1976
B. gulbenkianum Reis	Holotype, COI	Portugal, Confulcos, River Águeda; M.P. Reis & A. Santos, no. 456, 07.vii.1965
B. helminthosum Bory	Lectotype, PC0591734	France, Fougéres; Bory de St. Vincent, no collection date
B. testale Sirodot	Lectotype, PC0451869	France, near Montfort, Fontaine de Gaillardon; S. Sirodot, 24.iv.1882
B. transtaganum Reis	Holotype, COI 542	Portugal, Odemira, River Torgal; M.P. Reis no. 542A, 19.iv.1968
B. viride Sirodot	Lectotype, PC0451877	France, near Rennes, Ruisseaux de Gallet; S. Sirodot, 02.v.1882
B. viride-brasiliense Necchi & D.C. Agostinho	Holotype, SJRP 29845	Brazil, São Paulo State: Campos do Jordão, 'Ducha da Prata', M.O. Paiano, 15.xii.2009
B. vogesiacum T. G. Schultz *ex* Skuja	Lectotype, PC0591499	France, Laugerie-Haute; S. Sirodot, 01.viii.1880

1. Herbarium acronyms according to Thiers (2018).

10 (http://www.geneious.com, Kearse *et al.*, 2012). For phylogenetic analyses of the *rbc*L data, a GTR+I+G was determined to be the best-fit model of sequence evolution by the Akaike Information Criterion using jModelTest 2.1.4 (Darriba *et al.*, 2012). Two separate analyses were conducted based on: 1) a general matrix with sequences of representative members of the Batrachospermales; 2) a matrix with all sequences available for members of section *Virescentia* only. Maximum-likelihood (ML) topologies and bootstrap values from 1,000 replicates were inferred using Randomized Accelerated Maximum Likelihood graphic user interface (RAxMLGUI version 1.2;

Silvestro & Michalak, 2011). Bayesian analysis (BA) was performed in MrBayes using Geneious plugin, with three runs of five chains of Metropolis coupled Markov Chain Monte Carlo for 10 x10^6 generations. 500,000 chains were removed as burn-in prior to determining the posterior probabilities. The COI-5P and *rbc*L sequence data were not combined because there were few specimens in common between the two data sets of section *Virescentia* (Table S1), which would have considerably limited the number of specimens in the analysis.

RESULTS AND DISCUSSION

Molecular analyses

BA and ML analyses of the *rbc*L sequences revealed strong support for the section *Virescentia* as a monophyletic group (Fig. S1, Supporting information), corroborating results of previous studies (Vis *et al.*, 1998; Hanyuda *et al.*, 2004; Entwisle *et al.*, 2009; Agostinho & Necchi, 2014; Chiasson *et al.*, 2014). Within the section, there were five clades with high support, corresponding to four geographic regions (Fig. S2, Supporting information): Asia, Europe (two clades), North America (USA) and South America (Brazil). Each clade represented a species, as follows: Asia (*Batrachospermum* sp.), Europe (*B. helminthosum* and *B. vogesiacum*), North America (*Batrachospermum* sp.) and South America (*B. viride-brasiliense*). There was a remaining sequence from Asia (Japan) that did not form a close association with other specimens and may also represent a distinct species. This analysis corroborates previous studies with the recognition of two species from Asia (Japan), as found by Hanyuda *et al.* (2004), two species from Europe as reported by Chiasson *et al.* (2014), one species from North America (USA) and another one from South America (Brazil), as reported by Agostinho & Necchi (2014). The sequences from USA formed a clade with two groups showing sequence divergences within a group of 0-19 bp (0-1.5%) and between groups of 15-30 bp (1.2-2.4%).

The COI-5P NJ tree (Fig. S3, Supporting information) showed three well-supported groups, with clear geographical disjunction between clades of sequences from Europe (Norway), North America (USA) and South America (Brazil); no data for Asia were available. As shown by *rbc*L analysis, sequences of each clade represent a species, as follows: Europe (*B. helminthosum*), North America (*Batrachospermum* sp.) and South America (*B. viride-brasiliense*). These results match those reported by Agostinho & Necchi (2014). The sequences from USA formed a group with two minor groups, showing sequence divergences within each minor group of 0-28 bp (0-4.3%) and between groups of 36-46 bp (5.5-7.0%).

Morphological analyses

On the basis of the high support of *Batrachospermum* section *Virescentia* in the phylogenetic analyses (Vis *et al.*, 1998; Hanyuda *et al.*, 2004; Vis *et al.,* 2005; Entwisle *et al.*, 2009; Agostinho & Necchi, 2014; Chiasson *et al.*, 2014; this study) and to continue to rectify the paraphyly of the genus *Batrachospermum* by raising sections that form distinct clades within the genus *Batrachospermum* to the generic level, we propose this section as a genus.

Virescentia (Sirodot) Necchi, D.C.Agostinho & M.L.Vis**, gen. et stat. nov.**

Basionym: section *Virescentia* Sirodot*, Comptes-rendus hebdomadaires des séances de l'académie des sciences* 76: 1219 (1873).

Synonyms: section *Viridia* De Toni, *Sylloge Floridearum*, 60 (1897); section *Verts* Sirodot, *Batrachospermes,* 269 (1884); section *Claviformia* Reis, *Boletim da Sociedade Broteriana* 46: 209 (1972).

Revised description: Plants monoecious, dioecious or polyoecious, greenish, blue-greenish or less often brownish; branching irregular or pseudo-dichotomous; whorls well-developed, contiguous or separated, obconic, pear-shaped, spherical or barrel-shaped; cortical filaments of the main axis well-developed, formed by one or two layers of cylindrical cells; main axis sometimes brownish colored; primary fascicles formed by cells variable in shape along the fascicle, ranging from cylindrical to elliptical, obovoid or pear-shaped or audouinelloid (same shape along the fascicle, cylindrical or barrel-shaped), with 6-19 cell storeys; secondary fascicles few and sparse to abundant and covering the entire internode; spermatangia spherical or obovoid, on primary or secondary fascicles, 4-9 μm wide; carpogonial branches well differentiated from the fascicles, straight, rarely curved, developing from the periaxial, proximal cells of primary fascicles, rarely from distal cells of primary fascicles, secondary fascicles or cortical filaments, short or long, composed of 1-12(-20) disc- or barrel-shaped cells, 3.0-37.5(-68) μm long; involucral filaments, short, composed 1-4 cylindrical, elliptical or spherical cells; carpogonia 20-110 μm long, with stalked, rarely shortly stalked, cylindrical, sub-cylindrical, club-shaped or elliptical trichogynes; carposporophytes axial, hemispherical or spherical, inserted within the whorls, 1-2, rarely 3, per whorl, large, (55-)120-550 μm wide and (30-)100-420 μm in height; gonimoblast filaments densely arranged, composed of 2-7 cylindrical, elliptic or barrel-shaped cells; carposporangia obovoid, pear-shaped, club-shaped or elliptic, 10-35 μm long, 6.5-24 μm wide.

Type species: *V. helminthosa* (Bory) Necchi, D.C.Agostinho & M.L.Vis, comb. nov.

Remarks: members of the genus can be recognized by the combinations of characters: including presence of well-developed whorls; carpogonial branches well differentiated from the fascicles, straight, rarely curved; carpogonia with cylindrical, club-shaped or elliptical, stalked trichogynes; axial, large and dense carposporophytes. Members of *Virescentia* are comparable with those of section *Turfosa* in gross vegetative morphology and presence of large carposporophytes, but differ having only one type of gonimoblast filaments, which grow radially from the fertilized carpogonia forming a hemispherical or spherical structure. Members of the genus *Kumanoa* also have the same type of carposporophytes, but they have strongly curved or twisted carpogonial branches.

