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ORIGINAL ARTICLE

Sistotremastrum guttuliferum: a new species from the Macaronesian islands

M. Teresa Telleria · Ireneia Melo · Margarita Dueñas · Isabel Salcedo · Esperanza Beltrán-Tejera · J. Laura Rodríguez-Armas · María P. Martín

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Abstract Using morphological and molecular data, the new species *Sistotremastrum guttuliferum* is described from specimens collected in the Azores archipelago, Madeira and Canary Islands. Morphologically, this new species differs from *S. niveocremeum* and *S. suecicum* by the small oil drops in the cytoplasm of subicular hyphae and the spore size. An updated key of *Sistotremastrum* species is provided.

Keywords Basidiomycota · Trechisporales · Corticioid fungi · ITS nrDNA · LSU nrDNA · Oceanic islands · Taxonomy

Introduction

The small genus *Sistotremastrum* (Basidiomycota, Trechisporales) was described by Eriksson (1958) to include two taxa: *Sistotremastrum suecicum* Litsch. ex J. Erikss., as type species, and *Sistotremastrum niveocremeum* (Höhn & Litsch.) J. Erikss. Both species share resupinate, adnate and effused basidioma, whitish, ceraceous when living and brittle when dried, monomitic hyphal system with clamped hyphae, basidia subclavate to tubular, with four to six sterigmata, and spores narrowly ellipsoid or subcylindrical to suballantoid.

This genus resembles *Sistotrema* morphologically sharing similar basidioma and number of sterigmata, but differs by the shape of basidia and hyphal inclusions. Whereas *Sistotrema* has urniform basidia and rich oily inclusions in all hyphal elements, *Sistotremastrum* is characterized by the presence of subclavate to tubular basidia and lacks oily inclusions in the hyphal elements. According to Eriksson et al. (1984), one peculiarity of *Sistotremastrum* is the appearance of the postmature basidia with shrunken sterigmata more or less turned inwards, a characteristic of restricted taxonomic value that could, however, be useful to distinguish specimens of the two species, because it is more evident in *S. suecicum* than in *S. niveocremeum*. Boidin and Gilles (1994) enlarged the concept of the genus, describing its first cystidiate species, *Sistotremastrum lateclavigerum* Boidin & Gilles from France.

Within the framework of our studies about corticioid fungi from oceanic islands (Dueñas et al. 2008a, b; Melo et al. 2008; Telleria et al. 2008, 2009a, b, c, 2012) we collected several specimens in the Macaronesian region (Azores archipelago, Madeira and Canary Islands) whose characteristics lead us to include them in the genus *Sistotremastrum*. The aim of this study was to identify, characterize and analyze those specimens using morphological and molecular data; as result a new species is described.

M. T. Telleria (✉) · M. Dueñas · M. P. Martín
Departamento de Micología, Real Jardín Botánico, RJB—CSIC,
Plaza de Murillo 2,
28014 Madrid, Spain
e-mail: telleria@rjb.csic.es

I. Melo
Jardim Botânico (MNHNC), Universidade de Lisboa/CBA-FCUL,
Rua da Escola Politécnica 58,
1250-102 Lisbon, Portugal

I. Salcedo
Departamento de Biología Vegetal y Ecología (Botánica),
Universidad del País Vasco (UPV/EHU), Aptdo. 644,
48080 Bilbao, Spain

E. Beltrán-Tejera · J. L. Rodríguez-Armas
Departamento de Biología Vegetal (Botánica),
Universidad de La Laguna, 38071 La Laguna, Tenerife, Spain

Materials and methods

Morphological studies

The study was based on specimens collected during mycological forays to the Atlantic Archipelagos of Azores, Madeira and Canary Islands, in the Macaronesian region. The collections are deposited in LISU, MA, and TFC Herbaria. Dried specimens were used for light microscope study. Colors of dried basidioma are according to ISCC_NBS Centroid Color Charts (U.S. Dept. of Commerce, National Bureau of Standards. Washington, DC). Measurements and drawings were made from microscopic sections mounted in 3 % KOH solution and examined at up to ×1,250 with an Olympus BX51 microscope provided with a drawing tube. Lengths and widths of 30 spores and 10 basidia were measured from each sample.

Molecular analyses

Genomic DNA was extracted from three *Sistotremastrum* collections (10471MD, 11986MD, and 16566Tell.) using the E.Z.N.A.® Fungal DNA Miniprep Kit (Omega Biotek, Doraville, GA, USA) following the instructions of the manufacturers; samples were incubated in lysis buffer overnight at 55 °C, as indicated in Martín and Winka (2000). Total DNA was used for PCR amplification and sequencing of the internal transcribed spacer region (ITS nrDNA), 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 as described in Telleria et al. (2010b). The consensus sequences have been lodged in the EMLB-EBI database with the accession numbers JX310443 (10471MD), JX310444 (11986MD), and JX310445 (16566Tell.)

