

Molecular analyses confirm *Brevicellicium* in *Trechisporales*

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Abstract: The genus *Brevicellicium* encompasses wood-inhabiting corticioid fungi characterized by isodiametric subhymenial hyphae, short basidia, and smooth, often subangular spores with a distinct apiculus. Eight new LSU nrDNA sequences and 13 new ITS nrDNA of this genus, including the type species, were aligned with 47 and 42 accessions respectively of species of *Trechisporales* obtained from GenBank, and phylogenetic analyses were performed. The order *Trechisporales* was confirmed as a monophyletic group; the genera *Porpomyces*, *Sistotremastrum*, *Subulicystidium* and *Trechispora* form a highly supported clade where all *Brevicellicium* sequences are included. Our analyses also support that this genus belongs to *Hydnodontaceae*. A new species, *Brevicellicium atlanticum* from the Azores Archipelago, is described.

Key words:

Basidiomycota
Agaricomycetes
Corticioid fungi
ITS
LSU nrDNA
Phylogeny
Taxonomy

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INTRODUCTION

Brevicellicium was described by Larsson and Hjortstam (Hjortstam & Larsson 1978) to accommodate *Corticium exile*. At the time, two more species were transferred to the new genus, *Odontia olivascens* and *Athelopsis viridula*. The isodiametric subhymenial hyphae, short basidia and smooth, often subangular, spores with a distinct apiculus were emphasized as important morphological characteristics of this genus of wood-inhabiting corticioid fungi.

Twelve species have been placed in this cosmopolitan genus. *Brevicellicium exile*, originally described from Canada (Jackson 1950) as *Corticium exile*, seems to be a rare species in the Northern Hemisphere (Hjortstam 2001), and is known from north Europe (Hjortstam & Larsson 1978), France (Boidin & Gilles 1990), Spain (Telleria *et al.* 1993, Telleria & Melo 1995), and Colombia (Hjortstam & Ryvarden 1997). *Brevicellicium olivascens*, described from Italy by Bresadola (1892) as *Odontia olivacea*, is a cosmopolitan species, widely distributed in temperate areas and less frequent in tropical and subtropical regions (Hjortstam *et al.* 2005); it is common in Europe including the Iberian Peninsula (Telleria & Melo 1995, Bernicchia & Gorjón 2010) and is also reported from North America (Ginns & Lefebvre 1993), South America, Burundi, and India (Hjortstam 2001, Hjortstam & Ryvarden 2007) as well as from Iran (Hallenberg 1981), and Japan (Maekawa 1993). It should also be noted that *B. exile* and *B. olivascens* were also found in the Macaronesian region: Canary Islands and Azores Archipelago (Ryvarden 1976, Hjortstam & Larsson 1978, Telleria *et al.* 2009a, b). *Brevicellicium viridulum*, transferred to the genus when it was described, was considered by Hjortstam *et al.*

(1988) as a colour morph of *B. olivascens*. *Brevicellicium permodicum*, described from Canada by Jackson (1950) as *Corticium permodicum*, and also reported from New Zealand (Cunningham 1963, Hjortstam 2001), is the only species of the genus without clamps known today. Its inclusion in the genus is perhaps questionable. The other eight species have a tropical distribution: *Brevicellicium mellinum*, originally described from Brazil by Bresadola (1920) as *Corticium mellinum*, is reported from Puerto Rico and Venezuela (Hjortstam & Ryvarden 2007). *Brevicellicium allantosporum*, described from Tanzania (Hjortstam & Ryvarden 1980), is also known from Brazil, Colombia, Venezuela, Ecuador, and Borneo (Hjortstam 2001, Hjortstam *et al.* 2005, Hjortstam & Ryvarden 2008). *Brevicellicium flavovirens*, from Argentina and Brazil (Hjortstam 2001), is morphologically similar to *B. exile* but differs in basidiome colour and the shape and size of the spores. *Brevicellicium molle*, described from Tanzania (Hjortstam & Ryvarden 1980), is also reported from Colombia and Brazil (Hjortstam & Ryvarden 1997, Hjortstam 2001). Four species are only known from their type locality: *B. asperum* from Venezuela (Hjortstam *et al.* 2005), *B. udinum* from Brazil (Hjortstam 2001), *B. uncinatum* from Tanzania (Hjortstam & Ryvarden 1980, Hjortstam 2001), and *B. vulcanense* from Hawaii (Gilbertson *et al.* 2001). Complete or partial keys to *Brevicellicium* have been published by Hjortstam & Larsson (1978), Hjortstam & Ryvarden (1980), Hjortstam (2001), and Hjortstam *et al.* (2005).