Key to the species of the genus *Virescentia*

1a. Whorls composed of curved audouinelloid fascicles, with cylindrical or barrel-shaped cells ..... *V. crispata*
1b. Whorls composed of straight fascicles, with cylindrical, elliptical, obovoid or pear-shaped cells 2
2a. Carpogonial branches slightly curved ..... *V. helminthosa*
2b. Carpogonial branches straight 3
3a. Carpogonia short, 20-45 μm in length, known distribution in Europe 4

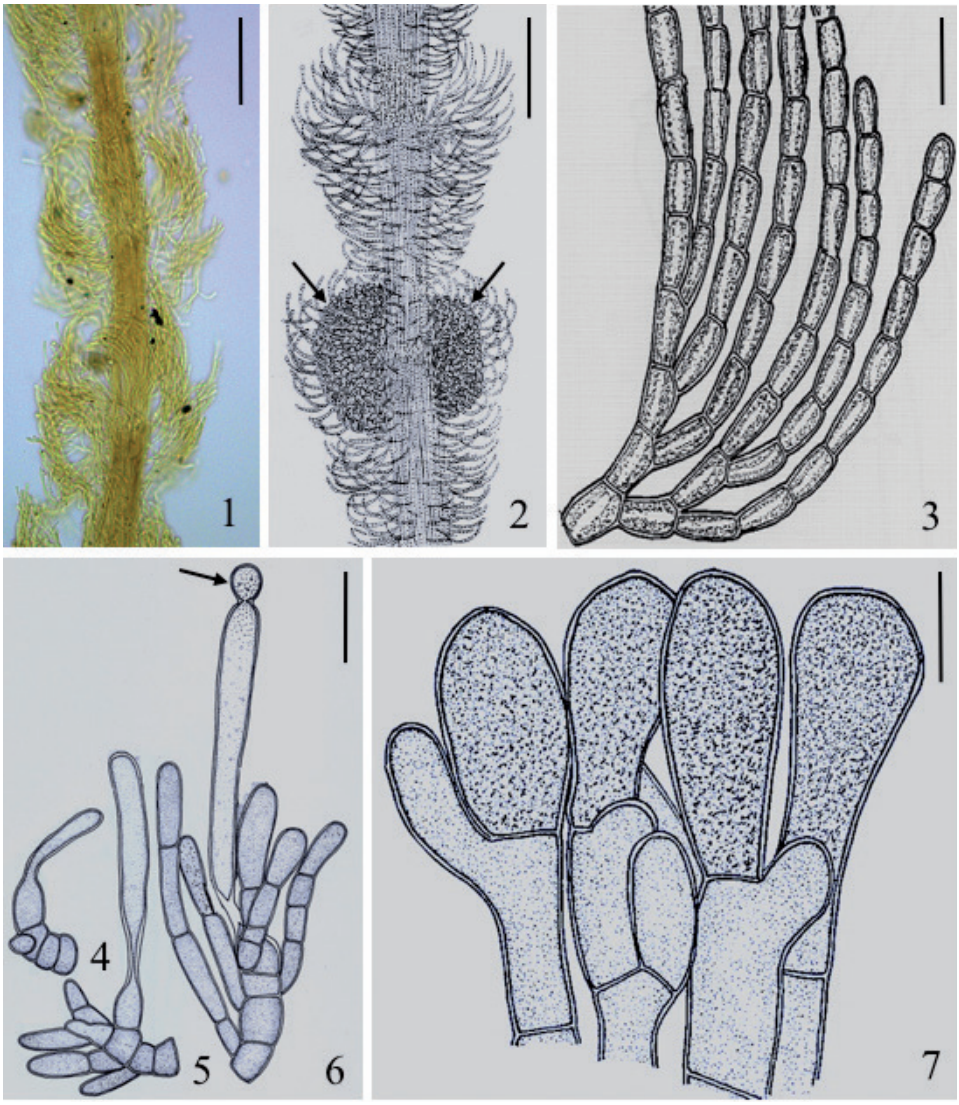
3b. Carpogonia long, (30-)40-110 μm in length, known distribution in the Americas 5
4a. Secondary fascicles few and sparse .....*V. gulbenkiana*
4b. Secondary fascicles abundant and covering ≥ half of the internode length ..... *V. vogesiaca*
5a. Known distribution in North America (USA).....*V. viride-americana*
5b. Known distribution in South America (Brazil)..... *V. viride-brasiliensis*

Virescentia crispata (Kumano & Ratnas.) Necchi, D.C.Agostinho & M.L.Vis, **comb. nov.** **Figs 1-7**

Basionym: *Batrachospermum crispatum* Kumano & Ratnas., *Japanese journal of phycology* 30: 18 (1982a).
Revised description: Plants dioecious or polyoecious; branching pseudo-dichotomous; whorls contiguous, obconic or pear-shaped, 150–350 μm wide; primary fascicles curved, unilaterally branched, with 6–13 cell storeys; secondary fascicles abundant and covering the entire internode; spermatangia spherical or obovoid, on primary or secondary fascicles, 6–8 μm wide; carpogonial branches slightly curved, composed of 3-4 disc- or barrel-shaped cells, developing from the periaxial or proximal cells of primary fascicles, short, 20-28 μm long; involucral filaments, short, composed of 2-4 cylindrical or elliptic cells; carpogonia 54-75 μm long, with stalked and cylindrical trichogynes; carposporophytes 1-2 per whorl, 140-300 μm wide and 100-250 μm high; gonimoblast filaments with 3-5 cylindrical cells; carposporangia club-shaped or obovoid, 14-30 μm long and 8-10 μm wide.
Distribution: Asia (Malaysia), known from the type locality only (Ratnasabapathy & Kumano, 1982a).
Remarks: This is the most easily distinguished species of the genus due to the presence of audouinelloid and curved primary and secondary fascicles, a character lacking in the remaining species.

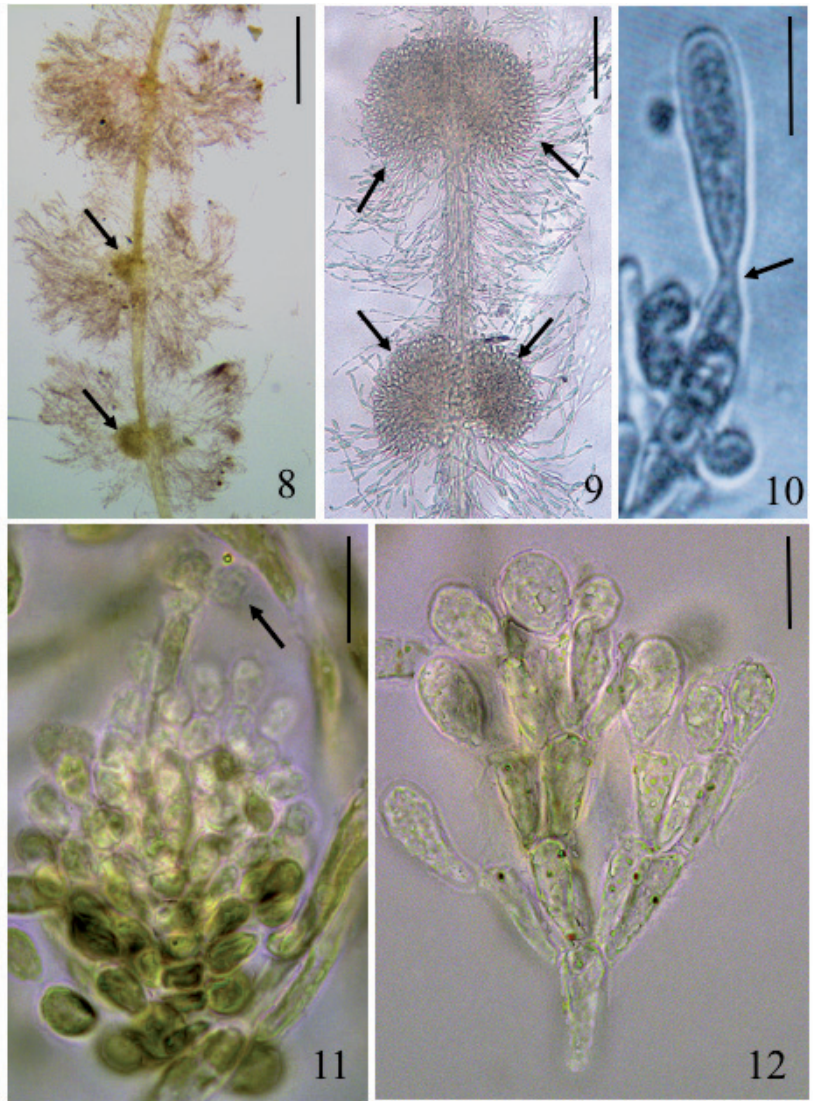
Virescentia gulbenkiana (Reis) Necchi, D.C.Agostinho & M.L.Vis, **comb. nov.** **Figs 8-12**