These sequences were aligned using Se-Al v2.0a11 Carbon (Rambaut 2002) for multiple sequences with Trechisporales sequences retrieved from the EMBL/GenBank/DDBJ databases (Cochrane et al. 2011). Maximum parsimony analysis (MP) using PAUP*Version 4.0b10 and Bayesian analyses using MrBayes 3.1 were carried out as indicated in Telleria et al. (2010a). The Bayesian 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 2.3 (Nylander 2004). 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. Moreover, from *Sistotremastrum* sequences, Kimura-2-parameter (K2P) pairwise distances were obtained using PAUP*Version 4.0b10.

Results

In both parsimony and Bayesian analyses, *Sistrotremastrum* sequences grouped in a separate clade from the other 49 Trechisporales sequences. Figure 1 shows the Bayesian 50 % majority rule consensus tree. The ITS sequences of 10471MD, 11986MD, and 16566Tell. appeared together in a well-supported clade (bootstrap value=99 %; posterior probability=0.70), as sister group of a sequence from GenBank deposited as *Sistotremastrum* sp. (AY805625), isolate olrim558 from a *Picea abies* wood disc from Sweden (Menkis et al. 2004). According to Kolaczkowski and Thorton (2004), in the real world gene, sequences are not identically distributed and evolve heterogeneously, and in these cases, maximum parsimony performs better than Bayesian analyses. Moreover, due to the Farris zone, in our analyses, it seems that the topology of maximum parsimony is favored over the Bayesian one (Philippe et al. 2005). The new sequences are also separated from the sequences of *S. niveocremeum* (AF347094) and *S. suecicum* (EU186667). On the other hand, the K2P pairwise distance among the six *Sistotremastrum* sequences (651 positions) included in Table 1 gave a low value (<0.00337) within the three sequences obtained in this study.

The similar morphological characters observed in these collections and the low genetic variability obtained among them, as well as the high genetic variability when comparing the sequences of these collections with those of *S. niveocremeum* and *S. suecicum*, led us to describe a new species:

Sistotremastrum guttuliferum Melo, M. Dueñas, Telleria & M.P. Martín, sp. nov. (Fig. 2)

MycoBank: MB 800173

Diagnosis This species differs from *Sistotremastrum niveocremeum* and *Sistotremastrum suecicum* by the small oil drops in the cytoplasm of its subicular hyphae and the spore size.

Holotype PORTUGAL, MADEIRA, Santana, Levada da Fajã da Nogueira, 32°45′1.59″N, 16°54′56.76″W, 780 m asl, on unidentified wood, 19.10.2006, 16566Tell. (MA-Fungi 82105).

