



## *Sistotremastrum chilensis* (Trechisporales, Basidiomycota), a new species from Chilean Patagonia

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### Abstract

*Sistotremastrum chilensis* sp. nov. is described and illustrated on the basis of morphological and molecular data. Specimens of this corticioid fungus were collected in Huinay Reserve, in the Valdivian temperate rainforest of the Chilean Northern Patagonian region. Affinities with other species of the genus are discussed.

### Resumen

Sobre la base de datos morfológicos y moleculares se describe e ilustra la especie nueva *Sistotremastrum chilensis*. Los especímenes de este hongo corticioide se recolectaron en la Reserva de Huinay, en el bosque húmedo valdiviano de la región chilena del norte de Patagonia. Se discuten las afinidades de esta especie con las del resto del género.

**Key words:** Chile, Comau fjord, ITS nrDNA, taxonomy, wood-inhabiting fungi

### Introduction

According to Gorjón & Hallenberg (2013) few studies about corticioid fungi have been focussed in the Chilean Patagonia and 94 species has been reported in their check-list. During an investigation in Huinay Reserve in the Chilean fjords region, we collected several specimens which correspond to *Sistotremastrum* Eriksson (1958: 62), but do not fit any described species of this genus.

*Sistotremastrum* is a small and cosmopolitan genus of corticioid fungi described to accommodate two species: *Sistotremastrum suecicum* Litsch. ex Eriksson (1958: 62), as type species, and *Corticium niveocremaum* Höhnelt & Litschauer (1908: 1117). Four species have been placed in this genus. *Sistotremastrum suecicum*, originally described from Sweden, is widely distributed in Europe, including the Macaronesian region, as well as Canada and USA (Eriksson *et al.* 1984, Telleria *et al.* 2008, 2009, Ginns & Lefebvre 1993); it has also been reported from China (Dai *et al.* 2004) and Southern Argentina (Greslebin 2001, Greslebin & Rajchenberg 2003). Additionally, Boidin & Gilles (1994a) studied three *Sistotremastrum* specimens (LY 11617, 14084, 14246) from Reunion Island which were identified as *Sistotremastum* cf. *suecicum*, differing by the hyphae, basidia and spores sizes.

*Sistotremastrum niveocremaum* (Höhnelt & Litschauer) Eriksson (1958: 62), originally described from Austria, is common in Europe including Macaronesian islands (Kotiranta & Saarenoksa 1990, Beltrán-Tejera 2004, Telleria *et al.* 2009), the formerly Soviet Union, Turkey, Caucasus region and Iran (Hallenberg 1978, Ghobad-Nejhad *et al.* 2009, Ghobad-Nejhad 2011, Ghobad-Nejhad & Hallenberg 2012); it has also been reported from China (Dai *et al.* 2004), India (Hjortstam & Ryvarden 2007) and Japan (Maekawa 1993), as well as North America (Ginns & Lefebvre 1993) and South America where it is known from Brazil (Hjortstam & Bononi 1987), Colombia (Hjortstam & Ryvarden 1997) and Southern Argentina (Greslebin & Rajchenberg 2003). The other two species present a more restricted distribution. *Sistotremastrum lateclavigerum* Boidin & Gilles (1994b: 217), described from France, is only known from its type locality, and *Sistotremastrum guttuliferum* Melo, M. Dueñas, Telleria & M.P. Martín in Telleria *et al.* (2013), described from Madeira Island, is also found in the Azores Archipelago and Canary Islands in the Macaronesian region.

The aim of this study was to identify, characterize and analyse the specimens of *Sistotremastrum* from Huinay Reserve referred to above on morphological and molecular grounds. As a result a new species is proposed and described.

## Materials and Methods

**Taxon sampling and morphological studies:**—Three specimens of *Sistotremastrum* from Huinay, Los Lagos Region, Chile, were studied. Vouchers are deposited in MA-Fungi herbarium. Measurements and drawings were made from microscopic sections mounted in 3% aqueous KOH and examined at magnification up to 1250 × using an Olympus BX51 microscope. The length and width of 30 spores and 10 basidia were measured from each sample. Line drawing was made with a Leyca DM2500 microscope with aid of a drawing tube by M. Dueñas.