Basionym: *Batrachospermum gulbenkianum* Reis, *Anuário da Sociedade Broteriana* 31: 31, 1965).
Synonyms: *Batrachospermum azeredoi* Reis, *Boletim da sociedade Broteriana* 41: 167, (1967); *B. ferreri* Reis, *Boletim da sociedade Broteriana* 41: 174 (1967); *B. gibbosum* Reis, *Boletim da sociedade Broteriana* 43: XXX (1969).
Revised description: plants monoecious or polyoecious; branching irregular; whorls contiguous or separated, barrel-shaped or spherical, 430-810 μm wide; primary fascicles straight, with 8-13 cell storeys; secondary fascicles few and sparse; spermatangia spherical, on primary fascicles, 4-6 μm wide; carpogonial branches straight, composed of 3-12 disc-shaped cells, developing from the periaxial or proximal cells of primary fascicles, short or long, 9-35 μm long; involucral filaments, short, composed of 1-3 cylindrical or elliptic cells; carpogonia 20-39 μm long, with stalked, club-shaped or cylindrical trichogynes; carposporophytes 1-3 per whorl, 120-300 μm wide and 60-150 μm high; gonimoblast filaments with 2-5 cylindrical cells; carposporangia obovoid or pear-shaped, 10-18 μm long, 6.5-10 μm wide.
Distribution: Europe, several localities in Portugal (Reis 1965, 1967, 1972, 1974).
Remarks: the distinction of *V. gulbenkiana* from *B. azeredoi*, *B. ferreri* and *B. gibbosum* was very unclear, broadly overlapping for most morphometric and morphological characters. Thus, we propose here the three later species to be synonyms of *V. gulbenkiana*. The proposal of section *Claviformia* to classify



Figs 1-7. *Virescentia crispata* (Kumano & Ratnas.) Necchi, D.C.Agostinho & M.L.Vis comb. nov. **1-2.** Whorls with carposporophytes (arrows); **3.** Detail of a primary fascicle showing audouinelloid cells and curved disposition; **4-6.** Details of carpogonia with curved carpogonial branches; **4-5.** Young carpogonia; **6.** Mature (fertilized) carpogonium with attached spermatium (arrow); **7.** Distal cells of gonimoblast filaments with carposporangia. Scale bars: 1-2 = 100 µm; 3-7 = 10 µm. Figs 2-7 redrawn from Ratnasabapathy & Kumano (1982a). Fig. 1 from the isotype (Table 1).

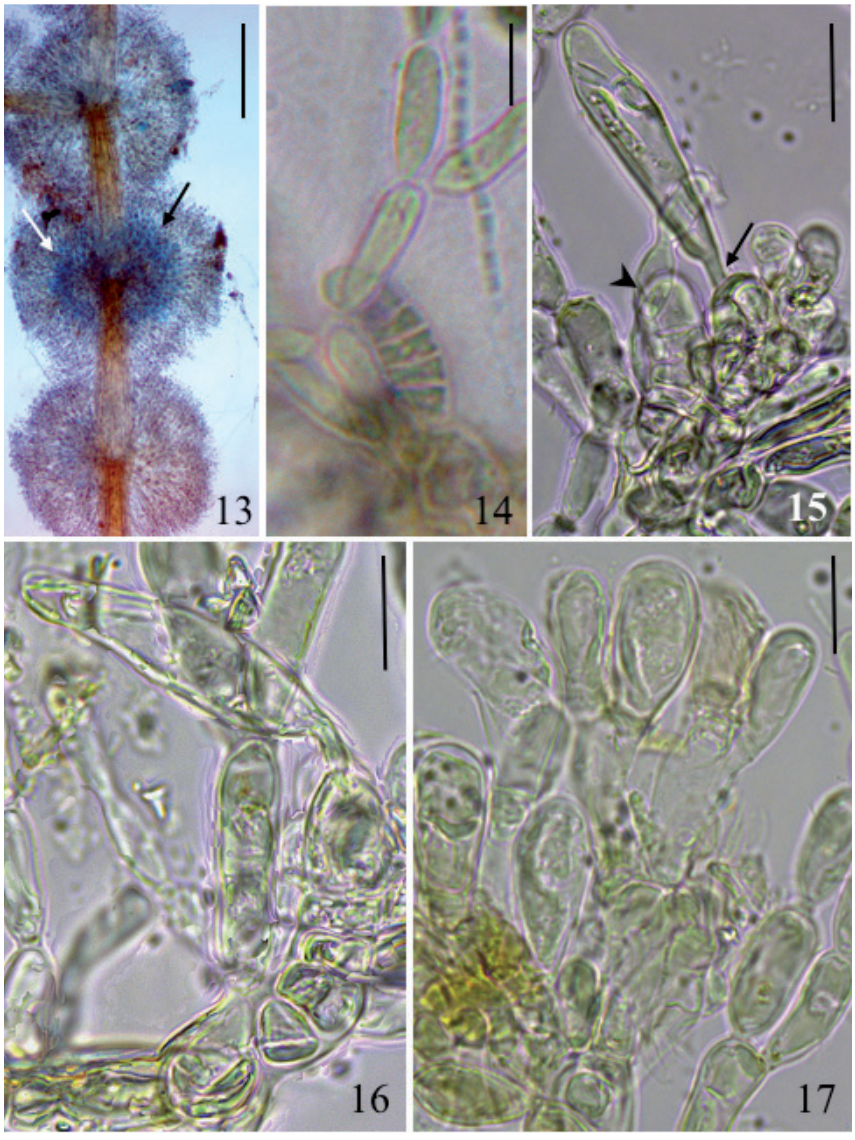
V. azeredoi and *V. ferreri* (Reis, 1972) has no basis, because the proposed characters (carpogonial branches short or long; carpogonia with club-shaped or cylindrical trichogynes; carposporophytes large, 1-2 per whorl) fit easily within the circumscription of *Virescentia.*



Figs 8-12. *Virescentia gulbenkiana* (Reis) Necchi, D.C.Agostinho & M.L.Vis comb. Nov. **8–9.** Whorls with carposporophytes (arrows); **10-11.** Details of carpogonia; **10**. Young carpogonium with shortly stalked trichogyne (arrow); **11.** Mature (fertilized) carpogonium with attached spermatium (arrow); **12.** Gonimoblast filaments with carposporangia. Scale bars: 250 µm for figure 8; 100 µm for figure 9; 10 µm for figures 10-12. Figure 10 modified from Reis (1967). Figs 8, 12 from the holotype of *V. ferreri*; Figs 9, 11 from the holotype of *V. gulbenkiana* (Table 1).

Virescentia helminthosa (Bory) Necchi, D.C.Agostinho & M.L.Vis**, comb. nov.**
Figs 13-17

Basionym: *Batrachospermum helminthosum* Bory, *Annales du muséum d'histoire naturelle* 12: 316 (1808).



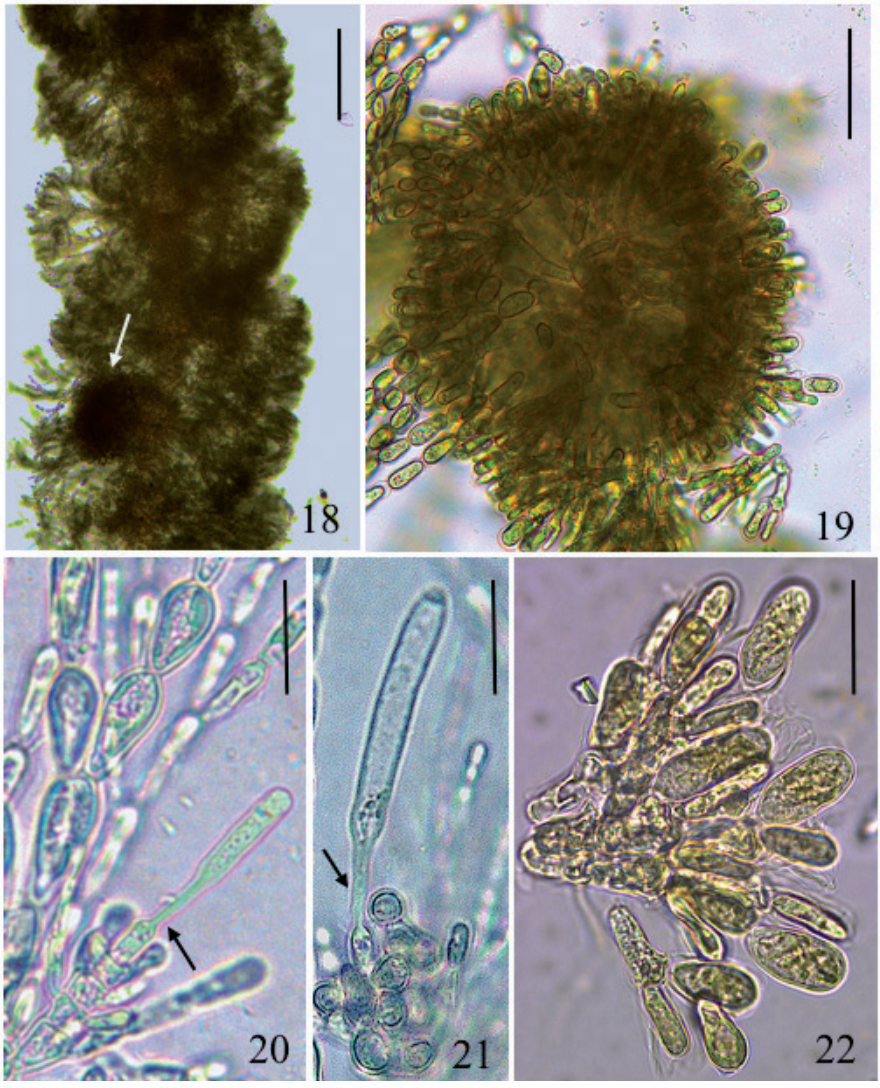
Figs 13-17. *Virescentia helminthosa* (Bory) Necchi, D.C.Agostinho & M.L.Vis comb. nov. **13.** Whorls with carposporophytes (arrows); **14.** Detail of a curved young carpogonial branch; **15-16.** Details of carpogonia; **15.** Curved young carpogonial branch (arrowhead) and a mature carpogonium with stalked trichogyne (arrow); **16.** Mature carpogonium with curved carpogonial branch; **17.** Gonimoblast filaments with carposporangia. Scale bars: 13 = 250 μm; 14-17 = 10 μm. Fig. 13 from the lectotype of *V. helminthosa* (Table 1); figs. 14-17 from BHO A-0936.