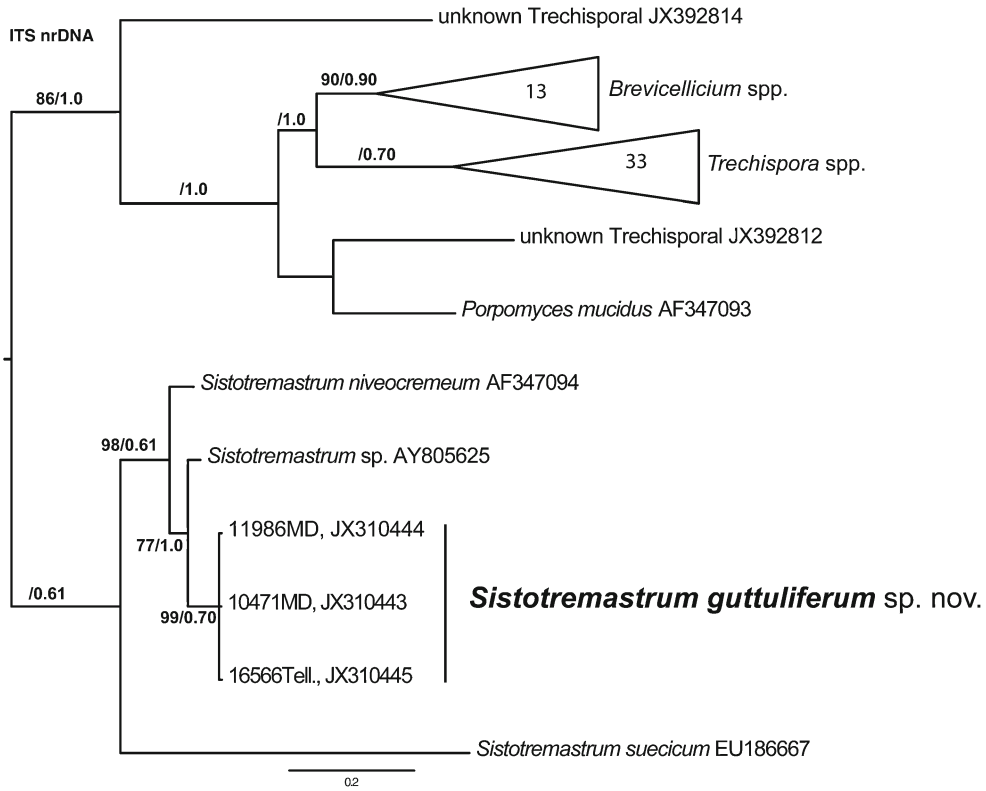

Fig. 1 The ITS nrDNA Bayesian 50 % majority rule consensus trees. In every collapsed clade, the number of sequences is indicated in the *triangle*. The percentages of bootstraps superior to 50 % (Felsenstein 1985) and the posterior probabilities superior to 0.50 (Ronquist and Huelsenbeck 2003) are indicated. The position of *Sistotremastrum guttuliferum* sp. nov. is highly supported (bs=90 %, pp=0.70)

Basidioma resupinate, effuse, adnate, thin, ceraceous to membranaceous; hymenophore whitish cream, when dry pale ochraceous, first almost smooth, under the lens porose, then more or less odontioid, after drying cracked transversally; margin indeterminate, gradually thinning out, in the periphery porose to pruinose. *Hyphal system* monomitic, hyphae with clamps, distinct, in the subiculum irregularly interwoven, thick-walled, 4–7(−10) μm wide, with small oil drops in the cytoplasm, in the subhymenium thinner, densely united, all with vertical hyphal direction. *Basidia* subclavate to subtubular, sometimes slightly constricted, walls thin but basally somewhat thickened, 15–20(−25)×(5–)6–7.5 μm, with six short sterigmata. *Spores* narrowly ellipsoid, thin-walled, 5–6.5×2.5–3 μm, without oil drops in the protoplasm, inamyloid, indextrinoid, and acyanophilous.

Material examined PORTUGAL, AZORES ARCHIPELAGO, Faial Island, Horta, Capelo, Cabeço Verde, Furna Ruim, 38°35′36.40″N, 28°48′9.55″W, 400 m asl, *Erica azorica*, 23. 2. 2005, 16097Tell. (MA-Fungi 76166); Horta, Ponta do Varadouro, 38°34′23.01″N, 28°46′43.04″W, 42 m asl, *Picconia azorica*, 21.2.2005, 10471MD (MA-Fungi 82100). Flores Island, St.ª Cruz das Flores, Vales, 39º26′ 48.8″N, 31º09′07.8″W, 245 m asl, *Pittosporum undulatum*, 26.3.2007, 17391Tell. (MA-Fungi 76283), 17392Tell. (MA-Fungi 76284); St.ª Cruz das Flores, Barrocas Altas, Chapel of N.ª Sr.ª das Flores, 39°26′17.24″N, 31°11′50.18″W, 580 m asl, *Cryptomeria japonica*, 27.3.2007, 10312IM, (LISU 211124), 10313IM, (LISU 211125), 17453Tell. (MA-Fungi 76285); St.ª Cruz das Flores, Alagoa, 39º28′ 26.7″N, 31º09′47.1″W, 180 m asl, *Pittosporum undulatum*, 27.3.2007, 11830MD, (MA-Fungi 76297), (TFCMic 18367); St.ª Cruz das Flores, Fazenda de St.ª Cruz, Ribeira da Fazenda, 39°27′21.56″N, 31°9′27.36″W, 215 m asl, *Acacia* sp., 28.3.2007, 10352IM, (LISU 211126), ibidem, *Pittosporum undulatum*, 28.3.2007, 11851MD (MA-Fungi

Table 1 Matrix of pairwise intra- and inter-specific Kimura-2-parameter (K2P) distance matrix among *Sistotremastrum* ITS nrDNA sequences

		1	2	3	4	5	6
1	*S. suecicum* EU186667	–					
2	*S. niveocremeum* AF347094	0.19553	–				
3	*Sistotremastrum* sp. AY805625	0.33214	0.05767	–			
4	*S. guttuliferum* 10471MD, JX310443	0.29736	0.07762	0.05405	–		
5	*S. guttuliferum* 16566Tell., JX310445	0.29393	0.07797	0.05615	0.00331	–	
6	*S. guttuliferum* 11986MD, JX310444	0.29912	0.08153	0.05601	0.00167	0.00337	–