**Molecular analysis:**—A portion of specimens 19594Tell./MA-Fungi 86366, and 19610 Tell./MA-Fungi 86368 were crushed on two FTA® Indicating Micro Cards (Cat N° WB120211, Whatman, Maidstone, England). A Harris punch was used to cut out a 2-mm plug and transferred into a thin-wall microcentrifuge tube. The extraction of the genomic DNA from the FTA was done as recommended by the manufacturer. Briefly, three washes with 200 µl of FTA purification reagent and two more washes with 1 × TE buffer (10 mmol/L Tris, 0.1 mmol/L EDTA, pH 8.0); later the FTA paper was air dried at room T for a minimum of one h, and the DNA was eluted from the plug with 50 µl pure sterile double distilled water by incubation at 95 °C for 15 min. The PCR was performed with one µl of the eluted DNA and one µl of each primer, ITS1F (White *et al.* 1990) and ITS4 (Gardes & Bruns 1993), using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) as described in Winka *et al.* (1998) following thermal cycling conditions in Martín & Winka (2000). Before sequencing, 20 µl of the amplifications products were cleaned with 8 µl of 1:10 ExoSAP-IT® (USB Corporation, OH, USA).

Sequences were compared with homologous *Sistotremastrum* sequences discussed in Telleria *et al.* (2013). Maximum parsimony analyses were performed using the program PAUP\* 4.0b10 for Macintosh (Swofford 2003). Exhaustive searches were conducted, without rooting the tree and gaps treated as missing data. To assess homoplasy levels, consistency index (CI) and retention index (RI) were calculated from each exhaustive search. Kimura-2-parameter (K2P) pairwise distances were obtained using PAUP\* 4.0b10.

The new consensus sequences have been lodged in the EMLB-EBI database with the accession numbers HG315520 (19594Tell./MA-Fungi 86366) and HG315521 (19610Tell./MA-Fungi 86368).

## Results

The two ITS nrDNA sequences obtained in this study were aligned with six *Sistotremastrum* sequences included in Telleria *et al.* (2013). A matrix of 651 unambiguously aligned nucleotide positions was produced (502 constant, 113 parsimony-uninformative, and 36 parsimony-informative). The number of trees evaluated by exhaustive search was 10395 and only two were retained (166 steps long, CI = 0.8542, RI = 0.8906). Topological differences between the two trees are minor, and only one is shown in Fig. 1; the two ITS sequences of the specimens from Huinay appeared together in a highly supported clade (BS = 100 %), as a sister group of the cluster formed by the three sequences of *Sistotremastrum guttuliferum* (Azores and Madeira), and one *Sistotremastrum* sp. (Sweden).

