Spirosphaera cupreorufescens sp nov., a rare aeroaquatic fungus

Article *in* Studies in Mycology · January 2004

CITATIONS
23

READS
119

1 author:

Hermann Voglmayr
BOKU - University of Natural Resources and Life Sciences Vienna
429 PUBLICATIONS **5,147** CITATIONS

SEE PROFILE

STUDIES IN MYCOLOGY 50: 221–228. 2004.

Spirosphaera cupreorufescens sp. nov., a rare aeroaquatic fungus

Hermann Voglmayr*

Institute of Botany, University of Vienna, Rennweg 14, A-1030 Wien, Austria

**Correspondence*: Hermann Voglmayr, hermann.voglmayr@univie.ac.at

Abstract: *Spirosphaera cupreorufescens* is described as a new aeroaquatic fungus. It inhabits leaves of deciduous trees submerged in small ditches or puddles in slightly minerotrophic bogs and mires. Molecular phylogenetic analyses of partial nLSU and of the ITS1-5.8S-ITS2 rDNA sequences are given to reveal its phylogenetic position. Although it is morphologically clearly attributable to the anamorph genus *Spirosphaera*, LSU rDNA sequence data show that it is not related to the type species of the genus, *Spirosphaera floriformis*. Whereas *Spirosphaera floriformis* is phylogenetically placed within the unitunicate *Leotiomycetidae sensu lato, Spirosphaera cupreorufescens* is embedded within the bitunicate *Dothideomycetes.* The genus *Spirosphaera* in its current circumscription is shown to be polyphyletic; however, lack of morphologically discriminating features prevent generic splitting, and the genus *Spirosphaera* is maintained as an anamorph form-genus. A key to all described species of *Spirosphaera* is provided.

Taxonomic novelty: *Spirosphaera cupreorufescens* Voglmayr sp. nov.
Key words: aeroaquatic, *Clathrosporium*, ITS, key, nLSU rDNA, *Spirosphaera.*

INTRODUCTION

Agathe van Beverwijk, a mycologist at the Centraalbureau voor Schimmelcultures, first coined the term aeroaquatic for amphibious fungi which decompose submerged plant litter, but can produce propagules only above the water level (Beverwijk 1951). These propagules enclose air, are well buoyant and dispersed by floating on the water surface. Subsequently, she described several new aeroaquatic species and genera, respectively; amongst these also the anamorph genus *Spirosphaera*, with *S. floriformis* Beverw. as type species (Beverwijk 1953). The genus was characterised by globose conidia formed by branching, coiled, loosely interwoven conidial filaments. Since then, seven additional *Spirosphaera* species have been described (Hennebert 1968, Abdullah *et al.* 1986, 1998, Voglmayr 1997, Marvanová & Bärlocher 1998, Udagawa & Uchiyama 1998), one from feathers and six from submerged litter.

During investigations of aero-aquatic fungi inhabiting leaf litter of ditches and puddles in bogs in Upper Austria and Norway, respectively, propagules of an undescribed fungus morphologically matching the genus *Spirosphaera* were detected. Despite some similarities to *Spirosphaera floriformis*, its features are clearly distinct, and there are no other similar species described. Therefore, these collections are described as a new species below.

MATERIALS AND METHODS

Sampling and documentation

Submerged leaves (mainly *Alnus* Mill., *Salix* L. and *Betula* L.) were taken from ditches or small puddles located in bogs and mires, respectively, packed into plastic bags and returned to the laboratory. The leaves were cut into small pieces, rinsed in water and spread on Petri dishes lined with moist filter paper, kept damp and exposed to natural light at room temperature. The Petri dishes were regularly examined under a dissecting microscope for the presence of propagules of aeroaquatic fungi. After spores had developed, they were transferred aseptically to 2 % malt extract agar (MEA; Merck) plates, where they germinated. To obtain sufficient sporulating material for morphological studies and herbarium specimens, respectively, fallen leaves of *Fagus sylvatica* L. were cut into pieces and placed in 250 mL culture flasks, submerged in 100 mL tap water, autoclaved twice, inoculated with mycelium and incubated for 1 mo at room temperature. The colonised substrate was spread on Petri dishes lined with moist filter paper, kept damp and exposed to natural light at room temperature. From about 5 d onwards, typical spores developed on leaves and exposed mycelial patches. Spores were examined under the light microscope, measured, and drawn using a drawing tube. Leaf fragments and agar cultures containing spores were air-dried and deposited at the herbarium of the Institute of Botany of the University of Vienna (WU). Living cultures were deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Scanning electron microscopy
For scanning electron microscopy (SEM), spores from natural substrate were prepared according to the methods of Voglmayr and Krisai-Greilhuber (1996) or Halbritter (1998). After preparation, the specimens were mounted on Cambridge stubs, sputter-coated with gold, and examined in a Jeol T 300 scanning electron microscope at 10 kV.

