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Lichenicolous fungi of the genus *Abrothallus* (Dothideomycetes: Abrothallales ordo nov.) are sister to the predominantly aquatic Janhulales

Sergio Pérez-Ortega · Ave Suija · Ana Crespo · Asunción de los Ríos

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Abstract This study provides new insights on the phylogenetic position of the lichenicolous fungal genus *Abrothallus* based on six molecular markers (nuSSU, nuLSU, mtSSU, RPB1, RPB2 and TEF-α). In a broad-scale analysis, we detected high support for inclusion of the genus within Dothideomycetes. A further analysis provided support for *Abrothallus* as a member of the subclass Pleosporomycetidae as a sister group of Jahnulales, an order of aquatic Dothideomycetes. Given the exclusive characters of this group of apotheciate fungi within the Dothidiomycetes, a new monotypic order Abrothallales is here introduced together with the new family Abrothallaceae. In a multi-locus analysis (based on the six loci indicated above plus ITS) restricted to 12 putative *Abrothallus* species, two clearly separated clades were observed: one comprising species growing on lichens of the families Parmeliaceae and Ramalinaceae, and the second including species that live on lichens of the order Peltigerales and the family Cladoniaceae.

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S. Pérez-Ortega (✉) · A. de los Ríos
Departamento Biología Ambiental, Museo Nacional de Ciencias Naturales (CSIC), c/Serrano 115 dpdo, 28006 Madrid, Spain
e-mail: sperezortega@mncn.csic.es

A. de los Ríos
e-mail: arios@mncn.csic.es

A. Suija
Institute of Ecology and Earth Sciences, University of Tartu, 40 Lai str., 51005 Tartu, Estonia
e-mail: ave.suija@ut.ee

A. Crespo
Facultad de Farmacia, Departamento Biología Vegetal II, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain
e-mail: acrespo@farm.ucm.es

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Introduction

Although many fungal species are known to live on lichen thalli (Lawrey and Diederich 2003; Arnold et al. 2009; Peršoh and Rambold 2012; U'Ren et al. 2012), only those that show recognizable sexual or asexual reproductive structures are denoted lichenicolous fungi. The heterogeneity of lichenicolous fungi is manifested through both their phylogenetic diversity and their life-strategies, ranging from aggressive parasitism to commensalism and saprophytism including several intermediate modes of life (Lawrey and Diederich 2003). Despite varying interest for lichenicolous fungi over the past two centuries, the introduction of new research techniques has prompted several studies designed to revisit these much-specialized enigmatic fungi (e.g. the comprehensive review by Lawrey and Diederich 2003). Within the past 10 years, the number of studies based on molecular methods has constantly grown and this has provided new insight into the origin of their life-strategies, speciation and population patterns, and host-specificity (e.g. Sikaroodi et al. 2001; Peršoh and Rambold 2002; Millanes et al. 2011; Lawrey et al. 2012; Werth et al. 2013).

Despite intensified research efforts, phylogenetic relationships remain obscure for numerous lichenicolous genera. One such case is the genus *Abrothallus*, which is one of the most easily recognizable fungal genera that exclusively grows on lichens (Fig. 1). The genus was introduced to accommodate the single species *A. bertianus* by De Notaris (1845), and although this species was first described as lichenized, its lichenicolous nature was soon to be established (De Notaris 1846; Tulasne 1852; Montagne 1856). The genus is well-characterized by its: (1) more-or-less globose apothecioid ascomata, often covered with green or golden pruina, (2) bitunicate asci with four to eight, 2- to

4-celled brown asymmetric ascospores with evident ornamentation, (3) ramified-anastomosed interascal filaments, (4) epiphymenial layer with granulose pigments, which commonly dissolve in potassium hydroxide, and (5) its pycnidial *Vouauxiomyces*-type anamorph (Diederich 2004; Suija 2006; Pérez-Ortega et al. 2011). Reports of the genus have spanned all continents except Antarctica, and representatives of the genus have been described to grow on a wide variety of foliose and fruticose lichens especially those belonging to the families Parmeliaceae and Lobariaceae, but also on species of Nephromataceae, Stereocaulaceae, Cladoniaceae, Ramalinaceae and Pannariaceae.

The genus *Abrothallus* shows no clear similarity to any other ascomycete genus, prompting several hypotheses about its phylogenetic relationships. Before its parasitic nature was discovered (De Notaris 1846; Tulasne 1852; Montagne 1856), the genus was classified together with lichens as a member of tribe Coccocarpiae of the order Gymnocarpi (Montagne 1851) or suborder Biatorinae of Lecideaceae (Körber 1855; Lindsay 1857). The subsequent authors ascribed it to the order Bulgariacei (= Bulgariaceae, Helotiales) (Montagne 1856) or suborder Phacidiae (= Phacidiaceae, Helotiales) (Saccardo 1889) based on its apparent lack of apothecial margin. Rehm (1896) considered it a member of the Patellariaceae (then Dermatales), a designation supported by several investigators (e.g. Lindau 1897; Vouaux 1913; von Keissler 1929; Kutorga and Hawksworth 1997). However, based on detailed ultrastructural studies, Bellemère et al. (1986) suggested its affinity to Arthoniales, as proposed earlier by Jatta (1911), who included the genus in the tribe Arthoniae, and later followed by Nannfeldt (1932). Fink (1935), who synonymized *Abrothallus* with *Buelliella*, proposed its relationship with Buelliaceae due to its dark-coloured ascospores. None of these hypotheses has been satisfactory addressed using molecular methods, and the status of the genus is currently *Ascomycetes incertae sedis* (Lumbsch and Huhndorf 2010). In preliminary work by Granberg (2001), the phylogenetic adscription of *Abrothallus* was tested using ribosomal DNA markers (nuSSU, nuLSU), yet results were inconclusive.