Revised description: Plants monoecious or polyoecious; branching irregular; whorls contiguous or separated, barrel-shaped, spherical or pear-shaped, 300-800 μm wide; primary fascicles straight, with 8-15 cell storeys; secondary fascicles varying from

few and sparse to abundant and covering the entire internode; spermatangia spherical, on primary or secondary fascicles, 5-7 μm wide; carpogonial branches slightly curved, composed of 3-11 disc-shaped cells, developing from the periaxial, proximal or distal cells of primary fascicles or cortical filaments of the main axis, short or long, 12-35 μm long; involucral filaments, short, composed of 1-3 cylindrical, elliptical or spherical cells; carpogonia 40-79 μm long, with stalked, cylindrical trichogynes, sometimes bifurcated or with knobs; carposporophytes 1-2 per whorl, 150-420 μm wide, 100-320 μm high; gonimoblast filaments with 3-7 cylindrical cells; carposporangia obovoid or pear-shaped, 14-20.5 μm long, 8.5-12.5 μm wide.
Distribution: Europe - Finland, France, Norway, Poland, Portugal, Spain, Sweden (Sirodot, 1884; Israelson, 1942; Reis, 1974; Eloranta *et al.* 2011; Chiasson *et al.*, 2014).
Representative DNA sequences: HQ412541, HQ412542 (COI-5P); KJ825955, KM593807, KM593841 (*rbc*L).
Additional specimens examined: France, Dirt road to Chappelle Saint Clair-de-Gout, off of D433, 44.237472, –0.058806, M. Vis, W. Chiasson & K. Chiasson 29.x.2011, BHO A-0936.
Remarks: this is the most widely reported species in the genus; however, recent studies based on molecular data (Hanyuda *et al.*, 2004; Rueness, 2010; Agostinho & Necchi, 2014; Chiasson *et al.*, 2014) indicate that it is an European species and all records outside Europe most likely represent other species. All species described by Sirodot (1884) were based on criteria that are currently regarded as having no taxonomic importance (color, sexuality, arrangement of primary fascicles, presence of hairs on fascicles, characters of the asexual 'Chantransia' stage). Thus, those species were placed as synonyms of *V. helminthosa*, reinforcing the taxonomic interpretations of previous authors (Sheath *et al.*, 1994; Vis *et al.*, 2001; Hanyuda *et al.*, 2004).

Virescentia viride-americana Necchi, D.C.Agostinho & M.L.Vis, **sp. nov.**
Figs 18-22

Description: Plants monoecious or dioecious or polyoecious; branching irregular; whorls contiguous or separated, barrel-shaped, spherical or pear-shaped, 280-940 μm side; primary fascicles straight, with 8-19 cell storeys; secondary fascicles varying from few and sparse to abundant and covering the entire internode; spermatangia spherical, on primary or secondary fascicles, 4-8 μm wide; carpogonial straight, composed of 1-7 disc-shaped cells, developing from the periaxial or proximal cells of primary fascicles, short or long, 6-30 μm long; involucral filaments, short, composed of 1-3 cylindrical or elliptical cells; carpogonia (30-)40-77 μm long, with stalked, club-shaped or cylindrical trichogynes; carposporophytes 1-2 per whorl, (130-)140-390 μm wide, 70-200(-240) μm high; gonimoblast filaments with 3-5 cylindrical cells; carposporangia obovoid or pear-shaped, 15-24 μm long, 8-12 μm wide.
Additional specimens examined: USA: Indiana, Otter Creek, M.L. Vis & M.M. Hall, 29.v.1999., BHO A-1177; Massachusetts, Poquoy Brook, M.L. Vis & M.M. Hall, 16.iv.1999, BHO A-1175; Michigan, Flemming Creek, M.L. Vis & W.B. Chiasson, 30.v.2000, BHO A-1473; Ohio, Five Mile Creek, M.L. Vis & R.G. Verb, 11.v.1998, BHO A-1179; Ohio, Sunfish Creek, M. Vis & D. House, 20.iv.2007, BHO A-0102; Rhode Island, Wood River tributary, M.L. Vis & M.M. Hall, 16.iv.1999, BHO A-1178; Rhode Island, Chipuxet River, no collector, no date, BHO A-1180; Tennessee, Scarboro Creek, M.L. Vis & M.M. Hall, 30.v.1999, BHO A-1176; Virginia, Carvin Creek, M. Vis & D. House, 22.iii.2007, BHO A-0128.
Holotype: USA, Big Run, Ohio. 4.2 miles east on Big Run Road (CR8S) from SR 329, 39.353813 N 81.842615 W, M.L. Vis & R.G. Verb, 29.v.1998, BHO A-1174.



Figs 18-22. *Virescentia viride-americana* Necchi, D.C.Agostinho & M.L.Vis sp. nov. **18.** Whorls with carposporophyte (arrow); **19.** Detail of an entire carposporophyte; **20-21.** Details of carpogonia; **20.** Young carpogonium with stalked trichogyne (arrow); **21.** Mature carpogonium with stalked trichogyne (arrow); **22.** Gonimoblast filaments with carposporangia. Scale bars: 18 = 100 µm; 19 = 25 µm; 20-22 = 10 µm. All figures from the holotype (Table 1).

Etymology: the species epithet indicates that the alga is a member of the *Virescentia* (from Latin *viride*) from North America (from Latin *americana*).
Distribution: North America – eastern, southern and mid-western United States (Sheath *et al.*, 1994; Vis *et al.*, 2001).