According to Hjortstam & Larsson (1978), *Brevicellicium* is morphologically close to the smooth-spored species of *Trechispora* (e.g. *Trechispora amianthina*, *T. cohaerens*, *T. confinis*, *T. byssinella*), differing in the absence of ampullate septa on the basal hyphae. Jülich (1982), placed both

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genera in *Hydnodontaceae*, and later Larsson (2007), in his phylogenetic classification of corticioid fungi, confirmed this arrangement and included the family in *Trechisporales*.

Recently, the genus *Brevicelopsis* has been segregated from *Brevicellicium* (Hjortstam & Ryvarden 2008), with *Brevicellicium allantosporum* as type species. Both genera share similar isodiametric subhymenial hyphae, but they can be distinguished by the hymenophore appearance and shape of the spores. In *Brevicellicium*, the hymenophore is granular to almost smooth, and the spores are subangular or short ellipsoid, whereas *Brevicelopsis* has a distinctly odontoid hymenophore and allantoid spores.

The aim of this study was to identify, characterize and analyze, using morphological and molecular data, 11 collections of *Brevicellicium* from the Iberian Peninsula (Spain and Portugal) and the Azores Archipelago, as well as to evaluate the phylogenetic circumscription of the genus. The ITS and LSU nrDNA sequences of all collections were compared with sequences of *Trechispora* and *Sistotremastrum* generated by our research group within the framework of other studies, and with sequences deposited in GenBank, in order to establish their phylogenetic relationships. As a result, a new species is described and the phylogenetic position of *Brevicellicium* as member of *Trechisporales* is confirmed (Larsson 2007).

MATERIALS AND METHODS

Sampling, morphological studies and line drawings

Twelve specimens of *Brevicellicium* from the Iberian Peninsula (Spain) and the Azores Archipelago, and two from Sweden, were studied (Table 1). Vouchers are deposited in MA-Fungi, LISU, TFCMic, and GB. Measurements and drawings were made from microscopic sections mounted in 3 % aqueous solution of potassium hydroxide and examined at magnifications up to $\times 1250$ using an Olympus BX51 microscope. The length and width of 30 spores and 10 basidia were measured from each sample. Colours of dried basidiomes are given according to the ISCC–NBS *Centroid Color Charts*. The drawing was made with aid of a drawing tube.

DNA isolation and sequencing

Genomic DNA was extracted from 13 collections (Table 1) using the E.Z.N.A® Fungal DNA Miniprep Kit (Omega Biotek, Doraville, USA) or the DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA), following the manufacturer's instructions; lysis buffer incubation was overnight at 55 °C.

Total DNA was used for PCR amplification of the 5'-1450-base region of the large subunit (LSU nrDNA) and the internal transcribed spacer region (ITS nrDNA) of the nuclear ribosomal gene. The primers LR0R (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) were used to amplify the

Table 1. Specimens of *Brevicellicium* studied with GenBank accession numbers.

Species/Specimen	Country/Locality	Habitat	Acc. no.	
			ITS	28S
<i>B. atlanticum</i> sp. nov.				
LISU 178590, 9090IM	Portugal, Azores Archipelago, Terceira Island	<i>Juniperus brevifolia</i> subsp. <i>azorica</i>	HE963775	HE963776
LISU 178566, 9065IM	Portugal, Azores Archipelago, Terceira Island	<i>Erica azorica</i>	HE963773	HE963774
<i>B. exile</i> (H.S. Jacks.) K.H. Larss. & Hjortstam				
MA-Fungi 76132, 16118Tell.	Portugal, Azores Archipelago, Pico Island	<i>Pittosporum undulatum</i>	HE963779	—
MA-Fungi 26554, 5217MD	Spain, Huesca	<i>Buxus sempervirens</i>	HE963777	HE963778
GB, KHL 12130	Sweden, Västergötland	conifer	—	HE963780
<i>B. olivascens</i> (Bres.) K.H. Larss. & Hjortstam				
MA-Fungi 75998, 17370Tell.	Portugal, Azores Archipelago, Flores Island	<i>Pittosporum undulatum</i>	HE963790	—
TFCMic 15272	Portugal, Azores Archipelago, Pico Island	<i>Pittosporum undulatum</i>	HE963791	—
MA-Fungi 19016, 7743Tell.	Spain, Asturias	<i>Quercus robur</i>	HE963789	—
MA-Fungi 13843, 3491MD	Spain, Asturias	<i>Castanea sativa</i>	HE963782	HE963783
MA-Fungi 5674, 239Tell.	Spain, Barcelona	<i>Fagus sylvatica</i>	HE963781	—
MA-Fungi 21444, 3881MD	Spain, Guadalajara	<i>Rosmarinus officinalis</i>	HE963784	—
MA-Fungi 41366, 6910MD	Spain, Madrid	<i>Corylus avellana</i>	HE963785	HE963786
MA-Fungi 23496, 4611MD	Spain, Toledo	<i>Ulmus</i> sp.	HE963787	HE963788
GB, KHL 8571	Sweden, Bohuslän	hardwood	HE963792	HE963793