The intra-generic taxonomy of the genus *Abrothallus* has also been a topic of controversy because of the different weight given to characters such as host-specificity or the reaction of somatic hyphae with Lugol's solution. Hence, the existence of both broad (Lindsay 1857; von Keissler 1929; Hawksworth 1983) and narrow definitions (Kotte 1909; Clauzade et al. 1989; Santesson et al. 2004) has lead to the recognition of certain taxa only by some authors. For instance, Suija (2006) showed that the colour of the crystalline layer above the hymenium, the pruinosity of the ascomata and Lugol's solution reacting somatic hyphae are the most reliable characters for species recognition among 2-celled species of the genus.

This study was designed to resolve the phylogenetic relationships of *Abrothallus* within the Ascomycota through multi-locus analysis. Once this had been established, we addressed relationships within the genus by examining seven loci.

Fig. 1 Characteristic morphological and anatomical features of the lichenicolous genus *Abrothallus*. **a** Greenish pruina on the apothecia of *A. secedens*; **b** Convex apothecia of *A. acetabuli*; **c** Cross-section of the apothecium of *A. acetabuli*; **d** Scanning electron microscope image of a cross-section of *A. parmeliarum* growing on *Parmelia saxatilis*; **e** Paraphyses of *A. parmeliarum* in phloxin; **f** Mature ascus with mature ascospores; **g** Young ascus; **h** Conidiogenous cells with conidiospores of *A. bertianus*; **i** Ascospore with one septum in *A. bertianus*; **j** Ascospore with three septa in *A. suecicus*; **k** Ascospores divided into two part-spores in *A. secedens*; **l** Surface view of pycnidia of *A. bertianus* on the thallus of *Melanelixia fuliginosa*; **m** Cross-section of a pycnidium of *A. bertianus* observed by scanning electron microscopy; **n** Detail of the rugose surface of ascospores of *A. parmeliarum* observed by scanning electron microscopy; **o** Conidiospores of *A. bertianus*. Scale bars: **a**, **b**, **c**, **d**, **l**: 100 μm; **e**, **f**, **g**, **o**: 10 μm; **h**, **i**, **j**, **k**, **n**: 5 μm

Materials and methods

Taxon sampling and morpho-anatomical characterization

This study was performed on 14 specimens of *Abrothallus* covering its widest possible host range: three specimens growing on Peltigerales (on *Pseudocyphellaria* and *Nephroma*), one specimen on *Cladonia*, two on *Ramalina* and eight on Parmeliaceae (*Parmelinopsis*, *Parmelia*, *Parmelina*, *Parmotrema*, *Pleurosticta* and *Usnea*). Voucher specimens were deposited in TU, NY and MA (Table 1).

Specimens were examined using standard microscopy techniques. For colour tests, we used c. 10 % potassium hydroxide (KOH; K) and c. 50 % nitric acid (HNO_3; N); Lugol's solution (I) was used to examine fungal structure reactions. Morphometric measurements were performed in water and values recorded as the minimum–(average)–maximum. Portions of air-dried thalli were gold sputter-coated and observed by electron microscopy with back-scattered electron imaging (FEI INSPECT) at the facilities of the MNCN (CSIC, Madrid). Nomarski differential interference contrast (DIC) micrographs were captured using a Zeiss® AX10 microscope and Zeiss® AxioCam digital camera.

Molecular methods

Molecular analysis

DNA extraction, amplification and sequencing For DNA extraction, apothecia were removed from the host thallus with the help of a razor blade or forceps and immersed in a sterile water drop. The lower section of each apothecium, including most of the hypothecium, was carefully removed using a razor blade under the dissecting microscope and transferred to a 1.5 ml test