Sample sources used for sequencing
Clathrosporium cf. *intricatum* Nawawi & Kuthub.: Austria, Upper Austria, distr. Schärding, comm. Engelhartszell, Kleines Kesselbachtal, 300 m s. m., small puddle at the bottom of a ravine in mixed deciduous forest (*Fagus* L., *Carpinus* L., *Fraxinus* L.), ex soaked rotten wood of a submerged decorticated twig, leg. 29 Mar. 2002, H. Voglmayr, source code A27, WU 24690, living culture CBS 115285; GenBank accession no. AY616235 (nLSU).
Helicodendron tubulosum (Riess) Linder: Austria, Upper Austria, distr. Schärding, comm. St. Willibald, Großer Salletwald, 460 m s. m., small pond in mixed forests (*Picea* A. Dietr., *Betula*), ex submerged leaf of *Alnus glutinosa* (L.) Gaertn., leg. 18 Sep. 1992, H. Voglmayr, source code AE2, WU 24691, living culture CBS 115157; GenBank accession no. AY616237 (nLSU).
Spirosphaera cupreorufescens: Austria, Upper Austria, distr. Braunau, comm. Franking, Frankinger Möser (western part of the Ibmermoos), 430 m s. m., oligotrophic peat ditch at the margin of a wooded peat bog, ex submerged leaf of *Alnus glutinosa*, leg. 30 Mar. 2002, H. Voglmayr, source code A20, WU 24684 (holotype), living culture CBS 115026; GenBank accession no. AY616232 (ITS), AY616236 (nLSU); Austria, Upper Austria, distr. Vöcklabruck, comm. Oberhofen/Irrsee, mire north of Irrsee, shallow ditches in small *Alnus glutinosa* and *Betula pendula* Roth stand, ex leaf of *Alnus glutinosa*, leg. 23 Oct. 1993, H. Voglmayr, source code A89, WU 24688, living culture lost; GenBank accession no. AY616233 (ITS); Norway, Oslo, east of Skullerud, shallow puddles within *Salix caprea* L. stand, ex submerged leaf of *Salix caprea*, leg. 14 Aug. 2002, H. Voglmayr, source code A1, WU 24689, living culture lost; GenBank accession no. AY616234 (ITS).
Spirosphaera floriformis: Austria, Upper Austria, distr. Schärding, comm. St. Willibald, Großer Salletwald, 460 m s. m., small puddle in mixed forests, ex submerged twig of *Picea abies* (L.) Karst., leg. 2 Mar. 2002, H. Voglmayr, source code A80, WU 24692, living culture CBS 115284; GenBank accession no. AY616238 (nLSU).

DNA extraction, PCR and sequencing
For sample preparation, small pieces were cut out at the margin of actively growing agar cultures and dried in 2 mL reaction-tubes filled with silica gel. The dried samples were subsequently placed into another 2 mL reaction-tube filled with several glass beads (2–3 mm diam) and ground in a Retsch 200 mixer mill for 10 min. Subsequently, DNA was extracted using the modified CTAB-protocol described in Riethmüller *et al*. (2002).

The complete ITS rDNA region was amplified with primers ITS4 and ITS5 (White *et al*. 1990), the D1, D2 region of the nLSU rDNA region with primers LR0R (Moncalvo *et al*. 1995) and TW14 (White *et al*. 1990). PCR products were purified using the QIAquick Kit (Qiagen) according the manufaturer's instructions. DNA was sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Warrington) with ITS4, ITS5 and LR0R and TW14, respectively, as primers and an automated DNA sequencer (ABI 377, Applied Biosystems).

Data analysis
For the nLSU rDNA analysis, a nucleotide blast search was performed at the database of GenBank (NCBI, http://www.ncbi.nlm.nih.gov) to get deposited sequences most similar to those of *Spirosphaera floriformis* and *S. cupreorufescens*, respectively. Then, additional sequences were selected according to the results of the phylogenetic analyses of Tehler *et al*. (2003) to obtain a representative taxon sampling. The GenBank accession numbers of the selected sequences are given in the tree, following the taxon names (Fig. 1). Similarly, appropriate GenBank sequences were selected using the ITS rDNA region of *S. cupreorufescens* for nucleotide BLAST search.

The sequence alignment was initially produced with ClustalX (Thompson *et al.* 1997), version 1.81, and visually checked and refined with BioEdit (Hall 1999), version 4.8.6. All characters of the resulting partial nLSU alignment were included in the subsequent phylogenetic analysis, whereas for analysis of the ITS1-5.8S-ITS2 region most of the ITS1 region was discarded due to alignment problems.

Maximum parsimony bootstrap analysis was performed with PAUP 4.0b10 (Swofford 2002), using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect), with 10 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate. Gaps were treated as missing data; a cost of 2 was assigned to transversions and a cost of 1 to transitions.

