The Lichenologist **42**(3): 291–296 (2010) © British Lichen Society, 2010
doi:10.1017/S0024282909990715

Acarospora rosulata in Europe, North America and Asia

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Abstract: *Acarospora rosulata* is revised and reported from North America and Mongolia. *Acarospora bullata* is not verified as occurring in South and North America and may be conspecific with *A. rugulosa.*

Keywords: *Acarosporaceae*, ITS nrDNA, lichens, taxonomy

Introduction

Acarospora is a large and cosmopolitan genus erected by Massalongo (1852) that contains over 200 species and is not monophyletic (Reeb *et al.* 2004; Crewe *et al.* 2006). The type species of the genus is the yellow, terricolous *Acarospora schleicheri* (Ach.) A. Massal. The genus is characterized by polyspory (50 or more ascospores per ascus), simple hyaline ascospores, usually aspicilioid or pseudo-lecanorine apothecia, and bitunicate but non-fissitunicate asci with a non-amyloid tholus (Knudsen 2007). *Acarospora* species are areolate to squamulose, brown or yellow, sometimes pruinose or effigurate, saxicolous or terricolous, incidentally and rarely lignicolous, with a small number of species lichenicolous with a parasitic juvenile phase (Magnusson 1929; Clauzade & Roux 1981; Knudsen 2007).

Many species, including several yellow taxa, have a simple chemistry of gyrophoric acid usually with lecanoric acid (Knudsen 2007). This is best studied under the microscope with the application of KOH followed by C, to mounted thin sections which will produce a pinkish-red reaction. Rarely is thin-layer chromatography necessary, but some species such as *Acarospora nicolai* B. de Lesd. often have very low concentrations of gyrophoric and lecanoric acid (Knudsen & Morse 2009).

Acarospora bullata Anzi is a brown species with a usually effigurate margin and contains gyrophoric acid. In our continuing studies of the genus *Acarospora* it became apparent that there were possible problems of heterogeneity with Magnusson's concept of *A. bullata* which included specimens from Asia, Europe and California (Magnusson 1929). It was also apparent that using an even broader concept of *A. bullata* that included South American specimens was unsatisfactory (Knudsen *et al.* 2008). Molecular phylogenetic analysis to verify heterogeneity was therefore an appropriate tool in this case. We sequenced selected specimens identified as *A. bullata* from Asia, Europe, North and South America and used sequences of *A. rugulosa* Körb (Crewe *et al.* 2006) for phylogenetic reconstruction and established these species as heterogenic.

Acarospora rosulata (Th. Fr.) H. Magn. is an effigurate to non-effigurate brown species with gyrophoric acid known previously only from a small area of Norway (Magnusson 1929). We discovered that specimens identified as *A. bullata* from

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TABLE 1. *Specimens of* Acarospora *sequenced in this study.*

Species	Abbreviation	Collection data (herbarium)	GenBank Accession number
A. bullata	ACABUL Aus1	Austria, *Obermayer* 1987-09-09 (GZU 1-90)	GU184108
A. bullata	ACABUL Ger1	Germany, *Huneck-Poelt* 21 v 1993 (GZU)	GU184110
A. bullata	ACABUL Ger2	Germany, *Sipman* 36213 (B60 0098080)	GU184111
A. bullata	ACABUL Ger3	Germany, *Huneck* 14 iv 1991 (60 0118983)	GU184112
A. bullata	ACABUL Ita1	Italy, *Reeb* and *Roux* VR 8-VII-98/6 (DUKE)	GU184114
A. bullata	ACABUL Ira1	Iran, *Maassoumi Safavi* 1850 (B 60 0133511)	GU184113
A. rosulata	ACABUL Mon1	Mongolia, *Reeb* and *Zavarzin* VR 17-VII-04/1 (DUKE)	GU184115
A. rosulata	ACABUL Mon2	Mongolia, *Reeb* and *Zavarzin* VR 30-VII-04/4 (DUKE)	GU184122
A. rosulata	ACABUL USA2	USA, California, *Knudsen* 929 (UCR)	GU184116
A. cf. *bullata*	ACABUL Bol1	Bolivia, *Flakus* 9547 (KRAM)	GU184109
A. rosulata	SAR31ua	USA, *Knudsen* 9509 (S)	GU184117
A. rosulata	SAR34	Norway, *Westberg* 08-193 (S)	GU184118
A. cf. *dissipata*	ACAYEL	USA, *Reeb* VR 12-X-97 St4.1 (DUKE)	GU184119
A. basidiofusca var. *basidiofusca*	ACABAD	France, *Reeb* and *Roux* VR 9-VII-98/11 (DUKE)	GU184120
A. basidiofusca	ACABAD2	Spain, *Reeb* and *Roux* VR 2-IX-00/ 23 (DUKE)	GU184121
A. macrospora ssp. *macrospora*	ACAMAC	Norway, *Timdal* 3186, 1982.04.03 (O, Lichens 33416)	GU184123
A. cervina	ACACER	France, *Reeb* and *Roux* VR 6-VII-98/11 (DUKE)	GU184124