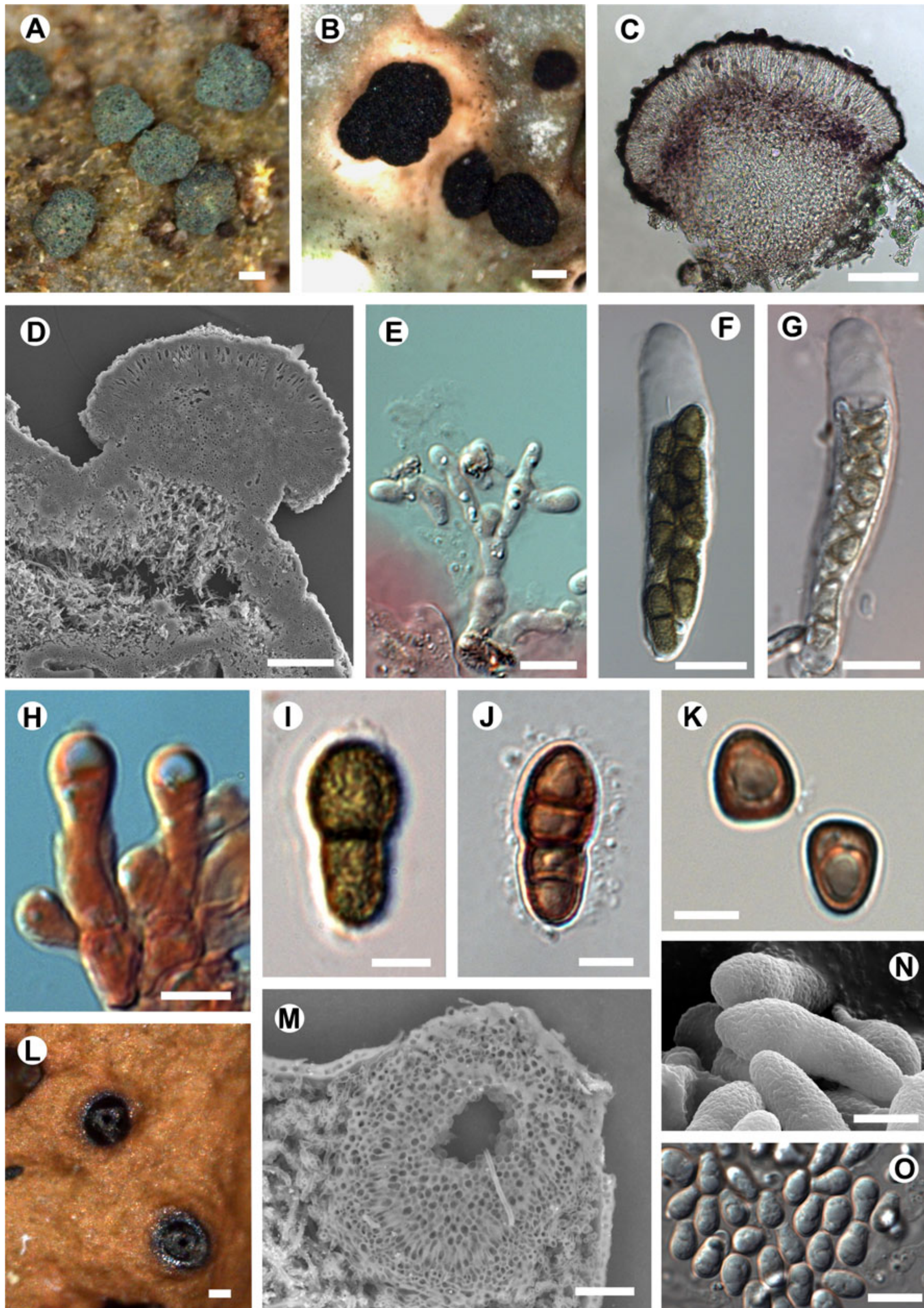
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Table 1 Accession numbers corresponding to the *Abrothallus* specimens sequenced in this study

Species	Origin	Lab Code	Host	ITS	nuLSU	nuSSU	RPB1	RPB2	TEF-α	mtSSU
Abrothallus parmeliarum	Scotland	AB36 (Kukwa 5747a)	*Parmelia saxatilis*	KF816172	KF816229	KF816222	–	KF816201	KF816182	–
Abrothallus parmeliarum	Estonia	AB57 (TU45857)	*Parmelia sulcata*	KF816178	KF816227	KF816221	–	KF816198	KF816180	KF816211
Abrothallus cladoniae	USA	AB53 (NY01041884)	*Cladonia cristatella*	KF816173	KF816228	KF816220	KF816190	KF816200	KF816181	–
Abrothallus nephromatis ad int.	Greenland	AB3 (TU65904)	*Nephroma parile*	KF816175	KF816230	KF816224	–	KF816203	KF816184	KF816213
Abrothallus parmotrematis	Switzerland	AB1 (TU65905)	*Parmotrema sp.*	KF816176	KF816231	KF816225	–	KF816204	KF816185	KF816214
Abrothallus suecicus	Estonia	AB56 (TU45449)	*Ramalina fraxinea*	KF816177	KF816226	–	–	KF816199	KF816179	KF816210
Abrothallus suecicus	Spain	SPO 304	*Ramalina cf. protecta*	KF816171	–	–	–	–	–	–
Abrothallus usneae	Canada	SPO 306	*Usnea lapponica*	KF816170	–	–	–	–	–	–
Abrothallus usneae	Portugal	AB20 (TU45810)	*Usnea rubicunda*	KF816174	–	KF816223	KF816189	KF816202	KF816183	KF816212
Abrothallus hypotrachynae	Portugal	SPO 302	*Parmelinopsis horrescens*	KF816167	KF816233	KF816218	–	KF816196	–	KF816208
Abrothallus buellianus	Spain	SPO 303	*Parmelina tiliacea*	KF816166	KF816234	KF816217	KF816191	KF816195	KF816186	KF816207
Abrothallus secedens	Chile	SPO 305	*Pseudocyphellaria* sp.	KF816169	KF816236	KF816216	–	–	–	KF816206
Abrothallus sp.	Russia	SPO 310	*Nephroma* sp.	KF816168	KF816235	KF816219	KF816193	KF816197	KF816187	KF816209
Abrothallus acetabuli	Spain	SPO 308	*Pleurosticta acetabulum*	KF816165	KF816232	KF816215	KF816192	KF816194	KF816188	KF816205

tube. The number of apothecia used for DNA extraction was 1 to 12. DNA was extracted using the DNEasy Plant Mini Kit (Qiagen®) and High Pure PCR Template Preparation Kit (Roche Applied Science®) following the manufacturer's instructions with minor modifications.