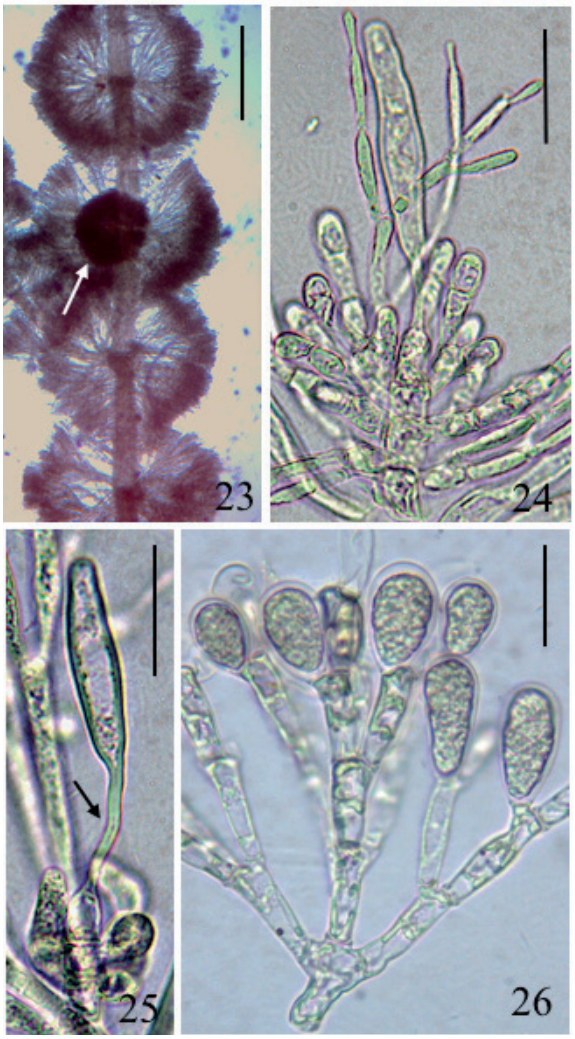
Representative DNA sequences: EU636733, EU636736, EU073836, EU073839, EU073845, EU073846, EU073847 (COI-5P); AF244110, AF244111, AF244115, AF244117, AF244118 (*rbc*L).
Remarks: this species has been previously reported as *V. helminthosa* (Sheath *et al.*, 1994, and references therein), which is now regarded an exclusively European species. Based on recent studies including molecular data (Hanyuda *et al.*, 2004; Agostinho & Necchi, 2014; Chiasson *et al.*, 2014), the sequences of North American specimens are genetically divergent from the those of Asia, Europe and South America, representing a distinct species, that is here described as new to science. The species is highly divergent from the South American *V. viride-brasiliensis* in sequence data of *rbc*L (36-57 bp, 2.9-4.5%) and COI-5P (57-69 bp, 8.6-10.4%). However, these two species are morphologically very similar with considerable overlap for all morphological characters. In addition to the unique *rbc*L and COI-5P sequences for these species, they have a disjunct geographic pattern that can be applied as an additional criterion. The North American specimens formed a clade with two subgroups in both the *rbc*L and COI-5P tree as noted above, but the sequence variation within and among these two groups overlapped. The morphology in all taxonomic characters among the populations was similar and there was no geographic disjunction between the two groups. Thus, we have chosen to describe a single species that is somewhat variable in DNA sequence.

Virescentia viride-brasiliensis (Necchi & Agostinho) Necchi, M.L.Vis & D.C.Agostinho, **comb. nov.** **Figs 23-26**

Basionym: *Batrachospermum viride-brasiliense* Necchi & Agostinho, *Phycologia* 53: 566 (2014).
Distribution: South America – southeastern and southern Brazil (Espírito Santo, Paraná, Rio Grande do Sul, São Paulo; Agostinho & Necchi, 2014).
Representative DNA sequences: KX452188 to KX452196 (COI-5P); KM078031 to KM078042 (*rbc*L).
Revised description: plants monoecious, dioecious or polyoecious; branching irregular; whorls contiguous or separated, barrel-shaped, spherical, pear-shaped or obconic, 280-1100 µm wide; primary fascicles straight, with 7-18 cell storeys; secondary fascicles varying from few and sparse to abundant and covering the entire internode; spermatangia spherical or obovoid, on primary or secondary fascicles, 4-9 µm wide; carpogonial straight, short, composed of 1-7 disc-shaped cells, developing from the periaxial or proximal cells of primary fascicles, short or long, 4-37.5 µm long; involucral filaments, short, composed of 1-3 cylindrical or spherical cells; carpogonia 40-110 µm long, with stalked, club-shaped or sub-cylindrical trichogynes; carposporophytes axial, inserted within the whorls, 1-2 per whorl, 200-550 µm wide and 100-300 µm in height; gonimoblast filaments with 3-6 cells cylindrical or barrel-shaped cells; carposporangia obovoid, pear-shaped or elliptic, 19-35 µm long, 10-24 µm wide.
Remarks: this species resembles *V. viride-americana* in all morphological features. However, based on the divergence in sequences of *rbc*L and COI-5P and the disjunct geographic distribution it was proposed a new species by Agostinho & Necchi (2014).

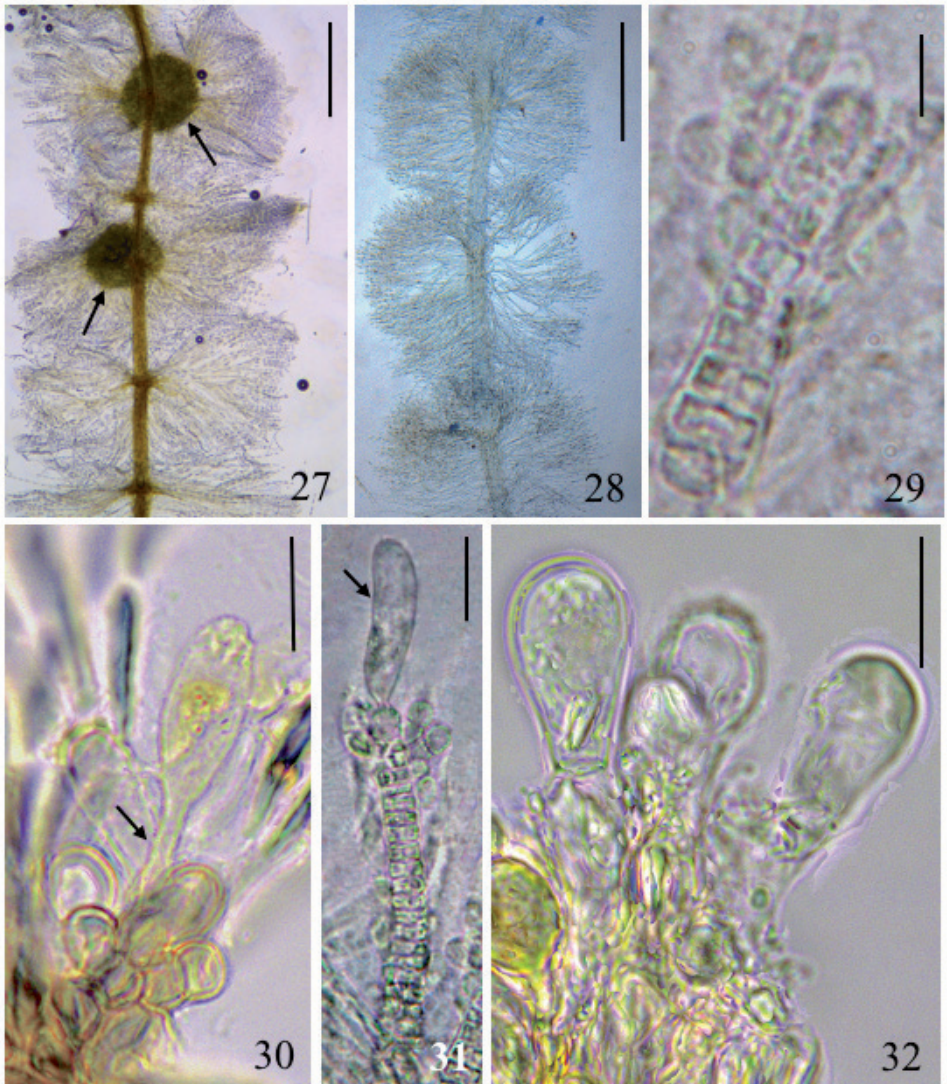
Virescentia vogesiaca (T.G. Schultz ex Skuja) Necchi, D.C.Agostinho & M.L.Vis, **comb. nov.** **Figs 27-32**

Basionym: *Batrachospermum vogesiacum* T. G. Schultz ex Skuja, *Archiv für Hydrobiologie* Suppl 15: 623 (1938).



Figs 23-26. *Virescentia viride-brasiliensis* (Necchi & D.C. Agostinho) Necchi, D.C.Agostinho & M.L.Vis comb. nov. **23.** Whorls with carposporophyte (arrow); **24-25.** Details of carpogonia showing long stalk trichogyne (arrow); **26.** Gonimoblast filaments with carposporangia. Scale bars: 23 = 250 µm; 24-26 = 10 µm. All figures from the holotype (Table 1).