western North America and Mongolia were conspecific with *A. rosulata*. *Acarospora rosulata* is revised in this paper.

Materials and Methods

Microscopy

Specimens were studied using standard microscopy and manually prepared sections. Measurements were made on material mounted in water. Specimens of *A. bullata, A. hellbomii, A. mendozana, A. montana, A. rosulata, A. rugulosa* and *A. subcastanea* were examined from B, DUKE, FH, GZU, KRAM, MSC, NY, S, UCR and UPS.

DNA preparation and sequencing

A total of 11 specimens identified as *A. bullata* from seven different countries (Austria, Bolivia, Germany, Iran, Italy, Mongolia, and USA) and one specimen of *A. rosulata* from Norway were sequenced over the internal transcribed spacer of the nuclear ribosomal DNA (ITS nrDNA), as well as five other specimens of *Acarospora* as outgroup (Table 1).

Lichen tissues were frozen in liquid nitrogen and thawed three times, prior to total genomic DNA extraction using the DNeasy kit (Qiagen, Santa Clarita, CA, USA) following the manufacturer's protocol for plant tissue. Amplification of the *c.* 600-bp ITS nrDNA was performed using the primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) and the Herculase® II Fusion DNA polymerase from Stratagene (Cat. #600677). PCR conditions were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 40 seconds, 52°C for 30 seconds, and 72°C for one and a half minutes, and a final extension period at 72°C for 5 minutes. PCR fragments were directly sequenced using the PCR primers and the BigDye™ terminator kit (PE-Applied Biosystems, Norwalk, CT, USA), then run on an ABI 3730 automated DNA sequencer at the Roy J. Carver Center for Comparative Genomics at the University of Iowa.

Phylogenetic analyses

Phylogenetic analyses were carried out on a 27-taxon data set including sequences generated from this study and sequences obtained from the NCBI public database (Fig. 1). The outgroup was determined using a broad phylogeny of 93 taxa for the *Acarosporaceae* (unpublished data). ITS sequences were aligned under MacClade v4.07 (Maddison & Maddison 2005). Ambiguously aligned regions that likely violate positional homology were assessed manually and excluded from further analysis. The model of sequence evolution for