To determine the phylogenetic position of *Abrothallus* and infer relationships among taxa within the genus, we amplified six nuclear genomic regions (ITS, nuLSU, nuSSU, TEF-α, RPB1 and RPB2) and one mitochondrial locus (mtSSU). The following primers were used: ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for ITS; nuSSU97A, nSSU131, nSSU1088r (Kauff and Lutzoni 2002) or NS1 (White et al. 1990) and NS24 (Gargas and Taylor 1992) for nuSSU; LR0R (Rehner and Samuels 1994), LR1R (Moncalvo et al. 1993), LR3r, LR7 and LR5 (Vilgalys and Hester 1990) for nuLSU; RPB1-Af (Stiller and Hall 1997) and RPB1-Cr (Matheny et al. 2002) or RPB1-Afasc and RPB1-6R1asc (Hofstetter et al. 2007) for RPB1; fRPB2-5F and fRPB2-7cR (Liu et al. 1999) for RPB2; TEF983F and TEF2218R (initially obtained from S. Rehner: http://ocid.nacse.org/research/deephyphae/EF1primer.pdf) for TEF-α; and mrSSU1 and mrSSU3R (Zoller et al. 1999) for the mtSSU region.

PCR reactions were prepared in a 25 μl final volume containing either 1.25 (10 μM) or 1 μl (20 μM) of each primer, 17.5 μl or 13 μl of distilled water and 5 μl or 10 μl of the DNA template. PuReTaq Ready-To-Go PCR beads (GE Health Care, Amersham Biosciences, 2004) were added to the mix according to the manufacturer's instructions.

PCR amplifications were performed in a GeneAmp PCR System 2400 (Applied Biosystems®) or Eppendorf Mastercycler® thermal cyclers. PCR (performed by SPO) conditions were as follows: an initial 4 min heating step at 94 °C, followed by 35 cycles of 1.15 min at 94 °C, 1.30 min at 52 °C (for RPB1, RPB2, nuLSU, nuSSU and mtSSU) and 1.45 min at 72 °C. After a final extension step of 7 min at 72 °C, the samples were stored at 4 °C. Conditions for amplification of the ITS and TEF-α regions were an initial step of 3 cycles at an annealing temperature of 54 °C, followed by 30 cycles with the annealing temperature set at 48 °C. PCR protocols (performed by AS) provided by Hofstetter et al. (2007) were used for RPB1; Liu et al. (1999) for RPB2; Rehner (http://ocid.nacse.org/research/deephyphae/EF1primer.pdf) for TEF-α, and Zoller et al. (1999) for mtSSU. For nuLSU and ITS, conditions were an initial 3 min heating step at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C; the final extension step was 10 min. at 72 °C followed by storage for 10 min at 6 °C. For nuSSU, conditions were an initial 1 min heating step at 95 °C, followed by 30 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C, and a final 5 min extension step at 72 °C. When needed, 'touchdown' (Don et al. 1991) preceded the PCR cycle. PCR products were purified using Exo-Sap enzymes (GE Healthcare, Freiburg, Germany) or Ultra Clean

PCR Clean-up (Mo-Bio, Ca U.S.A). Both complementary strands were sequenced in a Macrogen Inc. system (Seoul, Korea) using the same primer set as for PCR amplification with the exception of ITS for which ITS5 (White et al. 1990) was used instead of ITS1F in some cases. Contigs were assembled using Sequencher 4.7 software (GeneCodes Corp., Ann Arbor, MI, USA).

Sequence alignment and phylogenetic analysis In the first analysis, a six-locus (nuSSU, nuLSU, mtSSU, RPB1, RPB2 and TEF-α) dataset was compiled in which sequences from five specimens of *Abrothallus* were aligned with sequences retrieved from GenBank covering main groups of the phylum Ascomycota. As a starting point, we chose the dataset compiled by Schoch et al. (2009b), which is currently the largest dataset available for Ascomycota. 58 ingroup taxa (including five *Abrothallus* taxa) were used together with four outgroup taxa representing Basidiomycota (Supplementary Material, Table S1). For the second analysis, a dataset of five loci (nuSSU, nuLSU, RPB1, RPB2 and TEF-α) was built using *Abrothallus* sequences aligned with representatives of the best-known groups of Dothideomycetes following Schoch et al. (2009a). 126 ingroup taxa (including five species of *Abrothallus*) as well as six outgroup taxa (Arthoniomycetes) were used in this analysis (Supplementary Material, Table S2). For both datasets, we only used specimens with the highest number of available markers. For the third analysis, a seven-locus dataset (nuSSU, nuLSU, mtSSU, RPB1, RPB2, TEF-α and ITS) was used to reconstruct phylogenetic relationships within the genus. In total, 14 specimens of *Abrothallus* were used representing most of the lichen families on which the genus has been reported (Accession numbers are available in Table 1).

All alignments were carried out using Muscle v3.6 (Edgar 2004) and were manually optimized using Bioedit v.7.0.9 (Hall 1999). Gblocks 0.91b (Castresana 2000) was used to remove ambiguously aligned regions and large gaps. To select the best nucleotide substitution model for subsequent analyses, we used jModelTest (Posada 2008). The models selected for each genome region and dataset according to the Akaike Information Criterion (AIC, Akaike 1974) are provided in Supplementary Material, Table S3.