Description: Plants monoecious; branching irregular; whorls contiguous or separated, barrel-shaped, obconic, pear-shaped or spherical, 340-700 µm wide; primary fascicles straight, with 7-14 cell storeys; secondary fascicles abundant, covering the entire internode; spermatangia spherical, on primary or secondary fascicles, 5-8 µm wide; carpogonial branches straight, composed of (4-)8-20 disc-shaped cells, developing from the periaxial or proximal cells of primary fascicles, long or short, (13-)25-68 µm long; involucral filaments, short, composed of 1-3 elliptical or spherical cells; carpogonia 20-45 µm long, with stalked or shortly stalked, club-shaped or elliptical



Figs 27-32. *Virescentia vogesiaca* (T.G. Schultz) Necchi, D.C.Agostinho & M.L.Vis comb. nov. **27-28**. Whorls with carposporophytes (arrows); **29.** Detail of a young carpogonial branch; **30-31.** Details of mature carpogonia; **30.** Carpogonium with short carpogonial branch and stalked trichogyne (arrow); **31.** Carpogonium with long carpogonial branch and unstalked trichogyne; **32.** Gonimoblast filaments with carposporangia. Scale bars: 27-28 = 250 µm; 29-32 = 10 µm. Figs. 29, 31 from the lectotype (Table 1); figs 27-28, 30, 32 from BHO A-0926.

trichogynes; carposporophytes 1-2 per whorl, 140-330 µm wide, 95-175 µm high; gonimoblast filaments with 4-7 cylindrical or elliptical cells; carposporangia obovoid, 13-19 µm long, 8.5-13 µm wide.

Distribution: Europe - Belgium, France, Spain, Sweden (Sirodot, 1884; Israelson, 1942; Eloranta *et al.*, 2011; Chiasson *et al.*, 2014).

Representative DNA sequences: KJ825954, KU754497 (*rbc*L).
Additional specimens examined: France, River at St Jacques, 43.929083, –0.731194, M. Vis, W. Chiasson & K. Chiasson 26.x.2011, BHO A-0926.
Remarks: we confirmed the distinction of this species from *V. helminthosa* based on genetic divergences among sequences of *rbc*L (121-122 bp, 9.4-9.5%), that corroborates the previous finding by Chiasson *et al.* (2014). In addition, these species can be differentiated by the disposition of the carpogonial branches: slightly curved in *V. helminthosa* and straight in *V. vogesiaca.* This morphological character is consistent with the original description of each species by Sirodot (1884).

Excluded taxa

Batrachospermum bakarense Kumano & Ratnas., *Japanese journal of phycology* 32: 119 (1984)

Based on the characters described in the protologue and observed in the type specimen, i.e. club-shaped and unstalked trichogynes, loosely arranged carposporophytes and two types of gonimoblast filaments (prostrate and erect), we concluded that this species belongs to section *Turfosa*, which will be treated in a separate paper.

Batrachospermum desickacharyi Sankaran, *Phykos* 23: 169 (1984)

Based on the characters described in the protologue and by Kumano (2002), i.e. carpogonia short, carposporophytes stalked and small, we concluded that this species belongs to either *Batrachospermum* sensu stricto or *Sheathia*.

Batrachospermum gombakense Kumano & Ratnas., *Japanese journal of phycology* 30: 119 (1982b)

Based on the characters described in the protologue and observed in the type specimen, i.e. club-shaped and unstalked trichogynes, loosely arranged carposporophytes and two types of gonimoblast filaments (prostrate and erect), we concluded that this species belongs to section *Turfosa*, which will be treated in a separate paper.

Batrachospermum transtaganum Reis, *Memórias da sociedade Broteriana* 21: 23 (1970)

Based on the characters described in the protologue and observed in the type specimen, we concluded that this species is a synonym of *Kumanoa virgato-decaisneana* as described in Necchi & Vis (2012). Thus, it is here formally transferred to the genus *Kumanoa* as a synonym of *K. virgato-decaisneana*: *Kumanoa transtagana* (Reis) Necchi, D.C. Agostinho & M.L.Vis.

Virescentia spp.

These taxa were reported as *B. helminthosum* by Hanyuda *et al.* (2004) from Japan and based on genetic data (*rbc*L sequence analysis) and most likely represent two distinct, probably new, species. The authors described five distinct haplotypes, four belonging to a single species and a fifth one to a separate species. However, according to Hanyuda (pers. comm.) there are no voucher for those specimens required to describe morphological characters to propose new species. The status of these Japanese materials remains unresolved.

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SUPPORTING INFORMATION

Table S1. *rbc*L sequence data from additional taxa of Batrachospermales used for phylogenetic analyses.

Taxon	*GenBank Accession Number*
Atrophycus ater	KT802842
Atrophycus puiggarianus	FJ386462
Balliopsis prieurii	AY960688
Batrachospermum brasiliense	FJ386458
Batrachospermum cayennense	KM055245
Batrachospermum cayennense	AY423393
Batrachospermum dapsile	KM593855
Batrachospermum gelatinosum	GU810833
Batrachospermum macrosporum	EU106060
Batrachospermum macrosporum	EU106056
Batrachospermum naiadis	KM593857
Batrachospermum pozoazulense	KM593863
Batrachospermum shanxiense	KM593851
Batrachospermum turfosum	KJ825960
Kumanoa ambigua	AY423390
Kumanoa americana	JN589995
Kumanoa australica	JN589998
Kumanoa gudjewga	JN590002
Kumanoa holtonii	JN590004
Kumanoa louisianae	JN590005
Kumanoa nodiflora	AY423398
Kumanoa procarpa	FJ386464
Kumanoa vittata	JN590010
Lemanea fluviatilis	KM055243
Lemanea fucina	KJ825958
Lympha mucosa	KM593865
Nemalionopsis shawii	KM005141
Nocturama antipodites	FJ386456
Nothocladus campyloclonus	FJ386459
Nothocladus diatyches	KT802848
Nothocladus discors	KT802850
Nothocladus nodosus	AF029152
Nothocladus theaquus	KT802863
Nothocladus wattsii	KT802866
Paralemanea sp.	GQ285124
Petrohua bernabei	AY960690

Table S1. *rbc*L sequence data from additional taxa of Batrachospermales used for phylogenetic analyses *(continued)*.

Taxon	*GenBank Accession Number*
Psilosiphon scoparium	AF029155
Sheathia americana	JX669757
Sheathia confusa	DQ393133
Sheathia exigua	GU457345
Sheathia heterocortica	DQ393136
Sheathia involuta	KU672395
Sirodotia delicatula	DQ646476
Sirodotia huillensis	AF029157
Sirodotia suecica	JF344718
Thorea riekei	KM005140
Tuomeya americana	KM055244
Volatus carrionii	KM593864
Volatus personatus	KM593856
Volatus ulterior	KM593852

Table S2. Sequences of *rbc*L and COI-5P of *Batrachospermum* section *Virescentia* from GenBank used in the analyses.

Species	*Location*	*GenBank Acession Number*		*Reference*
		***rbc*L**	*COI-5P**	
Batrachospermum sp.	U.S.A., Michigan, Spring Brook	AF244109	EU636727 EU073847	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Michigan, Flemming Creek	AF244110	EU636727 EU636736 EU073847	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Ohio, Big Run Creek	AF244111	EU636733 EU073833 EU073838	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Ohio, Browning Creek	AF244112	EU073834 EU073837 EU073848 EU636732 EU636734	Vis *et al.* (2001)
Batrachospermum sp.	U.S.A., Connecticut, Mill Brook	AF244113	EU073834 EU636732	Vis *et al.* (2001)
Batrachospermum sp.	U.S.A., Louisiana, unnamed stream	AF244114	EU636729 EU073845	Vis *et al*. (2001)

* the COI-5P was sequenced from multiple specimens within a location such that there may be multiple sequences per location and the same COI-5P haplotype can occur in more than one location. These data are from either Sherwood *et al*. (2008) or House *et al*. (2008).

Species	*Location*	*GenBank Acession Number*		*Reference*
		***rbc*L**	*COI-5P**	
Batrachospermum sp.	U.S.A., Massachusetts, Poquoy Brook	AF244115	EU073839 EU636735	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., North Carolina, Cedar Fork	AF244116	EU073834 EU073836 EU073838 EU073840	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Tennessee, Scarboro Creek	AF244117	EU073842 EU073843 EU073844 EU073846 EU636728 EU636730 EU636731	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Indiana, Otter Creek	AF244118	EU073845 EU636729	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Rhode Island, Wood River tributary	AF244119	EU073833 EU073836	Vis *et al.* (2001)
Batrachospermum sp.	U.S.A., Ohio, Five Mile Creek	AF244120	EU073834 EU073838 EU636736	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Rhode Island, Chipuxet River	AF029142	---	Vis *et al*. (1998)
Batrachospermum sp.	U.S.A., Ohio, Yellow Springs	AY198417	EU073847 EU073848 EU636727	Chiasson *et al*. (2003)
Batrachospermum sp.	U.S.A., Ohio, Big Run		EU073833	Vis *et al*. (2001)
Batrachospermum sp.	U.S.A., Virgina, Carvin Creek	-----	EU073833 EU073835 EU073840 EU073843	
Batrachospermum sp.	U.S.A., Ohio, Sunfish Creek	-----	EU073841	
Batrachospermum sp.	Japan, Kagoshima, Oguchi	AB114642	-----	Hanyuda et al. (2004)
Batrachospermum sp.	Japan, Kyoto, Tanba	AB114643	-----	Hanyuda *et al*. (2004)
Batrachospermum sp.	Japan, Ehime, Komatsu	AB114644	-----	Hanyuda *et al*. (2004)
Batrachospermum sp.	Japan, Iwate, Takizawa	AB114645	-----	Hanyuda *et al*. (2004)

* the COI-5P was sequenced from multiple specimens within a location such that there may be multiple sequences per location and the same COI-5P haplotype can occur in more than one location. These data are from either Sherwood *et al*. (2008) or House *et al*. (2008).