Markov chain Monte Carlo analyses (MCMC; Larget & Simon 1999, Mau *et al.* 1999) were performed only with the nLSU data set, using the computer programme MrBayes (version 3.0b4; Huelsenbeck & Ronquist 2001). Analysis was based on the general time-reversible model of DNA substitution, additionally assuming a portion of invariant sites with

gamma-distributed substitution rates of the remaining sites (GTR+I+G; see Swofford *et al.* 1996). Four incrementally heated simultaneous Markov chains were run over one million generations from which every 100th tree was sampled. The trees before apparent stability of the cold chain were discarded (usually the first 300 stored trees). A 50 % majority rule consensus of the remaining trees was computed to obtain estimates for the probabilities that groups are monophyletic given the sequence data (posterior probabilities). Branch lengths were computed as mean values over the sampled trees. To confirm that stationarity of the MCMC processes had actually been reached (Huelsenbeck *et al.* 2002), the Bayesian analysis was repeated six times on a personal computer, always starting with random trees and default parameter values of the programme. The phylogenetic trees were rooted with *Peziza vesiculosa* Bull. according the results of Tehler *et al.* (2003).

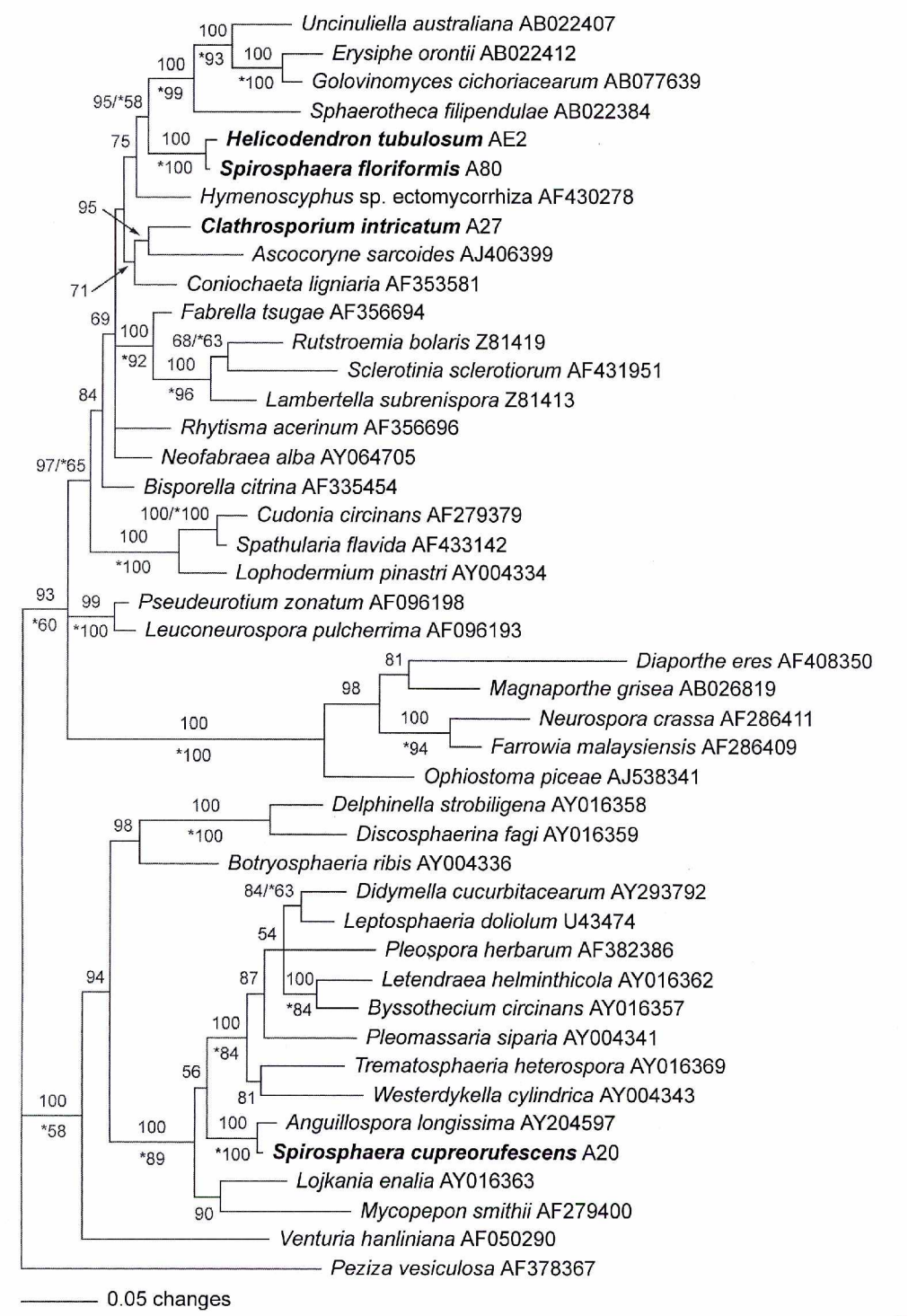


Fig. 1. Bayesian phylogenetic analysis using Markov chain Monte Carlo (MCMC) of 44 taxa of ascomycetes based on nuclear D1/D2 sequences (LSU rDNA). A 50 % majority rule consensus tree from a MCMC analysis over one million generations, in which every 100th tree was sampled, discarding the first 300 sampled trees; branch lengths are averaged over the sampled trees. Numbers on branches are estimates for posterior probabilities for the monophyly of the respective clades; numbers with asterisks MP bootstrap values. For taxa in bold, the sequences have been obtained in the present study.