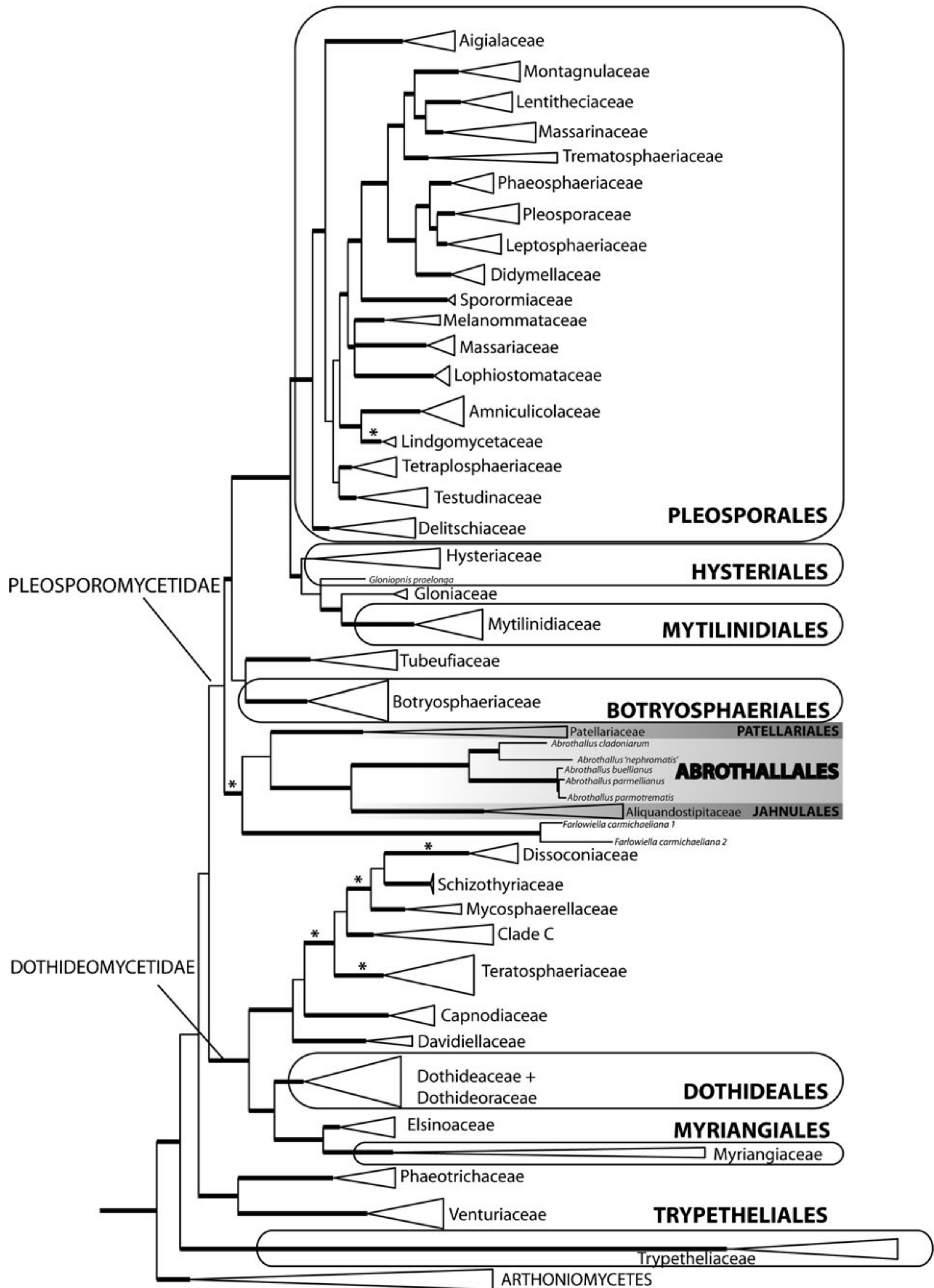
The maximum likelihood (ML) analysis was implemented in RAxML v7.3.2 (Randomized Accelerated Maximum Likelihood) (Stamatakis 2006) on the Cipres Science Gateway v3.3 webportal (http://www.phylo.org/index.php/portal/) (Stamatakis et al. 2008). ML searches were performed using the GTRGAMMA model; for each gene a different partition was set; for the protein coding markers (RPB1, RPB2, TEF-α), each position of the codon was set in a different partition. For the three datasets, 12, 11 and 13 partitions were used respectively. Bootstrap support was obtained through the rapid bootstrapping algorithm in 1, 000 replications. Bayesian analyses were those implemented in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Substitution models used for each partition and analysis are given in Supplementary Material, Table S3. Settings included two parallel runs with eight-chain runs over 8M generations. Sampling was performed after every 200th step; the first 10 % of saved data was discarded as ‘burnin’. Convergence of the parameters was established using Tracer v.1.4 (Rambaut and Drummond 2007). Phylogenetic trees were visualized in FigTree v. 1.3.1 (http://tree.bio.ed.ac.uk/) and Adobe Illustrator CS2® was used for artwork.