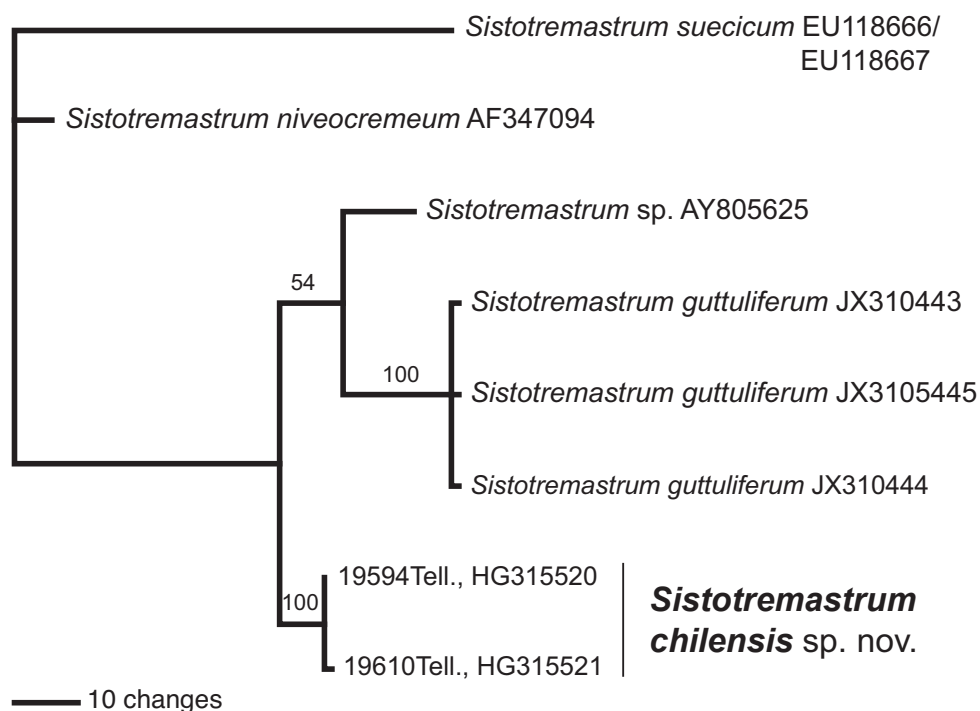
On the other hand, the K2P matrix distance (Tab. 1) among the eight *Sistotremastrum* sequences gave a low value (0.00165) between the two sequences from Huinay. The large genetic distance between the sequences obtained from 19594Tell./MA-Fungi 86366 and 19610 Tell./MA-Fungi 86368 specimens with those of the *S. guttuliferum* (0.05599–0.05809) and the sequence under *Sistotremastrum* sp. (0.05109–0.05298), led us to describe a new species.

## Taxonomy Treatment

*Sistotremastrum chilensis* Telleria, M. Dueñas & M.P. Martín, *sp. nov.* (Fig. 2)  
Mycobank MB804754

Type:—CHILE, Los Lagos (X Region), Palena province, Comuna Hualaihué, Comau fjord, Huinay Reserve, path to Cerro del Tambor, 100 m, 42°22'44.5"S 72°24'25.8"W, on unidentified wood, 26 April 2012, *M. Dueñas, M.P. Martín & M.T. Telleria*, 19610Tell. (holotype, MA-Fungi 86368!)

Diagnosis:—This new species is closely related to *S. guttuliferum* but differs by the shape of subicular and subhymenial hyphae and its unique ITS sequence.



**FIGURE 1.** One of the two trees for *Sistotremastrum* ITS nrDNA sequences obtained from an exhaustive search with PAUP\* 4.0b10. Parsimony bootstrap support ( $\geq 50\%$ ) indicated above branches.