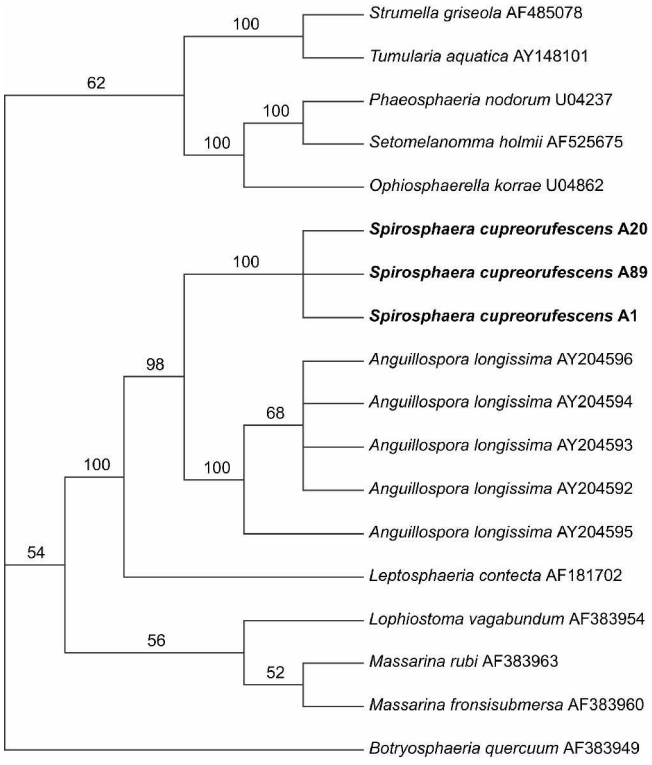


Fig. 2. Maximum parsimony bootstrap tree of of 12 taxa of *Dothideomycetes* based on partial ITS1-5.8S-ITS2 sequences. For taxa in bold, the sequences have been obtained in the present study.

RESULTS

The final alignments and the trees obtained were deposited in TreeBASE (http://www.treebase.org) and are available under study accession no. S1142.

Tree topologies of all six MCMC runs of the nLSU rDNA were identical, and the posterior probabilities were similar; one of the resulting six majority rule consensus trees is given in Fig. 1. As the MCMC and MP bootstrap trees were fully consistent, additionally the MP bootstrap values are listed in this tree (Fig. 1). As often observed, the posterior probabilities were usually significantly higher than the corresponding bootstrap values. *Spirosphaera floriformis* and *Clathrosporium* cf. *intricatum* are clearly members of the inoperculate *Leotiomycetes*/*Sordariomycetes* lineage, but are not closely related to each other. *Spirosphaera floriformis* is closely related to *Helicodendron tubulosum*, a common aeroaquatic fungus with doliiform conidia (100 % bootstrap support). Conversely, *Spirosphaera cupreorufescens* is embedded within the bitunicate *Dothideomycetes*, and is closely related to *Anguillospora longissima* (Sacc. & Syd.) Ingold, a common aquatic hyphomycete with long, sigmoid conidia.

Maximum parsimony bootstrap analysis of the partial ITS1-5.8S-ITS2 region (Fig. 2) confirmed that *Anguillospora longissima* is closely related to *Spirosphaera cupreorufescens* (98 % bootstrap support). Sister group relationship of the former clade to *Leptosphaeria contecta* Kohlm. is also highly supported (100 % bootstrap support).

TAXONOMIC PART

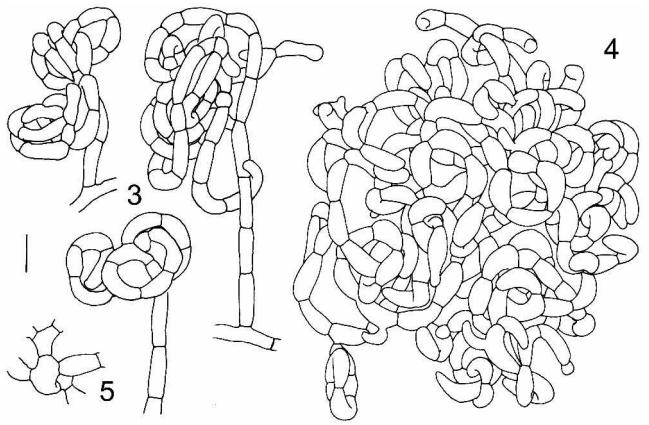
Spirosphaera cupreorufescens Voglmayr, **sp. nov.** MycoBank MB500063. Figs 3–11.