Results

Our six-locus based analysis of *Abrothallus* sequences within the framework of Ascomycota provided the first insight into the phylogenetic adscription of the genus (Supplementary Material, Fig. S1). The recovered relationships among higher taxa within Ascomycetes were similar to those reported by Schoch et al. (2009a, b). The most striking difference was the position of Eurotiomycetes, which rather than as a sister group to Lecanoromycetes + Lichinomycetes as in Schoch et al. (2009a, b) appeared basal to several classes including Dothideomycetes, Sordariomycetes, Leotiomycetes, Lecanoromycetes and Arthoniomycetes. These relationships were, however, not statistically supported in either study. The genus *Abrothallus* was included within Dothideomycetes with high statistical support (both BP and PP). In this first analysis, *Abrothallus* appeared as a sister group to *Botryosphaeria*, but with low statistical support.

Subsequent analyses provided further information on the systematic position of the genus. General relationships between the families and orders of Dothideomycetes were similar to those recovered by Schoch et al. (2009a). Only slight differences were detected in the relationships among some groups. For instance, the positions of Botryosphaeriaceae and Patellariales differed but, as before, these relationships were not statistically supported in either case. Our data assigned *Abrothallus* to a sister group of the order Jahnulales (Fig. 2) and this relationship was well-supported by PP and BP. In turn, Jahnulales and *Abrothallus* appeared as sister groups to Patellariales. Many authors have included *Abrothallus* in this latter order (Lindau 1897; Vouaux 1913; von Keissler 1929; Kutorga and Hawksworth 1997). The enigmatic taxon *Farlowiella carmichaeliana* (Berk.) Sacc., recovered in previous studies as basal to Jahnulales (Schoch et al. 2006), appeared as a well-supported (BP and PP) sister group to the clade made up of Jahnulales, *Abrothallus* and Patellariales.

In our seven-marker based phylogenetic reconstruction including 14 specimens of the genus, relationships among the 11 putative species showed an interesting pattern (Fig. 3). First, we observed a clear division of species based on host taxonomy. Two well-supported clades (both by PP and BP) were recovered. The first of these clades included species


Aigialaceae
Montagnulaceae
Lentitheciaceae
Massarinaceae
Trematosphaeriaceae
Phaeosphaeriaceae
Pleosporaceae
Leptosphaeriaceae
Didymellaceae
Sporormiaceae
Melanommataceae
Massariaceae
Lophiostomataceae
Amniculicolaceae
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Lindgomycetaceae
Tetraplosphaeriaceae
Testudinaceae
Delitschiaceae
PLEOSPORALES
Hysteriaceae
HYSTERIALES
PLEOSPOROMYCETIDAE
Gloniopnis praelonga
Gloniaceae
Mytilinidiaceae
MYTILINIDIALES
Tubeufiaceae
Botryosphaeriaceae
BOTRYOSPHAERIALES
Patellariaceae
PATELLARIALES
Abrothallus cladoniarum
Abrothallus 'nephromatis'
Abrothallus buellianus
Abrothallus parmellianus
Abrothallus parmotrematis
ABROTHALLALES
*
Aliquandostipitaceae
JAHNULALES
Farlowiella carmichaeliana 1
Farlowiella carmichaeliana 2
*
Dissoconiaceae
*
Schizothyriaceae
Mycosphaerellaceae
*
Clade C
DOTHIDEOMYCETIDAE
*
Teratosphaeriaceae
Capnodiaceae
Davidiellaceae
Dothideaceae +
Dothideoraceae
DOTHIDEALES
Elsinoaceae
MYRIANGIALES
Myriangiaceae
Phaeotrichaceae
Venturiaceae
TRYPETHELIALES
Trypetheliaceae
ARTHONIOMYCETES
0.2

◀**Fig. 2** Six-locus phylogeny (50 % majority rule consensus tree) depicting phylogenetic relationships among orders and families within Dothideomycetes including *Abrothallus* species. Branches in *bold* indicate PP≥95 % and ML bootstrap values≥75 %. *Asterisks* represent branches supported only in one of two analyses

living on lichens of the order Peltigerales: *A. secedens* Wedin & R. Sant. (Wedin 1994) on *Pseudocyphellaria* sp., and undescribed species growing on *Nephroma* species (Suija et al., in prep.). As sister to this clade appeared specimen NY01041884 whose characters matched those described for *A. cladoniae* R. Sant. & D. Hawksw. (Hawksworth 1990), i.e. small ascomata (135–(184.5)–270 µm, $n=10$) with small ascospores 6–(7.4)–8×2.5–(3.1)–3.5 µm ($n=19$) which form part-spores already within the ascus.

The second large clade included specimens growing on members of the lichen-forming family Parmeliaceae and on the genus *Ramalina*. The characters of specimen TU45449 (host *R. fraxinea*) and SPO 304 (host *R. cf. protecta*) matched those described for *A. suecicus* (Kirschst.) Nordin (Nordin 1964) including the presence of 4-celled ascospores (Fig. 1j). In turn this clade, whose synapomorphic characters are host of Parmeliaceae and the presence of 2-celled ascospores, is subdivided into two groups. The first of these groups includes two specimens growing on *Usnea* species. The characters of both specimens match with description of *A. usneae* var. *usneae* Rabenh. (Etayo and Osorio 2004; Etayo and van den Boom 2006) i.e. the ascospores are 8.4–(10.3)–11.7× 3.9–(4.5)–5.0 µm ($n=20$; TU45810) and (8–(9.7)–12× 3–(4.7)–6.0 µm ($n=20$; SPO306) in size, and the hymenium contains violet granules. The second clade includes specimens growing on several hosts (*Parmelia saxatilis*, *P. sulcata*, *Parmelina tiliacea*, *Parmelinopsis horrescens*, *Pleurosticta acetabulum*, and *Parmotrema* sp.). Molecular differences among these specimens were small as indicated by the short branches and low statistical support for relationships among them. However, differences were indeed detected in morphological-anatomical characters (size of the ascomata and ascospores, presence/absence of greenish pruina, somatic hyphae reactive to Lugol's, epihymenial pigments reactive to K, and conidiospore size).