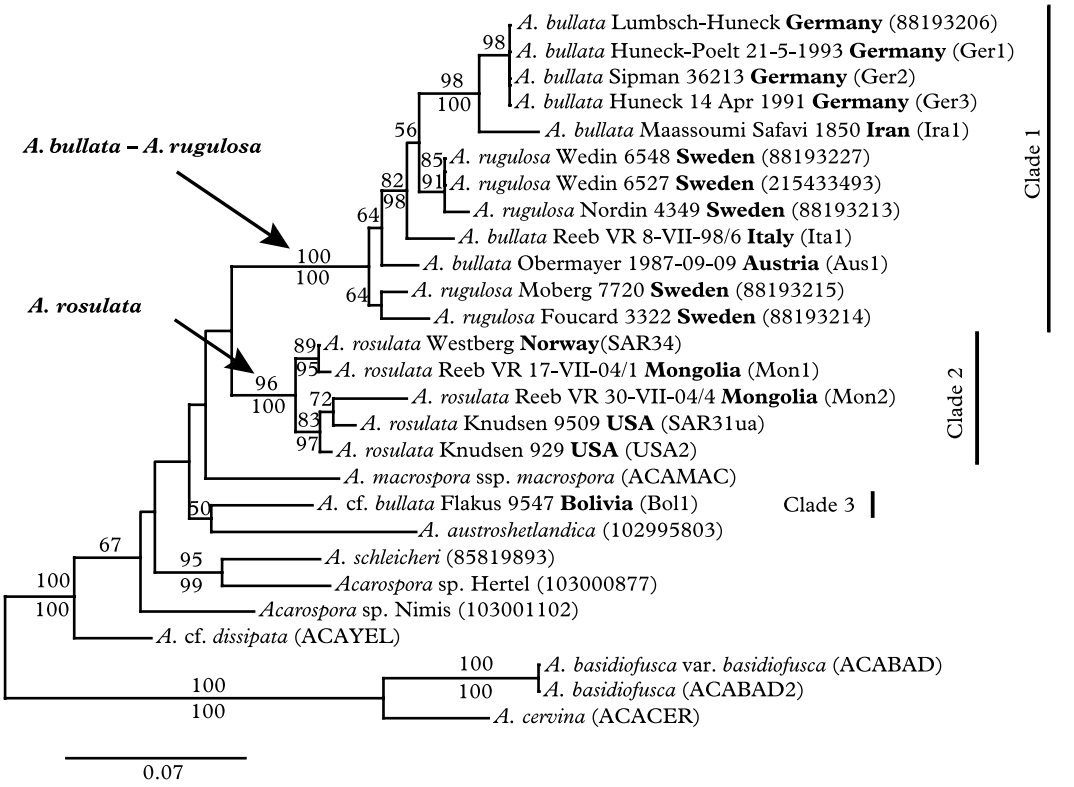

FIGURE. 1. Phylogeny of 27 *Acarospora* specimens inferred from maximum likelihood (RAxML) analysis of the internal transcribed spacer of the nuclear ribosomal DNA (ITS nrDNA). The phylogram shows the RAxML tree with the highest likelihood. Numbers above the branches are RAxML bootstrap proportions when ≥ 50%. Numbers below the branches indicate Bayesian posterior probabilities ≥ 95%. Numbers in parentheses correspond to NCBI GI numbers. Other terms in parentheses correspond to abreviations used for molecular work.

the 511 sites ITS dataset was determined using Modeltest 3.7 (Posada & Crandall 1998). Bayesian posterior probabilities (PP) were computed under the GTR + I + Γ model using the parallel version of MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). Two times four MCMCMC chains were run simultaneously for 2 million generations sampling trees every 100 generations. Stationarity in likelihood scores was determined by plotting the tree -lnL values against generations using Tracer v1.3 (Rambaut & Drummond 2005). All trees below the stationarity level were discarded (i.e., as 'burnin'). A majority rule consensus tree was generated from the post-burnin trees. A maximum likelihood phylogeny was also inferred using RAxML v 7.0.4 (Stamatakis *et al.* 2008) on the Cyber infrastructure for Phylogenetic Research (CIPRES) portal v 1.13 at http://www.phylo.org/. RAxML bootstrap analysis was done for 100 iterations using an estimated proportion of invariable sites. A majority rule consensus tree was calculated to determine the bootstrap proportion (BP) for each node.

Results

Based on the molecular sequence data, the specimens identified as *Acarospora bullata* included in this study are distributed among three distinct clades (Fig. 1). Clade no. 1 (BP = 100% and PP = 100%) includes *A. bullata* from Europe (Austria, Germany, and Italy) and Iran, intermixed with *A. rugulosa* from Sweden (Crewe *et al.* 2006). Clade no. 2 is also well supported (BP = 96% and PP = 100%), including specimens identified originally as *A. bullata* from the US and Mongolia, as well as *A. rosulata* from Norway. Finally, in Clade no. 3, a specimen identified as *A. bullata* from Bolivia forms a sister relationship with *A. austroshetlandica*, but without support. Only the Clades nos 1

https://doi.org/10.1017/S0024282909990715 Published online by Cambridge University Press