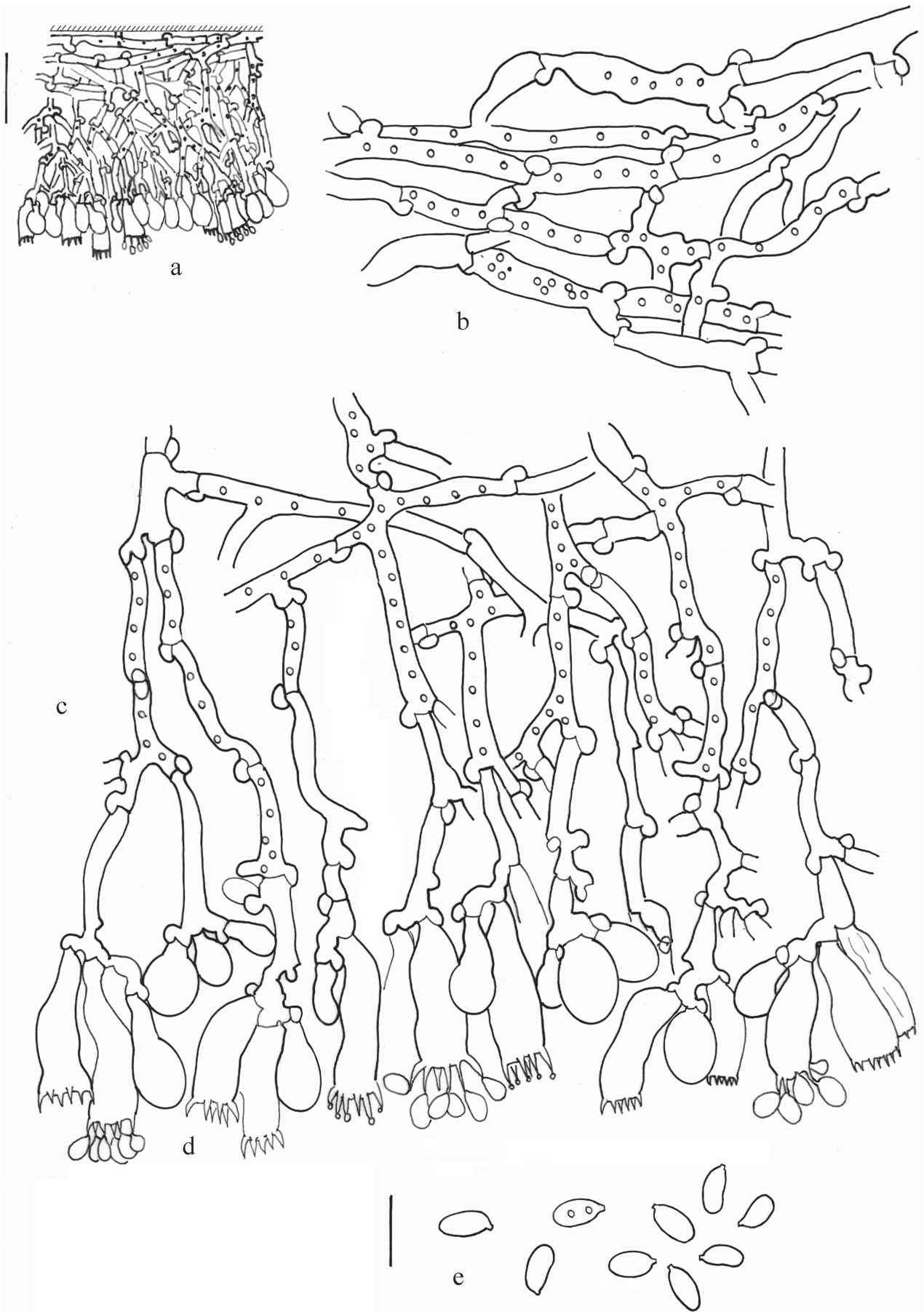
**TABLE 1.** Matrix of pairwise intra- and inter-specific Kimura-2-parameter (K2P) distance matrix among *Sistotremastrum* ITS nrDNA sequences.

	1	2	3	4	5	6	7	8
1 <i>S. suecicum</i> EU186667	-							
2 <i>S. niveocreameum</i> AF347094	0.19553	-						
3 <i>Sistotremastrum</i> sp. AY805625	0.33145	0.05767	-					
4 <i>S. guttuliferum</i> JX310443	0.29381	0.07762	0.05405	-				
5 <i>S. guttuliferum</i> JX310445	0.29039	0.07797	0.05615	0.00331	-			
6 <i>S. guttuliferum</i> JX310444	0.29545	0.08153	0.05601	0.00167	0.00337	-		
7 <i>S. chilensis</i> 19594Tell., HG315520	0.29803	0.05459	0.05298	0.05599	0.05795	0.05784	-	
8 <i>S. chilensis</i> 19610Tell., HG315521	0.30261	0.05469	0.05109	0.05612	0.05809	0.05786	0.00165	-