Etymology: Referring to the copper-brown conidia.

Coloniae in vitro tarde crescentes, in 2 % MEA primo colore candido, deinde fusco, saepe tinctae colore cupreo, centro obtextae mycelio coacto vel lanato, reversum ad marginem colore candido, in centro fuscescens, in 2 % MEA temperatura 20 °C ad 35–42 mm diametro post 3 hebdomades crescentes, sparsim vel abunde sporulantes in vitro. Mycelium velutinum vel lanatum, compositum ex hyphis hyalinis vel subhyalinis septatis 2–3.8 µm latis. Chlamydosporae absentes. Conidiophora semimacronematosa, mononematosa, subhyalina vel fusca, septata, non ramosa, 3.5–4 µm lata. Cellulae conidiogenae integratae, holoblasticae, terminales. Conidia colore cupreo, distincte irregulariter globosa, diametro 110–150 µm, formantur e filamentis spiraliter intertextis ramificantibusque. Filamenta conidialia fusca, distincte septata, formantia spiras irrgulares 20–35 µm diametro, 3–4 septa per spiram, ad septa constricta, 5-6 µm lata, septis 3–4 µm latis; cellulae 10–14 µm longae, distincte curvatae, saepe gerentes unum (raro dua) filamentum filiale.

Holotypus: **Austria**, Upper Austria, distr. Braunau, comm. Franking, Frankinger Möser (western part of the Ibmermoos), oligotrophic peat ditch at the margin of a wooded peat bog, on submerged leaf of *Alnus glutinosa*, leg. 30 Mar. 2002, H. Voglmayr, WU 24684 (**holotype**), living culture CBS 115026.

Colony growth rate *in vitro* slow, on 2 % MEA first cream-white, later becoming grey, often with coppery tinges, in centre covered by felty to woolly mycelium, reverse at the margins cream-white, at the centre becoming dark brown, reaching 35–42 mm diam at *ca*. 20 °C after 3 wk, sporulation *in vitro* scanty to abundant, often untypical. *Mycelium* velvety to felty, consisting of septate, hyaline to subhyaline hyphae 2–3.8 µm wide. *Chlamydospores* absent. *Conidiophores* semimacronematous, mononematous, subhyaline to fuscous, septate, unbranched, 3.5–4 µm wide. *Conidiogenous cells* integrated, holoblastic, terminal. *Conidia* coppery brown in mass, distinctly irregularly globose, 110–150 µm diam, formed by branched, loosely spirally interwoven filaments.



Figs 3–5. Line drawings of *Spirosphaera cupreorufescens*. 3. Three young conidia attached to the conidiophore (sporulating type culture). 4. Mature conidium; note that not every cell gives rise to a daughter filament, the unilateral branching and the spirally coiled conidial filaments (WU 24686). 5. Detail from a mature conidium, showing opposite (bilateral) branching (type). Scale bar = 10 µm.

Conidial filaments fuscous, distinctly septate, forming irregular spiral coils 20–35 µm in diam, 3–4 septa per coil, constricted at the septa, 5–6 µm in the middle of the cells, 3–4 µm at the septa; cells 10–14 µm long, usually strongly curved, often giving rise to one (rarely two) daughter filaments.

Additional specimens examined: **Austria**, Upper Austria, distr. Braunau, comm. Franking, Frankinger Möser (western part of the Ibmermoos), 430 m s. m., oligotrophic peat ditch at the margin of a wooded peat bog, ex submerged leaf of *Alnus glutinosa*, leg. 30 Mar. 2002, H. Voglmayr, WU 24685, living culture CBS 115025; *ibid*., ex submerged leaf of *Salix cinerea*, leg. 24 Oct. 1993, H. Voglmayr, WU 24686; distr. Vöcklabruck, comm. Oberhofen/Irrsee, mire north of Irrsee, 560 m. s. m., shallow ditches in small *Alnus glutinosa* and *Betula pendula* stand, ex submerged leaf of *Alnus glutinosa*, leg. 25 Oct. 1993, H. Voglmayr, WU 24687; *ibid.*, small puddles, ex leaf of *Alnus glutinosa*, leg. 25 Oct. 1993, H. Voglmayr, WU 24688. **Norway**, Oslo, east of Skullerud, shallow puddles surrounded by *Salix caprea* stand, ex submerged leaf of *Salix caprea*, leg. 14 Aug. 2002, H. Voglmayr, WU 24689.

Key to the species of *Spirosphaera*

All species described in *Spirosphaera* are included. Some species described in *Spirosphaera* do not conform to the current morphological concept of the genus; these are marked with inverted commas; if available, the appropriate form-genus is given in brackets. As *Spirosphaera* and *Clathrosporium* Hennebert in the strict (morphological) sense are already polyphyletic (Voglmayr, unpubl. data), no formal new combinations are proposed for these deviating species.