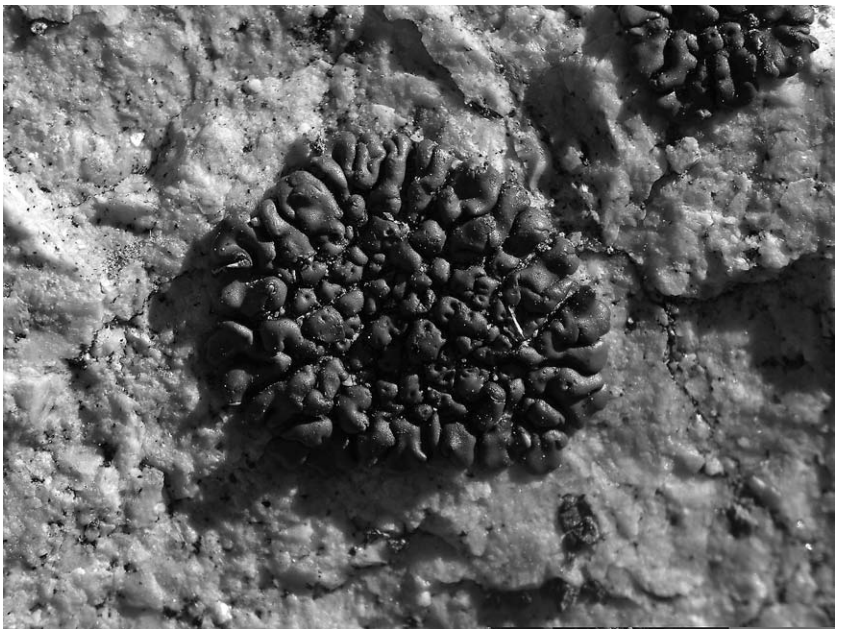

FIG. 2. *Acarospora rosulata* on granite, Eureka Peak, Joshua Tree National Park, California.

and 2 form monophyletic groups (without support), whereas the exact position of *A.* cf. *bullata* from Bolivia remains to be determined.

The Species

Acarospora rosulata (Th. Fr.) H. Magn.

Monogr. Scand. Acar. **28:** 121 (1924); type: Norway, Oppland, Vågå, Th. Fries, 1863 (UPS!—holotype)

Basionym: *Acarospora fuscata discreta* f. *rosulata* Th. Fr., *Lich. Scand.* **1:** 218 (1871).

(Fig. 2)

Thallus areolate, contiguous to dispersed, forming indeterminate to determinate patches to 7 cm, often confluent. *Areoles* round to angular, up to 3 mm diam. and 1·5 mm thick, broadly attached (over half of diameter), becoming subsquamulose, the mycelial base thickening, not forming a stipe, elevating the areoles (becoming gomphate), the areoles often lobulate, especially on the margin of thallus but also in the centre, the lobes generally round, 2 to 3 per areole, usually less than 1 mm long and less than 1 mm wide. Surface pale yellow brown to dark brown, usually shiny, smooth, epruinose. Upper and lateral cortices paraplectenchymatous to subparaplectenchymatous, 30–80 µm thick, cells 3–6 × 2·5–6 µm, globose to elongate, syncortex (*sensu* Knudsen 2007) 4–30 µm thick rarely with visible periclinal hyphae; eucortex with reddish brown upper layer to 20 µm thick, and thicker hyaline lower layer. Lower surface usually ecorticate and white or rarely brown. *Photobiont* chlorococcoid green alga up to 10 µm diam., forming a continuous stratum 60–100 µm thick, the algal cells sometimes arranged in distinct vertical columns, often thinning beneath apothecia. Medulla prosoplectenchymatous, up to 600 µm thick, continuous with attaching hyphae, hyaline, mostly 3–4 µm diam., cells mostly 4–5 µm long.

Apothecia one to many per areole, immersed and punctiform, sometimes expanding to 1 mm diam., usually remaining immersed, occurring also on marginal areoles, rarely with a thin parathecial ring visible

around the disc or elevated with thalline margin. *Disc* dark brown, epruinose, rough, round to irregular, rarely with umbo of sterile plectenchyma, sometimes reddish when wet. True exciple of radiating hyphae, 20–40 µm, expanding slightly around the surface of the disc, rarely forming parathecial crown. *Hymenium* 80–120 µm tall, epihymenium dark brown to reddish brown with diffused pigment, conglutinated, 10–20 µm thick; *paraphyses* lax in water, 1·5–2·5 µm diam., septate, sometimes with oil drops, apices often expanded, 2·5 – 4·0 µm wide, usually in brown pigmented caps. Subhymenium to 50 µm thick. *Hypothecium* 15–20 µm thick. *Asci* clavate, 60–100 × 15–28 µm, ascospores 100–200 per asci. *Ascospores* simple, hyaline, mostly 4–5 × 1·5–2 µm.