region of the LSU nrDNA and the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) were used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and flanking parts of the small subunit (SSU) and large subunit (LSU) nuclear ribosomal genes. Individual reactions to a final volume of 25 μm were carried out using illustra™ PuReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckingham) with a 10 pmol μL primer concentration following the thermal cycling conditions used in Martín & Winka (2000). When these pair of primers failed, the LSU nrDNA region was amplified in two parts using LR5 and LR3R (Viřgalys & Hester 1990), in the combination LR0R/LR5 and LR3R/LR7. The ITS1 nrDNA region and the beginning of 5.8S with primers ITS1F and ITS2 (White *et al.* 1990), and ITS2 nrDNA region and the end of 5.8S with primer ITS3 (White *et al.* 1990) and ITS4.

Negative controls lacking fungal DNA were run for each experiment to check for contamination. The reactions were run with the following parameters for the LSU nrDNA: initial denaturation at 94 °C for 5 min, then 36 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 1 min and 30 s, with a final extension at 72 °C for 10 min, and 4 °C soak; for the ITS nrDNA: initial denaturation at 95 °C for 5 min, then 5 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 1 min, followed by 33 cycles of denaturation at 72 °C for 1 min, annealing at 48 °C for 30 s, and extension at 72 °C, with a final extension at 72 °C for 10 min and 4 °C soak.

The PCR products were subsequently purified using the QIAquick Gel PCR Purification (Qiagen) kit according to the manufacturer's instructions. The purified PCR products were sequenced using the same amplification primers. When products were only faintly visible on agarose gels (less than 20 ng μL^{-1}), cloning was conducted with a pGEM®-T Easy Vector System II cloning kit (Promega Corporation, Madison, WI). From each cloning reaction, up to six clones were selected for sequencing. To confirm that the inserted product was correct, 2 μL of the purified plasmid DNA was digested with Eco RI prior to sequencing following the instructions of the manufacturers. Both strands were sequenced separately using vector specific primers T7 and SP6 at Secugen S.L. (Madrid, Spain) or Macrogen (Seoul, Korea).

Sequencher v. 4.2 (Gene Codes Corporation, Ann Arbor, MI) was used to edit the resulting electropherograms and to assemble contiguous sequences. BLAST searches with MEGABLAST option were used to compare the sequences obtained against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide databases (Altschul *et al.* 1997).

Sequence alignment and phylogenetic analyses

The LSU nrDNA and ITS nrDNA sequences obtained were aligned separately using Se-Al v. 2.0a11 Carbon (Rambaut 2002) for multiple sequences. The sequences were compared with homologous sequences retrieved from the EMBL/GenBank/DDBJ databases (Cochrane *et al.* 2011); many of the sequences were generated by our research group within the framework of other studies (*Sistotremastrum*: JX310442–JX310445; *Trechispora* and other *Trechisporales*: JX392812–

JX392856). In the LSU nrDNA analyses, *Sistotrema* and *Repetobasidium* (cantharelloid and hymenochaetoid clade respectively, Binder *et al.* 2005) sequences were included as outgroups. In order to root the ITS analyses, six *Sistotremastrum* sequences (*Sistotremastrum* family in Larsson 2007) were included as outgroups because they appear as the sister group of the clade formed by *Porpomyces*, *Subulicystidium*, and *Trechispora* in the trechisporoid clade (Binder *et al.* 2005, Larsson 2007). Where ambiguities in the alignment occurred, the alignment generating the fewest potentially informative characters were chosen (Baum & Sytsma 1994). Alignment gaps were marked “–”, unresolved nucleotides and unknown sequences were indicated with “N”.