1. Conidia with distinctly verrucose conidial filament 2
1. Conidia with smooth conidial filament 3

2. Conidia white to pale yellow, keratinophilous, terrestrial ..... ***Spirosphaera keratinophila*** Udagawa & Uchiy**.**
2. Conidia olive to blackish green, on submerged plant litter; conidial filament with consistently dichotomous, opposite branching (daughter filaments per cell usually two) ..... ‘***Spirosphaera*’ (*Clathrosporium*) *verruculosa*** Abdullah, Y. Horie & Udagawa

3. Conidia pure white when young, glistening in mass 4
3. Conidia light to dark fuscous, with reddish or greenish tinges 7

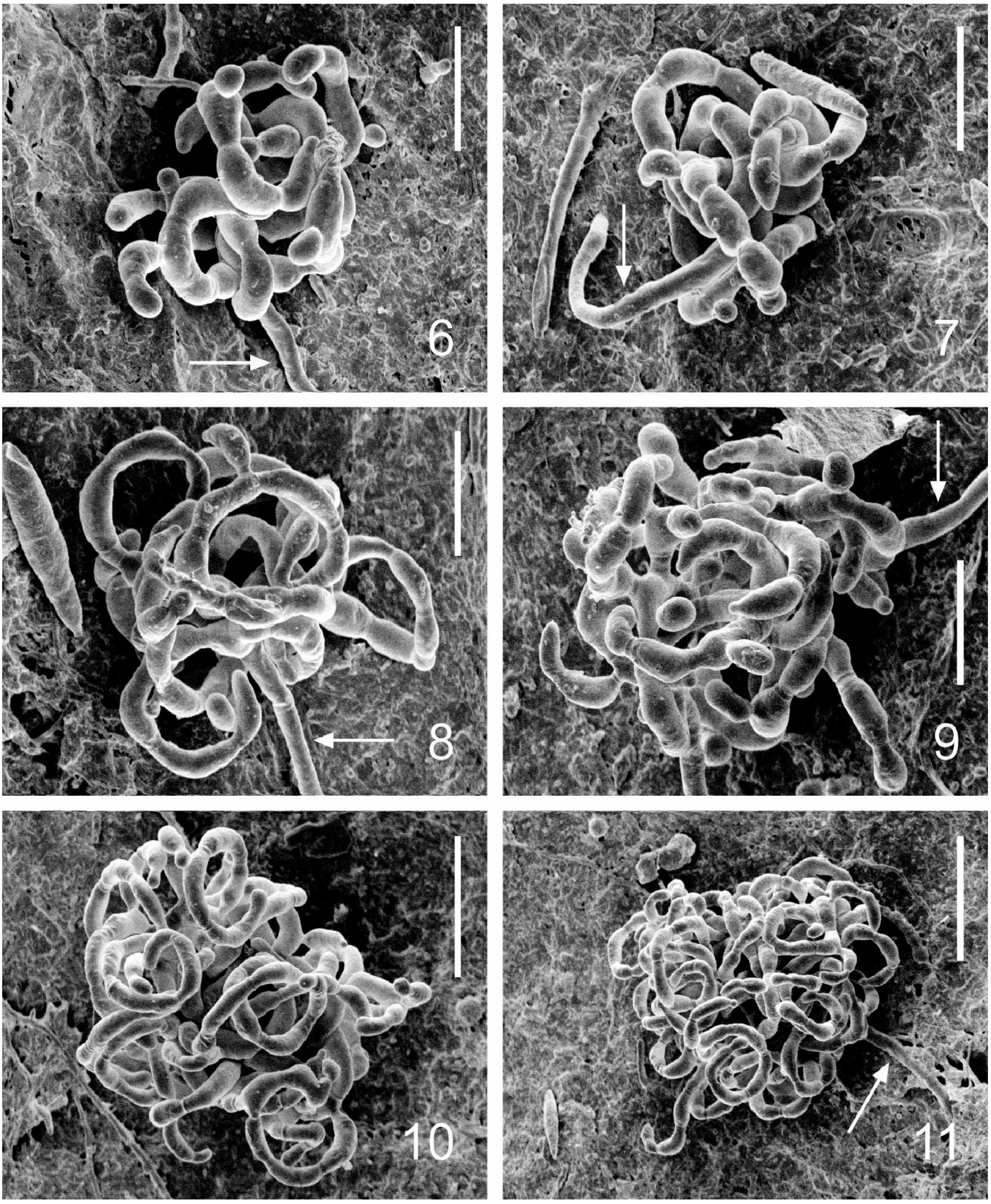
4. Conidial filament with consistently dichotomous, opposite branching (daughter filaments per cell usually two) ..... ‘***Spirosphaera*’ (*Clathrosporium*) *carici-graminis*** Voglmayr
4. Conidial filament usually with only one lateral branch (daughter filament) 5

5. Conidial filament 3.5–5.5 µm diam, sometimes becoming light fuscous at maturity; conidia 50–150 µm in diam. ..... ***Spirosphaera floriformis***
5. Conidial filament up to 2 µm diam, always hyaline; conidia 15–40 µm diam 6

