A new species of *Acanthostigma* (Tubeufiaceae, Dothideomycetes) from the southern hemisphere

R.M. Sánchez

*Laboratorio de Estudios Básicos y Biotecnológicos de Algas y Hongos, CERZOS-CCT-CONICET y U.N.S., Camino La Carrindanga Km 7, B8000FWB Bahía Blanca, Buenos Aires, Argentina*

A.N. Miller

*University of Illinois, Illinois Natural History Survey, 1816 South Oak Street, Champaign, Illinois 61820-6970, USA*

M.V. Bianchinotti

*Laboratorio de Estudios Básicos y Biotecnológicos de Algas y Hongos, CERZOS-CCT-CONICET y U.N.S., Camino La Carrindanga Km 7, B8000FWB Bahía Blanca, Buenos Aires, Argentina*

**Abstract:** A new species belonging to the Dothideomycete genus *Acanthostigma* is described from bark of two *Nothofagus* species from Argentina. Its identity as a new species is based on both morphology and molecular sequence data. *Acanthostigma patagonica* differs from other species in the genus by having larger ascomata and setae and wider, asymmetrical ascospores. An amended key to *Acanthostigma* species is provided along with a discussion of other species previously described from South America.

**Key words:** ITS, LSU, *Nothofagus*, phylogenetics, southern hemisphere, systematics

**INTRODUCTION**

In South America and particularly in Argentina several groups of ascomycetes have been insufficiently documented. The Andean Patagonian forests are considered one of the most interesting biogeographical formations of Argentina, however they have been categorized as one of the most poorly studied fungal reservoirs of the world (Lodge et al. 1995). Since 2005 the principal aim of our work has been to increase the knowledge of the biodiversity of ascomycetes on trees native to the Andean Patagonian forests. Many of the species we have found are related to North American and Australasian species, which occupy habitats with similar environmental conditions. In this work we considered the genus *Acanthostigma*, in which almost all the species have been described from the northern hemisphere. However, due to the small size of the ascomata, specimens of *Acanthostigma* and generally all Tubeufiaceae have been overlooked and their known distribution reflects more of the collecting activities of mycologists instead of their actual geographical distribution (Rossman 1987).

The systematic position of *Acanthostigma* de Not. (de Notaris 1863) has a long history that has yet to be resolved. After being transferred in and out of the family Tubeufiaceae several times (Saccardo 1883; Ellis and Everhart 1892; von Arx and Müller 1975; Barr 1980, 1990, 1993; Crane et al. 1998) it finally was redescribed and placed in this family by Réblova and Barr (2000). This placement was supported by molecular phylogenetic analyses (Tsui et al. 2006, 2007; Promputtha and Miller 2010).

The genus *Acanthostigma* was known previously only from the northern hemisphere (Table I) because most of the species have been described from Asia, Europe and North America. The distribution of *Acanthostigma* was extended to the southern hemisphere with the report of *Acanthostigma minutum* (Fuckel) Sacc. from Argentina (Sánchez and Bianchinotti 2010).

Through our work on the biodiversity of ascomycetes on trees native to the Andean Patagonian forests in Argentina a putative new species of *Acanthostigma* was found and herein is described, illustrated and compared morphologically and genetically to other known species in the genus. We also include in the phylogenetic analyses the other species reported from Argentina, *A. minutum*, and discuss its phylogenetic relationships with North American species. The key to all accepted species in the genus provided by Promputtha and Miller (2010) is amended to include the new species.

**MATERIALS AND METHODS**

**Morphological characterization.**—Samples were collected in forests of Los Alerces National Park (Chubut) and Lanín National Park (Neuquén) in the Andes of Patagonia (Argentina). The vegetation is composed mostly of native *Nothofagus* species together with some species of *Cupressaceae*, *Proteaceae*, ferns and mosses. Leaves, small branches and bark showing fungal growth when observed with a field magnifying lens were placed in paper bags and transported to the laboratory. The samples were dried at room temperature and deposited at Bahía Blanca Biology Herbarium (BBB). For collections made in 2009 a GPS eTrex Legend (Garmin Co., USA) was used to obtain map

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Corresponding author. E-mail: rsanchez@uns.edu.ar
coordinates. For microscopic examinations sections were cut freehand under a Leica EZ4 stereo microscope and mounted in water or 5% KOH with phloxine. A Leica DM2000 dissecting microscope with a Samsung NV10 digital camera was used to capture micromorphological images. At least 10 measurements were taken for each structure mounted in tap water. Averages for asci and ascospores were calculated, invariable sites and across site variation were optimized under these parameters: The above models were implemented in Modeltest 3.7 (Posada and Crandall 1998) as implemented in SeaView 4.1 (Gouy et al. 2010) and used for model selection. Maximum likelihood (ML) analyses were conducted with PhyML (Guindon and Gascuel 2003) as implemented in SeaView 4.1 (Gouy et al. 2010) using the remaining 9000 trees.
ACANTHOSTIGMA PATAGONICA

Anamorph. Scattered on bark of Nothofagus alpina in a mixed forest of N. alpina, N. dombei and N. obliqua and on bark of N. pumilio in a pure forest.

Habitat. Scattered on bark of Nothofagus alpina in a mixed forest of N. alpina, N. dombei and N. obliqua and on bark of N. pumilio in a pure forest.