Chemistry. Spot tests: cortex, C+, KC+ pinkish red (best seen in mount); gyrophoric acid.

Ecology and substratum. On granite and siliceous rocks, schist, sandstone, rarely on limestone or calcareous rocks.

Distribution. Europe (Oppland, Norway), western North America and Asia (Mongolia).

Discussion. The thallus of *Acarospora rosulata* can be determinate, especially in hot arid locations in the desert or on smooth hard rock surfaces, but the outer and inner areoles can be lobulate and its areoles regularly become elevated and subsquamulose. The surface is shiny and always smooth.

Acarospora rosulata was recognized as a species by Magnusson from a small area of Norway in Oppland. Specimens of *A. rosulata* from California were included in *A. bullata* by Magnusson (1929) and Knudsen (2007). We do not recognize *A. bullata* as occurring in North America.

The discs of Mongolian specimens were very rough owing to an uneven build-up of conglutinated pigment and could, superficially, be mistaken for the rugulose discs of *Acarospora bullata*. Specimens from Norway often produce many apothecia on a single areole, which subsequently begins the process of vegetative division into separate areoles. Western North American specimens often produce the largest areoles usually with a single apothecium.

Selected specimens examined. **Mongolia:** Khovad, Altai Mountain, west of Ulaantology, 46°54′32.0″ N 92°15′12.9″ E, 2098 m, 2004, *Reeb* [VR 17-VII-04/1] & *Zavarzin* (DUKE); Töv, Gokhi-Terelj Nature Reserve beside a river, 47°50′08.3″ N 107°28′42.6″ E, 1420 m, on cliff, 2004 *Reeb* [VR 30-VII-04/4] & *Zavarzin* (DUKE).—**Norway:** *Oppland*: Lom Parish, På muren vid kyrkan, 1952, *Per-Olaf Lindahl* (UPS); [*Krypt Exs*. No. 2867] *Magnusson* (S); *ibid*., 1948 *Ahlner* (S); *ibid.*, 2008, *Westberg* 08-156 (S); Vågå Parish, 5·5 km WSW of Vågå, 61° 52′ N, 7°99′ E, 580 m, 1985, *Tibell* 15834 (UPS); *ibid*., 2008, *Westberg* 08-192, 08-193, 08-238 (S).—**USA:** *California*: Inyo County, Little Lake Station, 1911, 2 specimens, *Hasse* (FH); near Bishop along US 395, 5400 ft, 1966, *Wetmore* 14871 (FH); Orange County, Santa Ana Mountains, Fremont Canyon, 33° 48′ 05″N, 117° 41′ 59″W, 387 m, on sandstone, 2007, *Knudsen* 9358 (UCR); Riverside County, east side of San Jacinto Mountains, Pinon Flats area, 33° 34′ 35″N, 116° 29′ 14″W, 1276 m, in full sun on granite, 2004, *Knudsen* 929 (NY, UCR); off Burnt Canyon Road, 33° 34′ 00″N, 116° 36′ 07″W, 1415 m, on granite boulder, 2008, *Knudsen* 9509 *w/ Lendemer* (S, UCR); San Bernardino County, east side of San Bernardino Mountains, Cactus Flats, 34° 18′ 22″N, 116° 47′ 43″W, 1858 m, on granite and limestone, 2005, *Knudsen* 3351 (UCR). *Colorado*: summit between Engineer & Gravel Mts., 1954, *Imshaug* 17082 (MSC). *Utah*: Sevier Co, 5 miles east of Salina, 1957, *Shushan* 14848 (FH); San Juna Co., La Sal Mts., summit of Mt. Mellenthin, 12890 ft., 1954 *Imshaug* 16899 (MSC).