Table S2. Sequences of *rbc*L and COI-5P of *Batrachospermum* section *Virescentia* from GenBank used in the analyses *(continued)*.

Species	*Location*	*GenBank Acession Number*		*Reference*
		***rbc*L**	*COI-5P**	
Batrachospermum sp.	Japan, Okinawa, Nago	AB114646	-----	Hanyuda *et al*. (2004)
B. helminthosum	Norway, Askerelva, county of Akershus	-----	HQ412541	Rueness (2010)
B. helminthosum	Norway, Askerelva, county of Akershus	-----	HQ412542	Rueness (2010)
B. helminthosum	France, Le Boulet Prioult, near Le Petit Boulet	KJ825955	-----	Chiasson *et al*. (2014)
B. helminthosum	Portugal, Portalegere, Termas de Nisa	KM593807	-----	Chapuis *et al*. unpublished
B. helminthosum	Spain, Galicia, A Coruña, Riachuelo das Brañas	KM593841	-----	Chapuis *et al*. unpublished
B. viride-brasiliense	Brasil, SP, Maringá, Pavão River	FJ386460	---	Entwisle *et al*. (2009)
B. viride-brasiliense	SP: Campos do Jordão, Ducha da Prata	KM259993	KM078048	Agostinho & Necchi (2014)
B. viride-brasiliense	SP: Paraibuna, Pousada Alto da Serra, Rio Negro	KM259994	KM097031	Agostinho & Necchi (2014)
B. viride-brasiliense	PR: Guarapuava, Rio das Pedras	KM259996	KM097033	Agostinho & Necchi (2014)
B. viride-brasiliense	PR: Guarapuava, Rio Lageado, Bacia do Rio Marrecas	KM259995	KM097032	Agostinho & Necchi (2014)
B. viride-brasiliense	ES: Domingos Martins, Pedra Azul, Rio Tucú	KM259998	KM097035	Agostinho & Necchi (2014)
B. viride-brasiliense	PR: Teixeira Soares, Floresta Nacional de Irati	KM259999	KM097036	Agostinho & Necchi (2014)
B. viride-brasiliense	PR: Teixeira Soares, Floresta Nacional de Irati	KM260000	KM097037	Agostinho & Necchi (2014)
B. viride-brasiliense	SC: São Domingos, Parque Estadual das Araucárias, rio Jacutinga	KM260001	KM097038	Agostinho & Necchi (2014)

* the COI-5P was sequenced from multiple specimens within a location such that there may be multiple sequences per location and the same COI-5P haplotype can occur in more than one location. These data are from either Sherwood *et al*. (2008) or House *et al*. (2008).

Species	*Location*	*GenBank Acession Number*		*Reference*
		***rbc*L**	*COI-5P**	
B. viride-brasiliense	SC: São Domingos, Parque Estadual das Araucárias	KM259997	KM097034	Agostinho & Necchi (2014)
B. viride-brasiliense	SC: São Domingos, Parque Estadual das Araucárias	KM260002	KM097039	Agostinho & Necchi (2014)
B. viride-brasiliense	SP: Brotas, Parque dos Saltos	KM260003	KM097040	Agostinho & Necchi (2014)
B. viride-brasiliense	RS: Cambará do Sul, Parque Nacional de Aparados da Serra, Arroio Camisas	KM260004	KM097041	Agostinho & Necchi (2014)
B. viride-brasiliense	RS: Cambará do Sul, Parque Aparados da Serra, Arroio Perdizes	KM260005	KM097042	Agostinho & Necchi (2014)
B. vogesiacum	France, river at St. Jacques	KJ825954	-----	Chiasson *et al*. (2014)
B. vogesiacum	Spain, Zamora, Río Tera	KU754497	-----	Chapuis *et al*. unpublished

* the COI-5P was sequenced from multiple specimens within a location such that there may be multiple sequences per location and the same COI-5P haplotype can occur in more than one location. These data are from either Sherwood *et al*. (2008) or House *et al*. (2008).

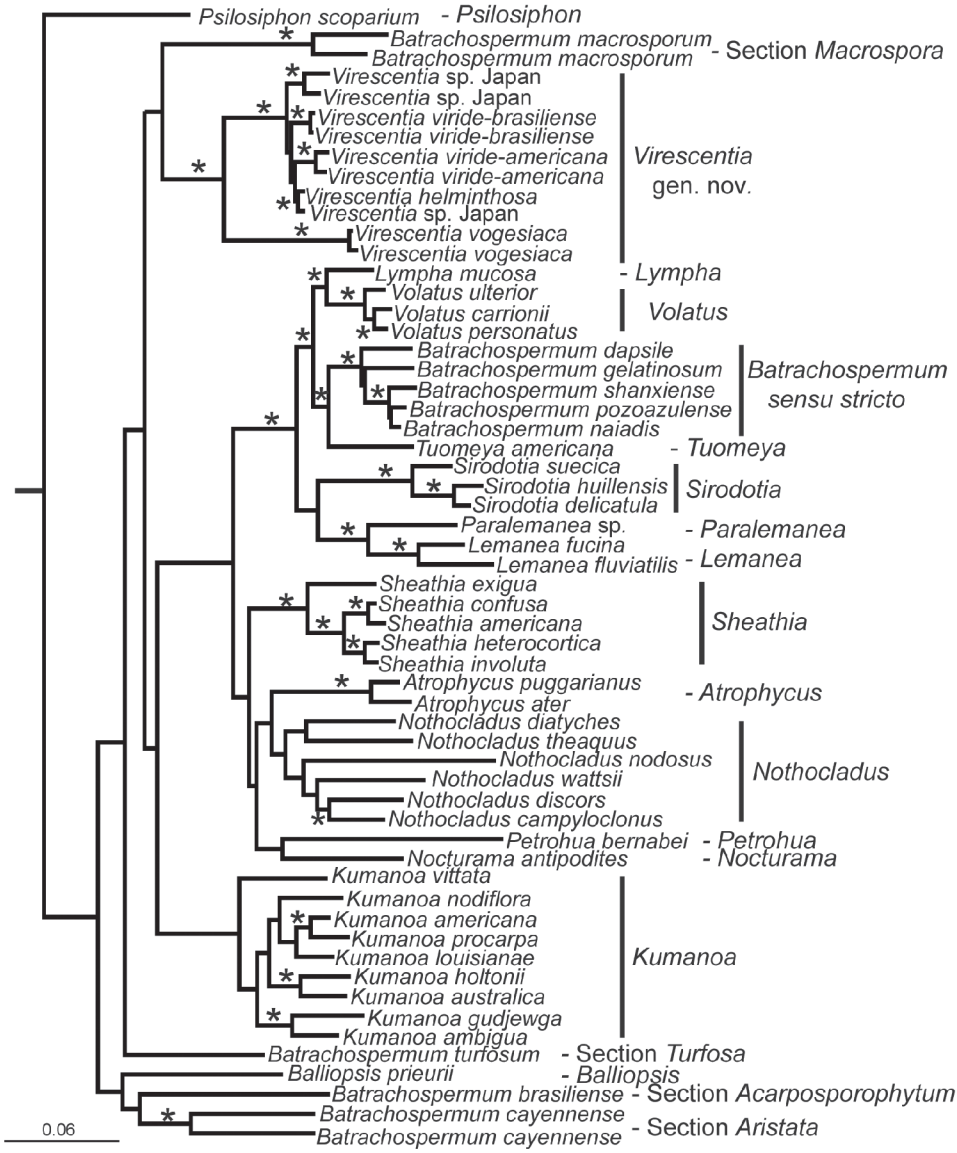

Fig. S1. Phylogenetic tree (maximum likelihood) showing relationship of *Virescentia* stat. nov. with other genera and sections of Batrachospermales based on *rbc*L sequence data. * = bootstrap support > 80 and posterior probability > 0.90; nodes without values indicate bootstrap values ≤ 80 and posterior probability ≤ 0.90. Information on the sequences are listed in Table S1 (Supporting Information). Scale represents substitutions per site.