Basidioma resupinate, adnate, very thin; hymenophore whitish, under the lens reticulate to porose; margin indeterminate. Hyphal system monomitic, hyphae with clamps, distinct; subicular hyphae up to 6  $\mu\text{m}$  wide, irregular and sometimes arranged parallelly next to the substrate; subhymenial hyphae thinner, 3–4  $\mu\text{m}$  wide, straighter and with vertical direction; both with small oil drops in the cytoplasm, and loosely interwoven. Cystidia absent. Basidia subclavate to subtubular, thin-walled, with a basal clamp, (16–)21–27  $\times$  6.5–8  $\mu\text{m}$ , and six to eight short, 3  $\mu\text{m}$  long sterigmata. Spores narrowly ellipsoid, thin walled, 5–6.5  $\times$  2.5–3  $\mu\text{m}$ , inamyloid, indextrinoid and acyanophilous.

**Etymology:**—Relating to Chile

**Known distribution:**—Valdivian temperate rainforest in Chilean Northern Patagonian region.



**FIGURE 2.** *Sistotremastrum chilensis* MA-Fungi 86368. a. Section through basidiome; bar = 25  $\mu$ m. b. Basal hyphae. c. Subhymenial hyphae. d. Detail of hymenium with basidia and probasidia. e. Spores. bar = 10  $\mu$ m (19610Tell., MA-Fungi 86368, holotypus)



**Additional specimens examined:**—CHILE. Los Lagos (X Region), Palena province, Comuna Hualaihué, Comau fjord, Huinay Reserve, path to Cerro del Tambor, 100 m, 42°22'44.5"S 72°24'25.8"W, on unidentified wood, 26 April 2012, *M. Dueñas, M.P. Martín & M.T. Telleria*, 19594Tell. (MA-Fungi 86366!). Los Lagos (X Region), Palena province, Comuna Hualaihué, Comau fjord, Huinay Reserve, 147 m, 42°22'9.0"S 72°24'42.7"W, on *Lophosoria quadripinnata*, 30 April 2012, *M. Dueñas, M.P. Martín & M.T. Telleria*, 19709Tell. (MA-Fungi 86367!).

## Discussion

From a morphological point of view, *Sistotremastum chilensis* is related to *S. guttuliferum*. Both share subicular hyphae with small oil drops in the cytoplasm and narrowly ellipsoid spores, 5–6.5 × 2.5–3 µm, but differ by the shape of basal and subhymenial hyphae, composed by rather short and wide cells in *S. guttuliferum* (Telleria *et al.* 2013) and long and thin ones in *S. chilensis*. Both also differ in their distribution: Macaronesian region, in the North Atlantic Ocean, and Chilean Northern Patagonia region respectively. The spores of *S. suecicum* are also narrowly ellipsoid but smaller, 4.5–6 × 1.5–2 µm, and *S. niveocremeum* has subcylindrical to allantoid spores, 6–9 × 2.5–3(–4) µm (Eriksson *et al.* 1984). *Sistotremastrum lateclavigerum* is the only species known in the genus with cystidia (Boidin & Gilles 1994b).

## Key to species

1. Cystidia present (leptocystidia)..... *S. lateclavigerum*
- Cystidia absent ..... 2
2. Subicular hyphae with oil drops in the cytoplasm. Spores narrowly ellipsoid, 5–6.5 × 2.5–3 µm ..... 3
- Subicular hyphae without oil drops in the cytoplasm ..... 4
3. Hyphae composed by rather short and wide cells. Subicular hyphae (10–)14–18 × (6–)7–9 µm and subhymenial hyphae 8–12 × 4–5 µm ..... *S. guttuliferum*
- Hyphae composed by long and thin cells. Subicular hyphae (14–)20–23 × 4–6 µm and subhymenial hyphae 15–23(–38) × 3–4 µm ..... *S. chilensis*
4. Spores subcylindrical to allantoid 6–9 × 2.5–3(–4) µm ..... *S. niveocremeum*
- Spores narrowly ellipsoid, 4.5–6 × 1.5–2 µm ..... *S. suecicum*

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