From each data set a maximum parsimony analysis (MP) was carried out; minimum length Fitch trees were constructed using heuristic searches with tree–bisection–reconnection (TBR) branch swapping, collapsing branches if maximum length was zero and with the MulTrees option on in PAUP v. 4.0b10 (Swofford 2003). Gaps were treated as missing data. Nonparametric bootstrap (bs) support (Felsenstein 1985) for each clade, based on 10 000 replicates using the fast–step option, was tested. The consistency index, CI (Kluge & Farris 1969), retention index, RI (Farris 1989), and rescaled consistency index, RC (Farris 1989) were obtained.

For each dataset a second analysis was done using a Bayesian approach (Larget & Simon 1999, Huelsenbeck *et al.* 2001) with MrBayes v. 3.1 (Ronquist & Huelsenbeck 2003). The analyses were performed assuming the general time reversible model (Rodríguez *et al.* 1990), including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTR+I+G) as selected by MrModeltest v. 2.3 (Nylander 2004). According to Rodríguez *et al.* (1990), only reversible models allow the calculation of the substitution rates. Two independent and simultaneous analyses starting from different random trees were run for 2 000 000 generations with four parallel chains and trees and model scores saved every 100th generation. The default priors in MrBayes were used in the analysis. Every 1 000th generation tree from the two runs was sampled to measure the similarities between them and to determine the level of convergence of the two runs. The potential scale reduction factor (PSRF) was used as a convergence diagnostic and the first 25 % of the trees were discarded as burn–in before stationary was reached. Both the 50 % majority–rule consensus tree and the posterior probability (pp) of the nodes were calculated from the remaining trees with MrBayes. Phylogenetic trees were drawn using TreeView (Page 1996).

RESULTS

In general, only amplifications in parts of the LSU nrDNA, with primers LR0R/LR5 and LR3R/LR7, and ITS nrDNA, with primers ITS1F/ITS2 and ITS3/ITS4 were successful. Weak products (faintly visible on agarose gels; less than 20 ng μL^{-1} after gel purification) or purified product, which sequences showed double peaks, were cloned. Thus, from LISU 178566, LISU 178590 and MA–Fungi 26554, good LSU sequences were obtained after cloning; the six cloned sequences from each fragment/collection were identical and only one

was selected for analyses. The BLAST search of the LSU nrDNA and ITS nrDNA sequences obtained (both direct or after cloning), excluding uncultured/environmental samples, showed more than 100 % and 85 % similarity respectively with *Trechisporales* sequences published in GenBank, mainly from Larsson *et al.* (2004). Sequences were located in EMBL/GenBank/DBJ and UNITE (Abarenkov *et al.* 2011, <http://unite.ut.ee/cite.php>) databases.

LSU nrDNA

Eight LSU nrDNA sequences generated for this study were aligned with 47 sequences downloaded from GenBank to produce a matrix of 1 358 unambiguously aligned nucleotide position characters. Among them, 908 positions were constant, 167 were parsimony-uninformative and 283 were parsimony-informative. In the maximum parsimony analysis under heuristic search, 100 most parsimonious trees (MPTs) were obtained (tree length=1085, consistency

index CI = 0.5512, retention index RI = 0.7105, rescaled consistency index RC = 0.3916). The trees obtained from the MP (strict consensus tree, data not shown) and the Bayesian analyses (Fig. 1) show similar topologies. In both analyses, the complete ingroup forms a highly supported monophyletic clade (bs = 78 %, pp = 1.0), and includes all *Brevicellicium* sequences. The *Trechisporales* clade is divided in two well-supported clades, one with the three *Sistotremastrum* accessions (bs = 78 %, pp = 1.0) and the other, with the remaining sequences (bs = 88 %, pp = 0.99). The latter are distributed over three subclades that either lack support or get support by the Bayesian analysis only. Subclade I (bs < 50 %, pp = 0.52) is formed by *Porpomyces* and *Subulicystidium*. Subclade II (bs = 58%, pp = 1.0) includes all *Brevicellicium* collections and is the sister group of subclade III (bs = 56, pp = 1.0) formed by 34 *Trechispora* spp. sequences. However, this sister-group relationship is not highly supported (bs < 50 % and pp =