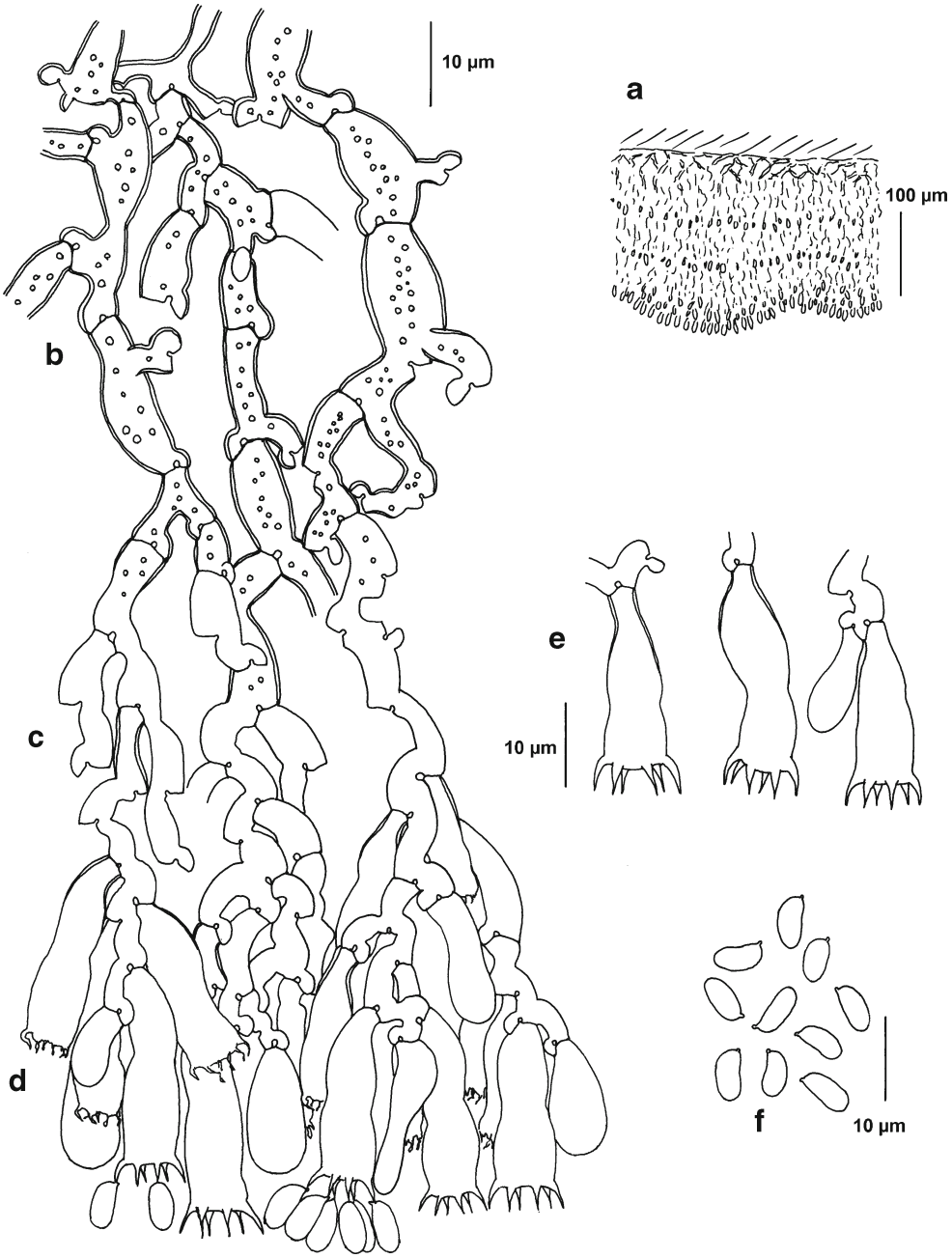

Fig. 2 *Sistotremastrum guttuliferum* (holotype, 16566Tell., MA-Fungi 82105). **a** Section through fruit-body; **b** subicular hyphae; **c** subhymenial hyphae; **d** hymenium; **e** basidia; **f** spores