Taxonomy

Acanthostigma patagonica R.M. Sánchez, A.N. Mill. et Bianchin., sp. nov. MycoBank MB561057


Type. ARGENTINA. NEUQUÉN PROVINCE: Pue. Nacional Lanín, on Provincial Route Number 48, before reaching Playas de Yuco, on bark of N. pumilio, 15-V-2007, M.V. Bianchiniotti, R.M. Sánchez, MVB574, BBB; NEUQUÉN PROVINCE: Pue. Nacional Lanín, Paso del Córdoba, 40° 35′ 44″S, 71° 08′ 37″W, 1245 m, on N. pumilio, 18-I-2009, M.V. Bianchiniotti, R.M. Sánchez, MVB776, BBB.

Etymology. Refers to the geographical place in Argentina where it was found.

Ascomata superficial to semi-immersed, globose, subglobose to conical, dark brown to bright black when dried, surface densely setose, 100–390 μm diam, 140–490 μm high. Setae mostly distributed on entire ascoma but sometimes only on the upper half, straight or curved, with acute tip, 0–4-septate, dark brown, 49–125 μm long, 5–13 μm wide at the base, 1–5 μm wide at the apex. Ostiole papillate, 30–113 × 50–100 μm. Ascomatal wall of textura angularis, two-layered in longitudinal section, inner layer composed of 4–6 rows of thin walled, flattened to angular, pale ochreous cells, 5–13 × 3–8 μm; outer layer composed of 5–7 rows of thick walled, elongated, dark brown cells, 8–15 × 4–10 μm. Pseudoparaphyses narrow, ramified, septate, forming a strong mesh, hyaline, 1–3 μm thick. Periphyses filling the entire papilla, undulated, hyaline. Ascii bitunicate, cylindrical to clavate, short stipitate, eight-spored, 85–139 × 15–33 μm (110 × 20 μm, n = 16). Ascosporae elongate fusiform, straight or slightly curved, ends asymmetrical, tapering at both ends, with one or two middle cells near the apical end slightly broader than the others, 9–18 septate, slightly constricted at the septa, smooth, hyaline, 62–111 × 5–10 μm (85 × 7.5 μm, n = 100). Anamorph not known.

Habitat. Scattered on bark of Nothofagus alpina in a mixed forest of N. alpina, N. dombei and N. obliqua and on bark of N. pumilio in a pure forest.


Results

The final ITS and LSU alignments contained 57 and 75 taxa and consisted of 325 and 536 bp respectively after the removal of missing data and ambiguous regions. The ML trees based on ITS and LSU data are provided (Figs. 3, 4 respectively). GenBank accession numbers are given after taxon names in the phylogenetic trees. In both analyses A. patagonica occurs closely related to Helicoma vaccinii in the moderately supported Tubeufia cerea clade and is highly supported as a sister taxon to H. vaccinii in the LSU tree. Acanthostigma patagonica and H. vaccinii possess 14 bp differences out of 407 bp (3.5%) in the unadulterated ITS dataset and nine bp differences out of 638 bp (1.4%) in the LSU dataset before the removal of highly conserved regions. The ITS data are based on the highly conserved 5.8S
region and the variable ITS2 region. Although the entire ITS region was successfully PCR amplified for the new species, the sequence quality of the ITS1 region unfortunately was poor so intraspecific comparisons of complete ITS sequences cannot be made at this time. No anamorph was found associated with *A. patagonica* on the substrates.

**DISCUSSION**

The new species of *Acanthostigma* was compared to all nine species currently accepted in the genus. *Acanthostigma patagonica* is most similar to *A. multisepatum* Promputtha & A.N. Mill. in that both species possess long ascospores with the greatest number of

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septa in the genus. However *A. patagonica* has slightly larger ascomata and setae and possesses wider ascospores that are asymmetrical. *Acanthostigma patagonica* also can be compared with *A. filiforme* Promputtha & A.N. Mill. and *A. scopulum* (Cooke & Ellis) Peck, which overlap with *A. patagonica* in ascospore length and number of septa. However both species can be distinguished from *A. patagonica* by their narrower ascospores. *Acanthostigma patagonica* is not closely related to any other *Acanthostigma* species in the phylogenetic analyses (Figs. 3, 4).

According to Sánchez and Bianchinotti (2010), from the species of *Acanthostigma* described by Spegazzini (1884, 1887, 1898, 1909), three (*A. 
Tubeufiaceae phylogeny (ln L = -1883.7) generated from a PhyML analysis of 57 ITS sequences showing the phylogenetic placement of *A. patagonica* (in boldface). Thickened branches indicate Bayesian posterior probabilities ≥ 95%, while numbers above or below branches refer to PhyML bootstrap values ≥ 50%. The tree is rooted with taxa in the *Tubeufia cerea* clade based on the LSU analysis.
Fig. 4. Tubeufiaceae phylogeny (ln L = −2619.3) generated from a PhyML analysis of 75 LSU sequences showing the phylogenetic placement of the newly sequenced taxa (in boldface) in this study. Thickened branches indicate Bayesian posterior probabilities ≥ 95%, while numbers above or below branches refer to PhyML bootstrap values ≥ 50%. Two species of *Botryosphaeria* are outgroups.
dimerosporioides, A. gnaphaliorum and A. imperspicuum) should be excluded because they possess three-septate ascospores and appear to have unitunicate asci. The fourth, A. guaraniticum, considered species dubious, differs from A. patagonica in having smaller, 5–6-septate ascospores (35 × 6–6.5 μm).

Sánchez and Bianchinotti (2010) reported A. minutum from Lanín National Park, Neuquén Province, Argentina, on Nothofagus dombeyi and N. pumilio. They concluded that their specimens morphologically agreed with A. minutum (Fuckel) Sacc., differing only in a lesser number of septa in the ascospores. However in the present phylogenetic analyses the material from South America does not group with the North American specimens (Fig. 4) suggesting this may be a new species. Additional specimens of putative A. minutum from Argentina should be included in further phylogenetic analyses before a new species is described.

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