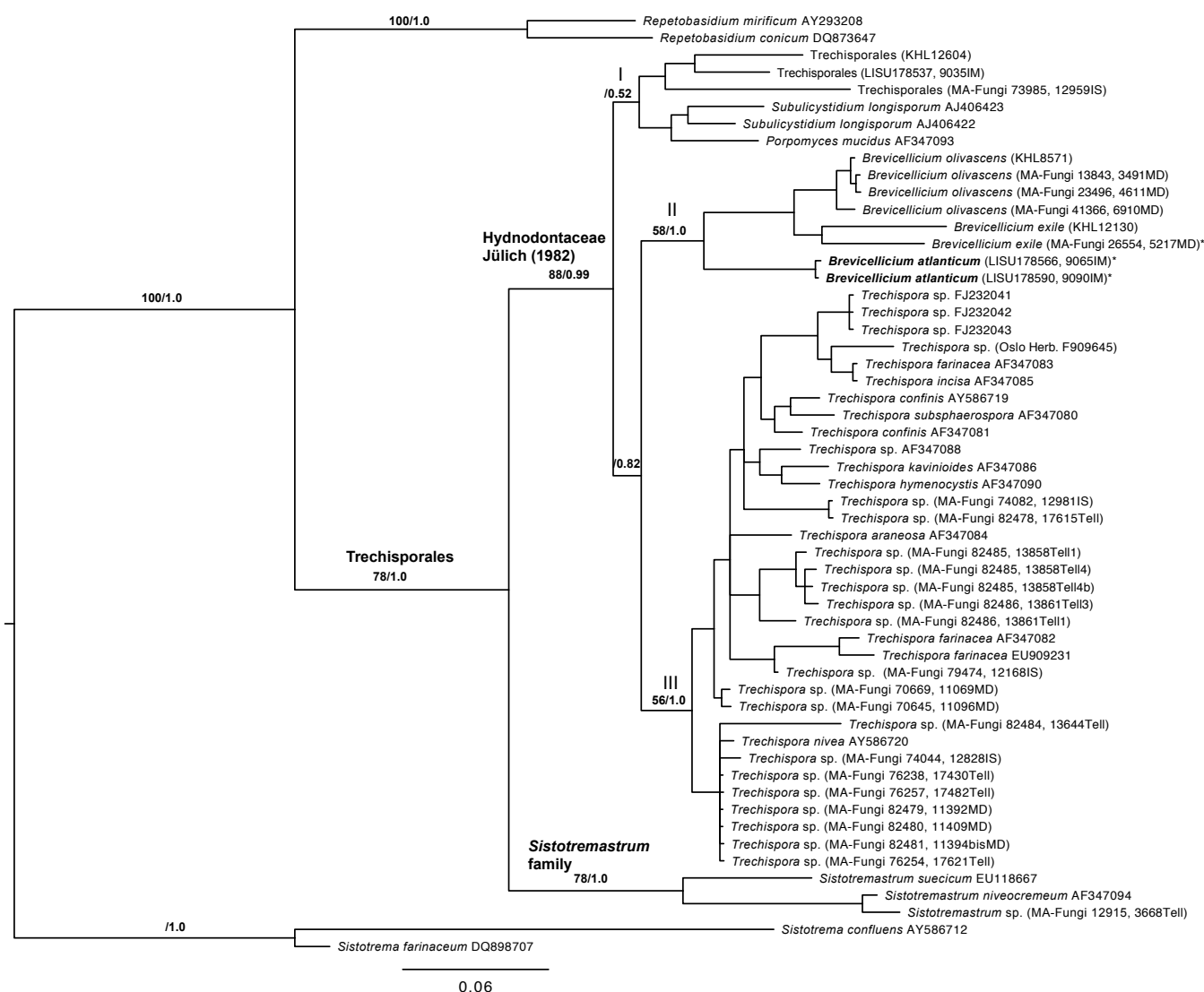


Fig. 1. The 50 % majority-rule consensus tree of Bayesian analysis based on nuclear D1/D2 sequences (LSU nrDNA). Bootstrap (> 50 %) and posterior probability values indicated on the branches to the main clades. The topology was rooted with *Sistotrema* and *Repetobasidium* species. The three main clades in Hydnodontaceae discussed in the text are designated I-III. The position of *Brevicellicium atlanticum* is indicated in bold. * Sequences obtained after cloning (from each sample, six identical sequences were obtained after cloning, but only one per sample was included in the analyses and sent to GenBank).

0.82). The *Brevicellicium* clade is separated in two strongly supported groups; one (bs = 100, pp = 1.0) that includes two collections from Terceira Island in Azores Archipelago (LISU 178566 and LISU 178590) and another (bs = 93, pp = 1.0) consisting of six accessions: four of *B. olivascens* clade (bs = 96 %, pp = 1.0) and two of *B. exile* (bs < 50 %, pp = 1.0).