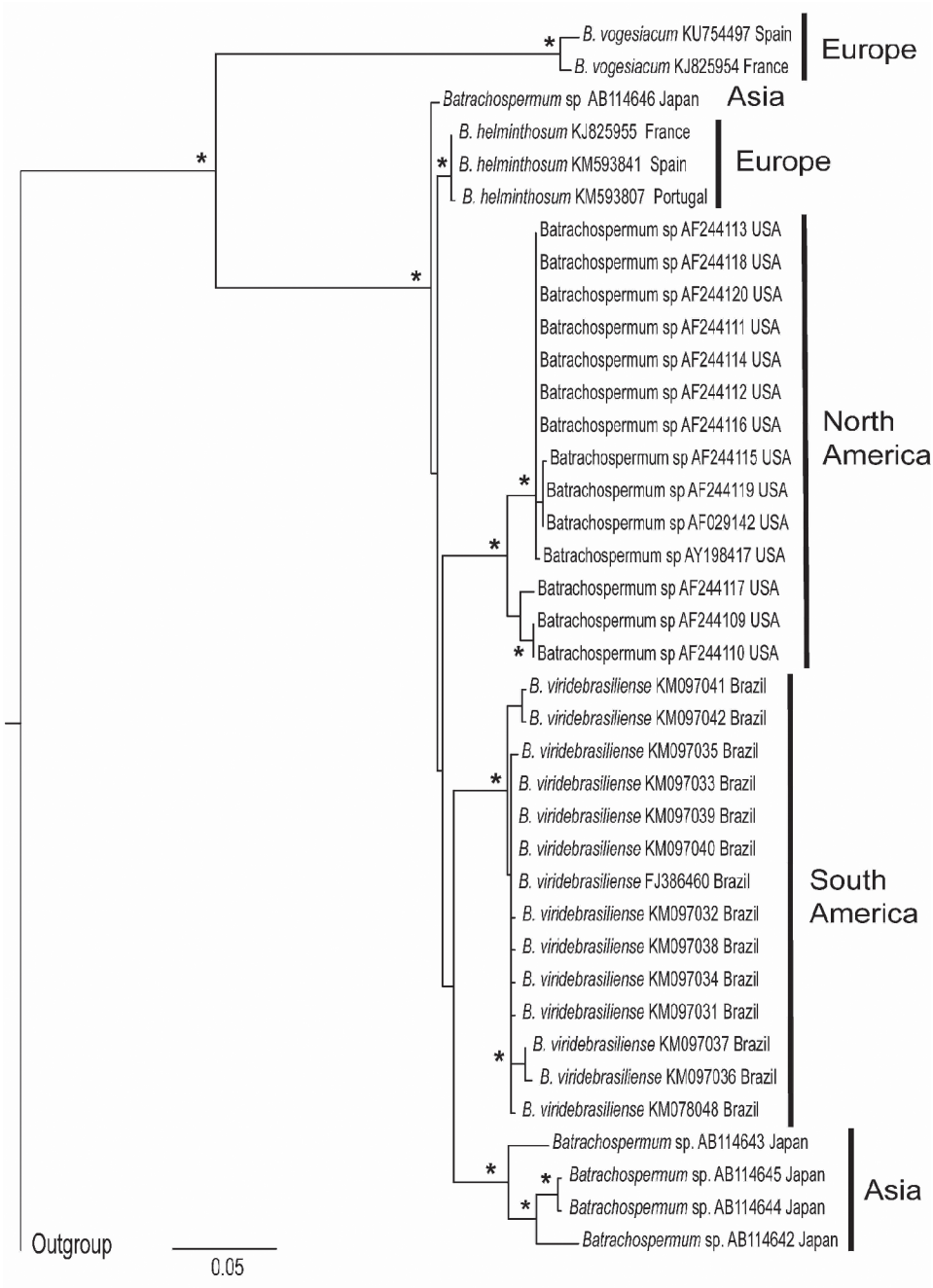

Fig. S2. Phylogenetic tree (maximum likelihood) based on *rbc*L sequences. * = bootstrap values > 90 and posterior probability > 0.90; nodes without values indicate bootstrap values 70 and posterior probability 0.70. Information on the sequences are listed in Table S2 (Supporting Information). Scale represents substitutions per site.

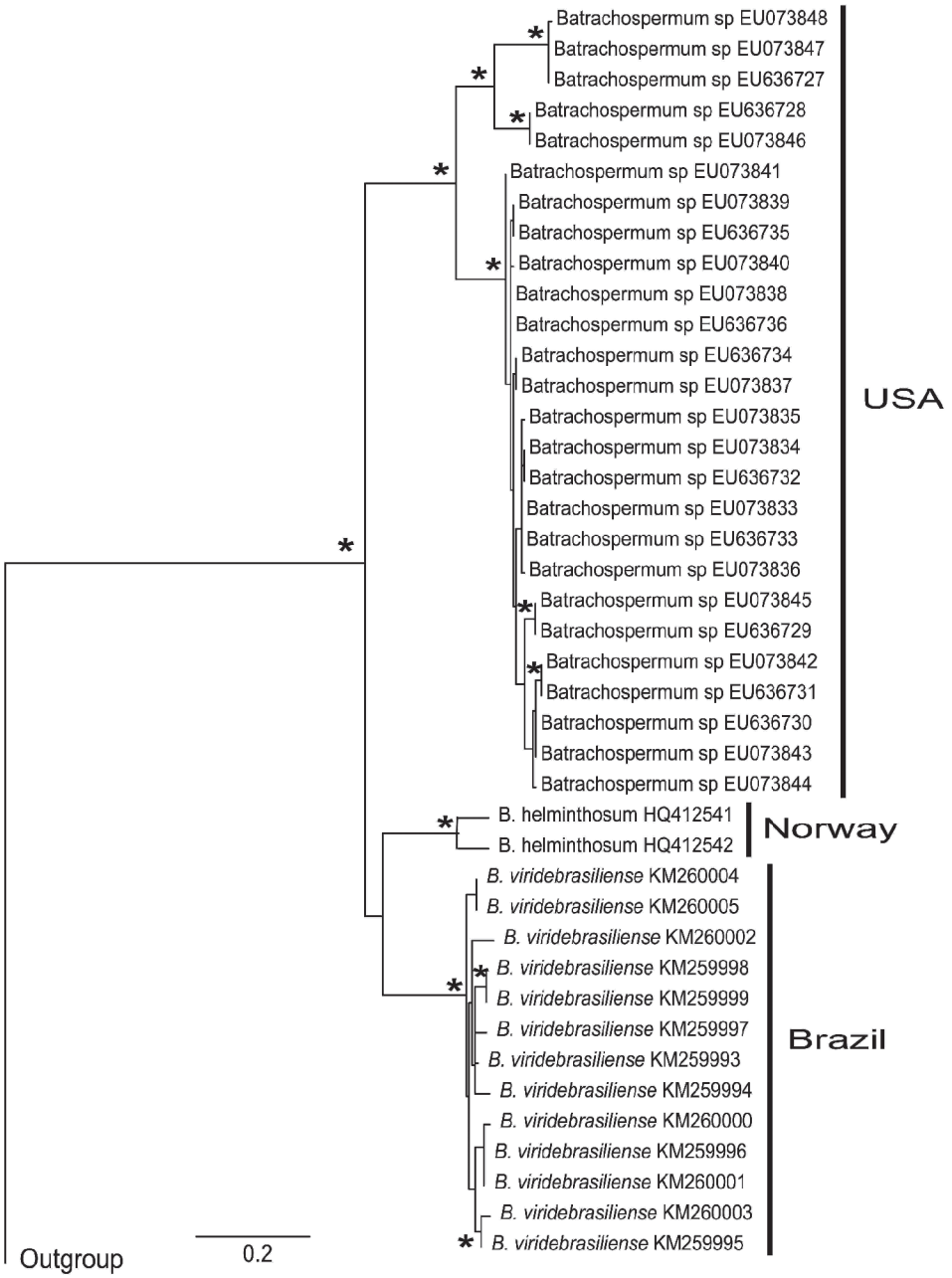

Fig. S3. Distance tree (neighbor-joining) based on COI-5P sequences. * = bootstrap values > 95; nodes without values indicate bootstrap values ≤ 70. Information on the sequences are listed in Table S2 (Supporting Information). Scale represents substitutions per site.