6. Cultures with *Lambdasporium* synanamorph; branching of conidial filament pseudodichotomous; rare ..... ***Spirosphaera dimorpha*** Marvanová & Bärl.
6. Cultures without *Lambdasporium* synanamorph; branching truly lateral; very common ..... ***Spirosphaera minuta*** Hennebert

7. Conidial filament regularly coiled, not to slightly constricted at the septa, conidia of irregular shape ..... ***Spirosphaera beverwijkiana*** Hennebert
7. Conidial filament distinctly constricted at the septa 8

8. Branching of the conidial filament truly unilateral, distinctly spirally coiling; conidia coppery red-brown.....***Spirosphaera cupreorufescens***
8. Branching of the conidial filament dichotomous; conidial filaments straight to slightly bent but not spirally coiling..... '***Spirosphaera' lignicola*** Abdullah, Gené & Guarro



Figs 6–11. SEM of conidia of *Spirosphaera cupreorufescens* (type). Arrows denote conidiophores. 6, 7. Young conidia. 8, 9. Premature conidia. 10, 11. Mature conidia. Note the loosely intertwining spiral conidial filaments, the long cells of the conidial filaments and the irregular appearance of the mature conidia. Scale bars: 6–9 = 20 µm; 10 = 30 µm; 11 = 40 µm.

DISCUSSION

Spirosphaera cupreorufescens shows the features considered typical for the genus: a spirally coiled, interwoven conidial filament, the cells of which give rise to one daughter filament which is also coiled and interwoven, resulting in a large, irregular, globose conidium (Hennebert 1998). However, very rarely also opposite branching of the conidial filament has been observed, resulting in two daughter filaments. Consistent opposite branching is considered characteristic for the genus *Clathrosporium*, the conidia of which are otherwise similar to *Spirosphaera* (Hennebert 1998).

The main distinctive features of *Spirosphaera cupreorufescens* comprise the conspicuous copper-brown conidia, which are rather irregular and loose. The conidial filament is only loosely coiled and shows comparatively rare branching, and usually only every 3rd to 5th cell gives rise to a daughter filament. Therefore, under the dissecting microscope the conidia are quite similar to the conidial heaps of some *Helicodendron* Peyronel species (e.g. *H. multicatenulatum* Beverw., *H. praetermissum* Voglmayr). However, under the compound microscope, except for the earliest stages (Fig. 3), conidia of *S. cupreorufescens* have no resemblance to *Helicodendron* at all.

From the phylogenetic analysis of the nLSU rDNA (Fig. 1) it becomes evident that *Spirosphaera cupreorufescens* is not closely related to the type species of the genus, *S. floriformis*. In addition, it also has no affinity to the type species of *Clathrosporium*, *C. intricatum*. However, it should be noted that in the present publication *C. intricatum* is used in the sense of Hennebert (1998). Unfortunately, no type culture is available for *C. intricatum*, and thorough studies of the holotype (slide IMI 312389) revealed some substantial morphological differences between the type from tropical Malaysia on the one hand, and specimens of the present study and of Hennebert (1998) on the other hand, which originate from temperate regions (Austria, Canada, Italy). Therefore, the culture used in the present study may not be conspecific with *C. intricatum* but could represent a yet undescribed species. However, also the type specimen of *C. intricatum* is morphologically substantially different from *Spirosphaera cupreorufescens*. Preliminary data from the ITS region of some other *Spirosphaera* and *Clathrosporium* species indicate that both genera *Spirosphaera* and *Clathrosporium* in their present conception are highly polyphyletic and have to be considered as pure (morphological) form genera, and no other species of the two genera shows close affinities to *Spirosphaera cupreorufescens* (Voglmayr, unpubl.). This is not surprising as the features characteristic for the genera (coiling and characteristic branching, interwoven conidial filaments producing spherical propagules) are rather simple, resulting in parallel aquisition of these structures in several ascomycete lineages as an adaptation to the aeroaquatic niche. Whereas polyphyly of the genus *Spirosphaera* is quite undesirable, the paucity of features at the moment precludes the erection of new anamorph genera. Therefore, it seems best to describe the new species in a morphologically suitable (but already polyphyletic!) genus *Spirosphaera*. Consequently, in the present publication the current concepts of the form genera *Spirosphaera* and *Clathrosporium* are maintained to avoid unnecessary nomenclatural complications.

In the phylogenetic analysis of the LSU rDNA (Fig. 1), *Spirosphaera cupreorufescens* is unequivocally placed within the *Dothideomycetidae*. Interestingly, *Spirosphaera cupreorufescens* is closely related to *Anguillospora longissima*, a common aquatic hyphomycete. According to Willoughby & Archer (1973), the teleomorph of *Anguillospora longissima* is an undescribed species of *Massarina* Sacc. (*Lophiostomataceae*). In addition, for *Tumularia aquatica* (Ingold) Descals & Marv., which is also distantly related to *S. cupreorufescens* according to the ITS data (Fig. 2), Webster (1965) described *Massarina aquatica* J. Webster as teleomorph. However, *Massarina* has been recently shown to be polyphyletic (Liew *et al*. 2002), and the generic attribution of the teleomorphs mentioned above needs further studies. This is evident from the highly supported sister group relationship of *Leptosphaeria contecta* to the *S. cupreorufescens*/*A. longissima* clade. Further thorough analyses are necessary within these teleomorph genera before affiliation to a teleomorph genus can be presumed for *S. cupreorufescens*.