Discussion

Dothideomycetes is the largest class within Ascomycota comprising more than 19, 000 species in 105 families, including saprotrophic, plant pathogenic, fungicolous, and symbiotic fungi (Kirk et al. 2008; Hyde et al. 2013). While in the traditional sense, the class was reserved for taxa with closed (perithecioid) ascolocular fruitbodies and I– bitunicate asci, in the era of molecular taxonomy, the concept of this class has been

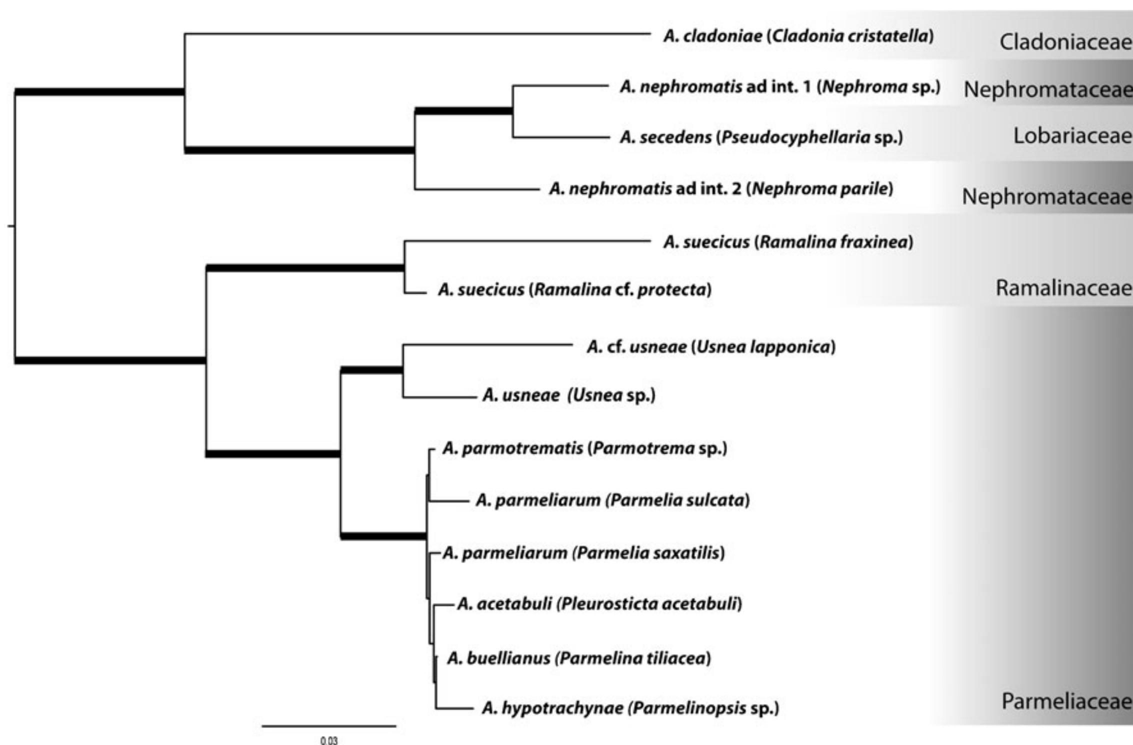

Fig. 3 Seven-locus phylogeny (50 % majority rule consensus tree, midpoint rooting) depicting phylogenetic relationships among 12 *Abrothallus* species (14 specimens). Branches in *bold* indicate PP≥ 95 % and ML bootstrap values≥75 %. Hosts are provided within brackets and host families are shown on the right of the figure

expanded (e.g. Hyde et al. 2013; Schoch et al. 2006, 2009a). Today, the Dothideomycetes include a small proportion of fungi bearing other types of ascomata – apothecia, hysterothecia, thyriothecia or cleistothecia (e.g. Kirk et al. 2008; Schoch et al. 2009a; Hyde et al. 2013) indicating convergency in fruiting body evolution (e.g. Berbee and Taylor 1992; Hibbett et al. 2007; Lumbsch and Huhndorf 2007; Schoch et al. 2006, 2009a; Zhuang and Liu 2012). Our multi-locus analysis revealed that the obligately lichenicolous genus *Abrothallus*, whose phylogenetic position has been much debated (e.g. Nannfeldt 1932; Bellemère et al. 1986), is one of the few genera with apothecioid ascomata within the Dothideomycetes. 'Discothecium' is a special term which was introduced to designate such ascomata which mimic apothecia but contain bitunicate asci (Korf 1973).