ITS nrDNA

Thirteen new ITS nrDNA sequences were aligned with 42 sequences available in GenBank including six *Sistotremastrum* sequences serving as outgroup. The resulting matrix consisted of 871 unambiguously aligned nucleotide position characters. Among them, 314 positions were constant, 127 were parsimony-uninformative and 410 were parsimony-informative. In the maximum parsimony analysis under exhaustive search, 100 most parsimonious trees (MPTs) were obtained (tree length = 1407, CI = 0.6041, RI = 0.8113, and RC = 0.491). The trees obtained from the MP (strict consensus tree, data not shown) and Bayesian

analyses show similar topologies (Fig. 2). Similar to the LSU analyses the *Brevicellicium* sequences form a clade (bs = 90 %, pp = 0.90), in a sister-group relationship to all *Trechispora* sequences (bs < 50 %, pp = 1.0). Sequences from LISU 178566 and LISU 178590 form a highly supported clade (bs = 100, pp = 1.0), sister group of *B. exile* and *B. olivascens* clade (bs = 90 %, pp = 1.0). The nine *B. olivascens* sequences form a highly supported clade (bs = 99 %, pp = 1.0), with low genetic variability (uncorrected “p” distances from 0.0 to 0.018), whereas the two *B. exile* sequences do not group together and show a high genetic variability (uncorrected “p” distance equal 0.139); apparently here are more taxonomical problems hidden that needs to be addressed.

Since LISU 178566 and LISU 178590 also have unique morphological characters we find reasons to describe them as a new species: *Brevicellicium atlanticum*.