Conclusion

Our study, while revising *A. rosulata*, does not revise *A. bullata* and *A. rugulosa* in Europe and Asia. The types of *A. rugulosa* could not be found at Leiden (G. Thijsse, pers comm.). The German specimens of *A. bullata* have rugulose thalli, the types of *A. bullata* are smooth and are similar to determinate and indeterminate specimens identified as *A. rugulosa* in Fennoscandia. Specimens in the *bullata-rugulosa* Clade no. 1 identified as *A. rugulosa* (Crewe *et al.* 2006) have indeterminate thalli with no lobes or rudimentary lobes and are apparently conspecific with Magnusson's concepts of *A. montana* H. Magn. and *A. hellbomii* H. Magn. (Magnusson 1929). The only characters uniting all specimens we studied from Europe were a rugulose disc, usually with a

parathecial crown and a distinct thalline margin and thin paraphyses, mostly 1 µm wide, not wider than 2 µm, usually branching, lax in water, apices not expanded or slightly expanded to 2 µm. Multi-gene analysis is needed to verify the results that *A. bullata* and *A. rugulosa* are conspecific and a neotype for *A. rugulosa* needs to be selected.

The first author synonomized *A. subcastanea* (Nyl.) Hue and *A. mendozana* H. Magn. from South America with *A. bullata* (Knudsen 2007) but we do not recognize this synonymy as valid. We think *A. mendozana* is probably synonymous with the older name *A. subcastanea* based on type material and specimens from S and H. Nonetheless we had only one specimen from South America collected by Adam Flakus in Bolivia which we could sequence and which was most similar to specimens identified as *A. mendozana*. This specimen was not related to *A. bullata* or *A. rosulata.* At this time, the Antarctic endemic *A. austroshetlandica* (C.W. Dodge) Øvstedal (Øvstedal & Lewis Smith 2001) appears distinct from the Flakus collection from Bolivia but probably related to the South American taxon. The revision of effigurate or subeffigurate species of brown *Acarospora* with gyrophoric acid in South America needs additional study and more specimens included in molecular phylogenetic analyses.

We would like to thank the curators of B, DUKE, FH, GZU, KRAM, L, MSC, S and UPS for sending specimens. We also thank G. Thijsse (L) for help in trying to locate the types of *A. rugulosa* and R. Muertter for the photograph in Fig. 2. We are grateful for the constructive comments of Christian Printzen and an anonymous reviewer. This research was partly supported by a grant to DB from the National Science Foundation Assembling the Tree of Life program (EF 04-31117).

References

Clauzade, G. & Roux, C. (1981) [1982] Les *Acarospora* de l'Europe occidentale et de la region méditerranéenne. *Bulletin du Musée d'Histoire Naturelle de Marseilles* **41:** 41–93.

Crewe, A. T., Purvis, O. W. & Wedin, M. (2006) Molecular phylogeny of Acarosporaceae (Ascomycota) with focus on the proposed genus *Polysporinopsis*. *Mycological Research* **110:** 521–526.

Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2:** 113–118.

Knudsen, K. (2007) [2008]. *Acarospora*. In *Lichen Flora of the Greater Sonoran Desert Region Vol. 3* (T. H. Nash III, C. Gries & F. Bungartz, eds): 1–38. Tempe: Lichens Unlimited, Arizona State University.

Knudsen, K. & Morse, C. A. (2009) *Acarospora nicolai* (Acarosporaceae), a rediscovered species. *Bryologist* **12:** 147–151.

Knudsen, K., Elix, J. A. & Reeb, V. (2008) A preliminary study of the genus *Acarospora* in South America. *Opuscula Philolichenum* **5:** 1–22.

Maddison, D. R. & Maddison, W. P. (2005) *MacClade 4.05*. Sunderland: Sinauer.

Magnusson, A. H. (1929) A monograph of the genus *Acarospora*. *Kongl. Svenska Vetenskaps Akademiens Handlingar, ser. 3*, **7:** 1–400.

Øvstedal, D. O. & Lewis Smith, R. I. (2001) *Lichens of Antarctica and South Georgia: A Guide to Their Identification and Ecology*. Cambridge: Cambridge University Press.

Posada, D. & Crandall, K. A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Rambaut, A. & Drummond, A. (2005) *Tracer, MCMC Trace Analysis Tool Version 1.3*.

Reeb, V., Lutzoni, F. & Roux, C. (2004) Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Molecular Phylogenetics and Evolution* **32:** 1036–1060.

Ronquist, F. & Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* **57:** 758–771.

White, T. J., Bruns, T. D., Lee, S. B. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. New York: Academic Press.

Accepted for publication 26 *November* 2009