Ecologically, *S. cupreorufescens* apparently is confined to oligotrophic ditches and small puddles in wooded bogs and mires. However, it is absent from extremely oligotrophic habitats with very low pH, and its habitats always show some minerotrophic influences. Up to date, it has only been found on submerged leaves of *Alnus glutinosa* or *Salix* spp. Despite many bogs have been investigated within ten years by the author, the species has been found only at few sites which indicates that it is a rare species, but the records from Austria and Norway demonstrate that it should be widely distributed at least in temperate to boreal Europe.

ACKNOWLEDGEMENTS

Cordial thanks are due to Dr Walter Till for handling herbarium loans and to the herbarium curators of CBS, IMI and MUCL for sending herbarium specimens.

REFERENCES

Abdullah SK, Gené J, Guarro J (1998). New and interesting aero-aquatic mitosporic fungi from Italy. *Mycotaxon* **66**: 267–272.

Abdullah SK, Horie Y, Udagawa S (1986). New or interesting aero-aquatic conidial fungi from Japan. *Nova Hedwigia* **43**: 507–513.

Beverwijk A van (1951). Zalewski's '*Clathrosphaera spirifera*'. *Transactions of the British Mycological Society* **34**: 280–290.

Beverwijk A van (1953). Helicosporous hyphomycetes. I. *Transactions of the British Mycological Society* **36**: 111–124.

Halbritter H (1998). Preparing living pollen material for scanning electron microscopy using 2,2-Dimethoxypropane (DMP) and critical-point drying. *Biotechnic & Histochemistry* **73**: 137–143.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Hennebert GL (1968). New species of *Spirosphaera*. *Transactions of the British Mycological Society* **51**: 13–24.

Hennebert GL (1998). New species of the aeroaquatic hyphomycete genus *Clathrosporium* and their relationship with *Strumella* Sacc. *Canadian Journal of Botany* **76**: 1596–1607.

Huelsenbeck JP, Larget B, Miller RE, Ronquis, F (2002). Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* **51**: 673–688.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Larget B, Simon DL (1999). Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16**: 750–759.

Liew ECG, Aptroot A, Hyde KD (2002). An evaluation of the monophyly of *Massarina* based on ribosomal DNA sequences. *Mycologia* **94**: 803–813.

Marvanová L, Bärlocher F (1998). Hyphomycetes from Canadian streams. IV. *Spirosphaera dimorpha* sp. nov. *Mycotaxon* **68**: 33–40.

Mau B, Newton MA, Larget B (1999). Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* **55**: 1–12.

Moncalvo JM, Wang HH, Hseu RS (1995). Phylogenetic relationships in *Ganoderma* inferrred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* **87**: 223–238.

Riethmüller A, Voglmayr H, Göker M, Weiß M, Oberwinkler F (2002). Phylogenetic relationships of the downy mildews (*Peronosporales*) and related groups based on nuclear large subunit ribosomal DNA sequences. *Mycologia* **94**: 834–849.

Swofford DL (2002). *PAUP*: Phylogenetic analysis using parsimony* (**and other methods*). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM (1996). Phylogenetic inference. In: *Molecular systematics* (Hillis DM, Moritz C., Mable BK, eds). Sinauer Associates, Mass., U.S.A.: 407–514.

Tehler A, Little DP, Farris JS (2003). The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi. *Mycological Research* **107**: 901–916.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.

Udagawa S, Uchiyama S (1998). Three new hyphomycetes isolated from soil and feather debris. *Canadian Journal of Botany* **76**: 1637–1646.

Voglmayr H (1997). *Helicodendron praetermissum* sp. nov. and *Spirosphaera carici-graminis* sp. nov., aero-aquatic fungi on monocotyledonous debris. *Canadian Journal of Botany* **75**: 1772–1777.

Voglmayr H, Krisai-Greilhuber I (1996). *Dicranophora fulva*, a rare mucoraceous fungus growing on boletes. *Mycological Research* **100**: 583–590.

Webster J (1965). The perfect state of *Pyricularia aquatica*. *Transactions of the British Mycological Society* **48**: 449–452.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A guide to methods and applications* (Innis MA, Gelfand DA, Sninsky JJ, White TJ, eds). Academic Press, San Diego, CA, U.S.A.: 315–322.

Willoughby LG, Archer JF (1973). The fungal spora of a freshwater stream and its colonization pattern on wood. *Freshwater Biology* **3**: 219–239.