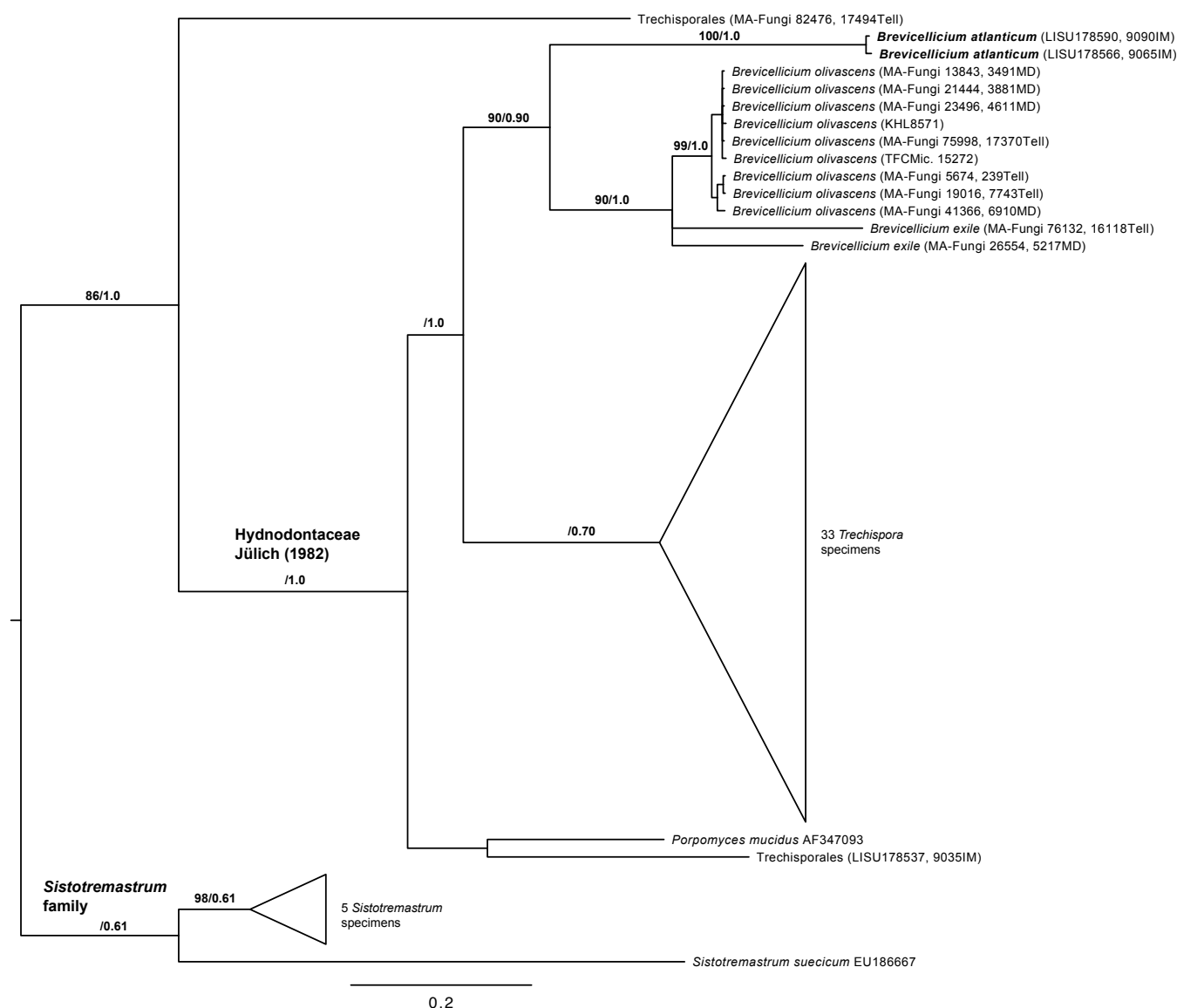


Fig. 2. The 50 % majority-rule consensus tree of Bayesian analysis based on ITS nrDNA sequences. Bootstrap (> 50 %) and posterior probability values indicated on the branches. The topology was rooted with *Sistotremastrum* species. *Trechispora* and *Sistotremastrum* clades not discussed in the text are indicated as triangles. The position of *Brevicellicium atlanticum* is indicated in bold.

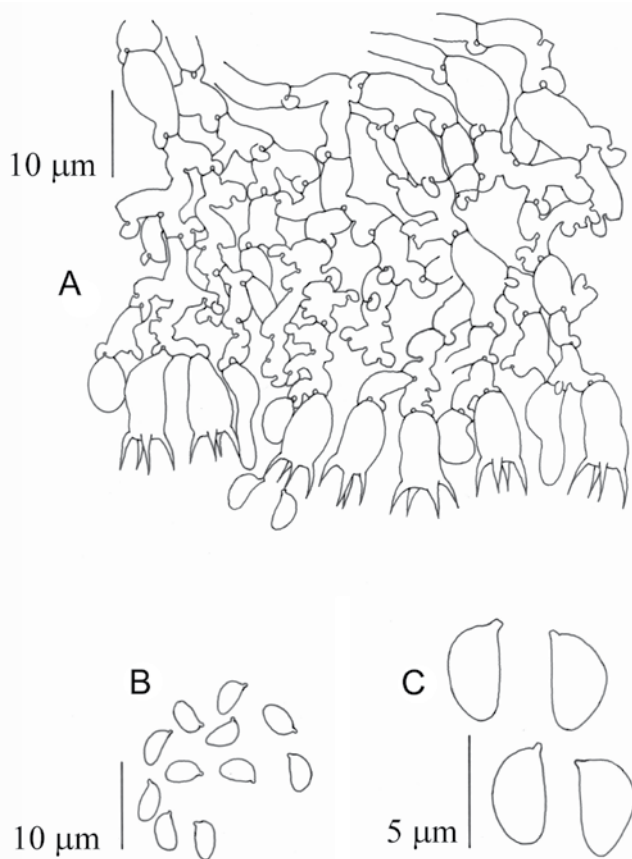


Fig. 3. *Brevicellicium atlanticum* (LISU 178566). a. Vertical section through basidiome; b–c, spores.

TAXONOMY

Brevicellicium atlanticum Melo, Telleria, M. Dueñas & M.P. Martín, **sp. nov.**

MycoBank MB800016

(Fig. 3)

Etymology: The Azores Archipelago is situated in the middle of the North Atlantic and *atlanticum* is derived from Atlantic Ocean.

Diagnosis: *Basidiome* resupinate, membranaceous, smooth, whitish. *Hyphal system* monomitic, hyphae with clamps, subhymenial hyphae to 6.0 µm diam. *Basidia* clavate to short cylindrical, 8.0–10.0 × 4.5–5.5 µm, with 4 sterigmata. *Basidiospores* short ellipsoid with a prominent apiculus, (3.8–) 4.0–4.5 × (2.0–) 2.3–2.5 µm.

Type: **Portugal:** Azores: Terceira, Angra do Heroísmo, Terra-Chã, Matela de Baixo, 26SMH7783, 470m asl, on live trunk of *Erica azorica*, 10 Mar. 2005, I. Melo & J. Cardoso 90651M (LISU 178566 – **holotype**).