75895); Lajes das Flores, next to Fazenda de Lajes, 39º24′ 08.7″N, 31º11′17.5″W, 400 m asl, *Erica scoparia*, 27.3.2007, 17417Tell. (MA-Fungi 76282). Pico Island, São Roque, Mistério da Prainha, 38°29′0.40″N, 28°14′ 27.96″W, *Pittosporum undulatum*, 25.2.2005 (TFCMic 15275); São Roque, Meia Encosta de Santa Luzia, Travessa de Cima, 38°30′49.96″N, 28°22′51.32″W, 600 m asl, *Myrica faya*, 26.2.2005, 10590MD (MA-Fungi 76101), (TFCMic 15302); ibidem, *Pittosporum undulatum*, 26.2.2005, (TFCMic 15290). S. Miguel Island, Nordeste, Outeiro Alto, Miradouro da Tronqueira, 37º37′06.1″N, 25º11′54.2″W, 386 m asl, *Laurus azorica* (TFCMic 18508); Vila Franca do Campo, Água de Alto, Ribeira da Praia, 37°43′57.15″N, 25°28′10.26″W, 215 m asl, *Acacia* sp., 29.3.2007, 10382IM (LISU 211127); Ribeira Grande, near Caldeiras, 37°48′1.21″N, 25°29′23.94″W, 220 m asl, *Acacia* sp., 30.3.2007, 10417IM (LISU 211128), 10421IM (LISU 211129); ibidem, *Pittosporum undulatum*, 31.3.2007, 17533Tell. (MA-Fungi 76287); Ponta Delgada, Capelas, near Pico do Cedro, 37°49′6.74″N, 25°42′11.36″W, 310 m asl, *Acacia* sp., 1.4.2007, 10525IM (LISU 211130), 10532IM (LISU 211131); ibidem, *Pittosporum undulatum*,

11986MD (MA-Fungi 82107). Terceira Island, Angra do Heroísmo, Monte Brasil, 38°38′43.12″N, 27°13′22.26″W, 140 m asl, *Pittosporum undulatum*, 2.3.2005, 16228Tell. (MA-Fungi 76140), 9109IM (LISU 178604); Angra do Heroísmo, near Algar do Carvão, Terra Brava, 38°44′ 22.41″N, 27°12′24.70″W, 670 m asl, unidentified wood, 1.3.2005, 16212Tell. (MA-Fungi 76128). – MADEIRA, Porto Moniz, Fanal, 32°48′52.58″N, 17°08′51.09″W, 1135 m asl, *Ocotea foetens*, 22.10.2006, 9480IM (LISU 211313); Santana, Levada da Fajã da Nogueira, 32°45′1.59″N, 16°54′ 56.76″W, 780 m asl, *Erica scoparia*, 19.10.2006, 16565Tell. (MA-Fungi 82104), 16567Tell. (MA-Fungi 82106); Machico, Portela, from Portela to Funduras, 32°45′2.95″N, 16°48′ 41.64″W, 600 m asl, *Pinus pinaster*, 18.10.2006, 16353Tell. (MA-Fungi 82103); ibidem, unidentified wood, 16337Tell. (MA-Fungi 82102). – SPAIN. CANARY ISLANDS, La Gomera Island, Hermigua, Parque Nacional de Garajonay, Monte de El Cedro, Las Mimbreras, 28º07′36.53″N, 17º13′16.74″W, 947 m asl, *Laurus novocanariensis*, 2.2.2007, 10105IM (LISU 240482), 10104IM (LISU 240481). Tenerife Island, El Rosario, road La Laguna to Las Cañadas del Teide, pista del Bailadero, next to Las Lagunetas, 28º24′57.58″N, 16º24′ 09.52″W, 1,400 m asl, *Pinus canariensis*, 31.1.2007, 11550MD (MA-Fungi 82101).

Etymology Referring to oil drops in the cytoplasm of hyphal elements.

Known distribution Macaronesian region: Azores Archipelago, Madeira and Canary Islands.

From a morphological point of view, the new species is characterized by the small oil drops in the cytoplasm of subicular hyphae. It differs from *Sistotremastrum niveocremeum* by the size and shape of spores, subcylindrical to allantoid, 6–9×2.5–3(−4) μm in this last species and narrowly ellipsoid, 5–6.5×2.5–3 μm in *Sistotremastrum guttuliferum*. The spores of *Sistotrematrum suecicum* are also narrowly ellipsoid but differ by size, 4.5–6×1.5–2 μm.

Key to species

1. Cystidia present (leptocystidia)	*S. lateclavigerum*
1. Cystidia absent	2
2. Subicular hyphae with oil drops in the cytoplasm. Spores narrowly ellipsoid, 5–6.5×2.5–3 μm	*S. guttuliferum*
2. Subicular hyphae without oil drops in the cytoplasm	3
3. Spores subcylindrical to allantoid, 6–9×2.5–3(−4) μm	*S. niveocremeum*
3. Spores narrowly ellipsoid, 4.5–6×1.5–2 μm long	*S. suecicum*

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