The ontogeny of the ascomata in *Abrothallus* is ascolocular (Schaechtelin and Werner 1927) which together with having 'jack-in-the-box'-type asci supports the inclusion of the genus into Dothideomycetes. Moreover, the genus clusters within the subclass Pleosporomycetidae, which is distinguished from the subclass Dothideomycetidae according to the presence of pseudoparaphyses in a hamathecium (Lumbsch and Lindemuth 2001; Schoch 2006, 2009a). To date, the nature of interascal filaments in the hamathecium of *Abrothallus* remains unclear. They have often been described as paraphyses (e.g. de Notaris 1846; Kotte 1909; Diederich 2004; Suija 2006), but in a few descriptions they have been designated paraphysoids (Nordin 1964; Hawksworth 1983; Hafellner 1994; Wedin 1994) or have not been named at all (Hafellner et al. 2008). Therefore the development of the hamathecium in this genus requires further investigation.

Our results clearly rule out prior hypotheses suggesting an inclusion of *Abrothallus* within the order Arthoniales (= Arthoniomycetes) or Patellariales (e.g. Rehm 1896; Bellemère et al. 1986). In an early attempt to resolve the phylogenetic relationships of *Abrothallus*, the nuSSU marker pointed to an unsupported relationship with Dothideales. However, in the combined analysis of nuLSU and nuSSU, in which unfortunately representatives of Dothideomycetes were not included, an ambiguous relationship was observed. Thus, *Abrothallus* appeared either basal to Arthoniales plus Sordariomycetes or basal with respect to a large group of Ascomycetes (Granberg 2001). Our results show that within Dothideomycetes, the genus is sister to the order Jahnulales, which comprises mainly aquatic (both freshwater and marine) saprotrophic ascomycetes (Pang et al. 2002). Jahnulales are characterized by having stalked or sessile dimorphic ascomata, asymmetric 2-celled hyaline to brown ascospores filled with lipid guttules and with gelatinous appendages or sheaths (Pang et al. 2002; Shearer et al. 2009). Judging from the descriptions and illustrations by Pang et al. (2002), several species of Jahnulales have ascospores which by their external appearance resemble ascospores of *Abrothallus*. In the recent revision of the class Dothideomycetes by Hyde et al. (2013), the Jahnulales were related with the newly decribed orders Dyfrolomycetales, Strigulales, and Acrospermales. None of the orders share phenotypical and ecological characters similar to *Abrothallus*. Therefore, given its different anatomical, morphological and ecological characters, and that the genus *Abrothallus* constitutes a distinct phylogenetic lineage, our proposal of a new monotypic order Abrothallales and family Abrothallaceae seems justified.

Taxonomy

Abrothallales Pérez-Ortega & Suija ord. nov

MycoBank no. MB806050

Type genus: *Abrothallus* De Not., Mem Reale Accad Sci Torino ser. 2 10:351–355 (1845)

Diagnosis: The monotypic order which contains lichenicolous species with apothecioid ascomata belongs to the Dothideomycetes subclass Pleosporomycetidae based on the multi-locus analysis. The inclusion to the Dothideomycetes is supported by the ascolocular type of ascoma ontogeny (Schaechtelin and Werner 1927) and by the presence of bitunicate asci. The new order is sister to the Jahnulales, which comprises mainly aquatic species. Differences between the new order and Jahnulales rely on the different ascoma type and ecologically by different nutrition mode and substrate type.

Description: *Mycelium* immersed in the host thallus, somatic hyphae either I+ violet or I–. *Ascomata* apothecioid, protruding through the host cortex, sessile or partly immersed, spherical to flattened, often with a greenish or yellowish pruina (Fig. 1a, b, d). *Excipulum* much reduced, composed of short, dichotomously ramified hyphae. *Epihymenium* brown- or redgranulose (Fig. 1c); granules usually dissolve in K. *Hypothecium* light to dark brown, consisting of oblong cells covered with a brown pigment. *Hamathecium* composed of thick-walled, unequally dichotomously branched and anastomosed, septate interascal filaments (possibly pseudoparaphyses *sensu* Kirk et al. 2008), sometimes slightly swollen at the apex (Fig. 1e); hymenial gel I–. *Asci* bitunicate, functionally fissitunicate, broadly to narrowly clavate, I–, comprised of four to eight ascospores (Fig. 1f, g); in some species these break into part-spores (Fig. 1k) inside the ascus. *Ascospores* initially hyaline, later brown, often verruculose, one-, two- or three-septate, asymmetric in shape (Fig. 1i, j). *Anamorph* common, pycnidial, black, immersed or semi-immersed in host thallus, with a small ostiole (Fig. 1l, m), *Vouauxiomyces*-type; wall of the pycnidium *textura angularis*, composed of thick-walled isodiametric cells. *Conidiophores* missing. *Conidiogenous cells* percurrently proliferating, ampulliform to lageniform (Fig. 1h), lining the cavity of the pycnidium, hyaline, smooth-walled. *Conidia* holoblastic, clavate to obpyriform, hyaline (Fig. 1n, o), smooth to very slightly echinulate, in muscilage.

Distribution: cosmopolitan

Ecology: obligately lichenicolous; hosts belong to a variety of fruticose and foliose macrolichens.

The new order Abrothallales includes the single family Abrothallaceae.

Abrothallaceae Pérez-Ortega & Suija fam. nov.

MycoBank no. MB806051

Type genus: *Abrothallus* De Not., Mem Reale Accad Sci Torino ser. 2 10:351–355 (1845)

Characters as in Abrothallales.

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