Description: *Basidiome* resupinate, effused, adnate, very thin, porose, membranaceous; hymenophore smooth, whitish; margin indeterminate. *Hyphal system* monomitic, hyphae with clamps, subiculum very thin, consisting of a few thin-walled, uniform, 2.5–3.5 µm diam. hyphae,

subhymenial hyphae richly branched, wider, some isodiametric and up to 6.0 µm diam. *Cystidia* absent. *Basidia* short clavate to short cylindrical, basally clamped, 8.0–10.0 × 4.5–5.5 µm, with 4 sterigmata up to 4.5 µm long. *Basidiospores* short ellipsoid with a prominent apiculus, smooth, thin-walled, (3.8) 4.0–4.5 × (2.0) 2.3–2.5 µm, inamyloid, indextrinoid, acyanophilous.

Substratum: On live trunk of *Erica azorica* and on decayed branch of *Juniperus brevifolia* ssp. *azorica*, both endemic plants from the Azores Archipelago.

Additional specimen examined: **Portugal:** Azores: Terceira, Angra do Heroísmo, Mistérios Negros, 26SMH7587, 630 m asl, on decayed branch of *Juniperus brevifolia* ssp. *azorica*, 2 Mar 2005, I. Melo & J. Cardoso 90901M (LISU 178590).

Notes: Overall, *Brevicellicium atlanticum* is morphologically most similar to *B. exile*, but the latter has wider basidia and larger spores, 9–11 × 5–6.5 µm and 4.5–5 × 2.5–3.5 µm respectively (Jackson 1950); 9–12 × 5.5–8 µm and 4.5–6 × 2.5–3.5 µm in specimens from the Iberian Peninsula (Telleria & Melo 1995) or 10–15 × 5–6.5 µm and 5–6.5 × 3.5–4 µm from the Azores Archipelago (Telleria et al. 2009 a, b). Also, *B. flavovirens* and *B. udinum* have a similar spore morphology, but the former has a yellowish grey basidiome and wider spores (4.5–5.0 (–5.5) × 3.0–3.5 µm). In *B. udinum* the basidiome is thick, cracked when dry, and the spores are narrowly ellipsoid, 5.0–5.5 (–6.0) × 2.5–2.75 µm.

Boidin & Gilles (1990) reported a specimen morphologically similar to *Brevicellicium exile* from France, Landes, Carcen-Ponson, on *Alnus glutinosa*, LY 13883, but differing in the narrower basidia (8–14 × 4–5 µm) and smaller spores (3.5–4.5 × 2–2.5 µm). This material was not available to us but could well represent *B. atlanticum*.

DISCUSSION

Trechisporales is a rather small order described by Larsson (Hibbett et al. 2007) and placed in the subphylum *Agaricomycotina*, class *Agaricomycetes*, with three exemplar genera included in the original description: *Trechispora*, *Sistotremastrum*, and *Porpomyces*. In his molecular phylogenetic classification of the corticioid fungi, Larsson (2007) included sequences of *Trechispora farinea* (AF347089), *T. hymenocystis* (AF347090), *Subulicystidium* (AY463468/AY586714), *Porpomyces mucidus* (AF347091) and *Sistotremastrum niveocreum* (AF347094), and preliminarily recognized two families in the order: *Hydnodontaceae* (Jülich 1982) with the genera *Brevicellicium*, *Fibriellum*, *Fibrodontia*, *Luellia*, *Porpomyces*, *Subulicystidium*, *Trechispora* and *Tubulicium*; and the *Sistotremastrum* family with the genus *Sistotrematrum*. Besides, he listed the genera *Dextrinocystis*, *Dextrinodontia* and *Litchauerella* as possible candidates to be included in *Hydnodontaceae*. The new genus *Brevicellopsis*, segregated from *Brevicellicium* (Hjortstam & Ryvarden 2008), could be another possible candidate to be included in this family.

The molecular phylogenetic analyses of the present study support *Trechisporales* as a monophyletic group with the species of *Porpomyces*, *Sistotremastrum*, *Subulicystidium*, and *Trechispora* forming a highly supported monophyletic clade where all *Brevicellicium* sequences are included. Our results also support the two families of this order: *Hydnodontaceae* where *Brevicellicium* and *Trechispora* are included and the *Sistotremastrum* family (Jülich 1982, Larsson 2007). Most species of *Brevicellicium* have yet to be included in molecular phylogenetic analyses. Only then can outstanding issues like the independent status of *Brevicellopsis* and the uncertain position for *Brevicellicium permodicum* be resolved.

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