

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

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**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 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

ascospores, 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 Réblová 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

TABLE I. Ecological information on the accepted species of *Acanthostigma*

Taxa	Known hosts	Distribution
<i>A. ellisii</i>	On <i>Abies alba</i> and on stromata of <i>Diatrype stigma</i> .	<i>Europe</i> : Germany, Norway, Poland, Slovakia (Robak 1976). <i>North America</i> : USA (Réblová and Barr 2000).
<i>A. filiforme</i>	Decorticated wood on ground of coniferous-deciduous forest.	<i>North America</i> : USA (Promputtha and Miller 2010).
<i>A. longisporum</i>	<i>Calluna vulgaris</i> , <i>Dryas octopetala</i> , <i>Rhododendron ferrugineum</i> , <i>R. hirsutum</i> , <i>Rhodothamnus chamaecistus</i> , <i>Vaccinium myrtillus</i> , <i>V. uliginosum</i> .	<i>Europe</i> : Austria, Norway (Réblová and Barr 2000).
<i>A. minutum</i>	<i>Fagus sylvatica</i> , <i>Gaultheria shallon</i> , <i>Nothofagus pumilio</i> , <i>Populus</i> sp., <i>Quercus</i> sp. and on old xylariaceous stromata on <i>Nothofagus dombeyi</i> .	<i>Asia</i> : China, Taiwan. <i>Europe</i> : France, Germany, Switzerland. <i>North America</i> : Canada, USA (Réblová and Barr 2000). <i>South America</i> : Argentina (Sánchez and Bianchinotti 2010).
<i>A. multiseptatum</i>	Decorticated wood on ground of coniferous-deciduous forest.	<i>North America</i> : USA (Promputtha and Miller 2010).
<i>A. patagonica</i>	On bark of <i>Nothofagus alpina</i> and <i>N. pumilio</i> .	<i>South America</i> : Argentina (this paper).
<i>A. perpusillum</i>	On inner side of bark of <i>Cerasus</i> sp., on <i>Elletaria</i> sp. and on decayed wood of <i>Quercus</i> sp.	<i>Asia</i> : Java. <i>Europe</i> : Austria, France, Italy, Switzerland. <i>North America</i> : Canada, USA. <i>South America</i> : Grenada, Venezuela (Réblová and Barr 2000).
<i>A. revocatum</i>	<i>Quercus</i> sp. and <i>Salix vitellina</i> .	<i>Europe</i> : Italy (Réblová and Barr 2000).
<i>A. scopulum</i>	<i>Fagus sylvatica</i> , <i>Pinus</i> sp., <i>Tsuga</i> sp.	<i>Asia</i> : China, Taiwan (Chang 2003, Kodsueb et al. 2004). <i>Europe</i> : Austria. <i>North America</i> : USA (Réblová and Barr 2000).
<i>A. septoconstrictum</i>	Decorticated wood on ground of coniferous-deciduous forest.	<i>North America</i> : USA (Promputtha and Miller 2010).

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 are in parentheses. Material was mounted in calcofluor 0.5% for examination under a Nikon Eclipse 80i fluorescence microscope with a Nikon DXM 1200F camera system.

**DNA extraction and sequencing.**—The extraction, amplification and sequencing of DNA followed that of Promputtha and Miller (2010). The 5.8S and internal transcribed spacer 2 (ITS) region along with the first 600 bp of the 5' end of 28S large subunit (LSU) nrDNA, which contains the variable D1 and D2 regions, were sequenced.

**Phylogenetic analyses.**—The ITS and LSU datasets, which were amended from Promputtha and Miller (2010), were aligned in Sequencher 4.9 (Gene Codes Corp., Ann Arbor, Michigan) and manually corrected by eye. Six and two ambiguous regions consisting of 82 and 25 bp were delimited by eye and excluded from the ITS and LSU datasets respectively. The first 66 bp in the 5' end and the last 11 bp in the 3' end of LSU also were excluded due to missing characters in most taxa. The Akaike information criterion (AIC) (Posada and Buckley 2004) as implemented in

Modeltest 3.7 (Posada and Crandall 1998) was used to determine the best-fit model of evolution for each dataset, which was the HKY model (Hasegawa et al. 1985) for the ITS dataset and the SYM model (Zharkikh 1994) for the LSU dataset. Both models included a proportion of invariable sites with the remaining sites subjected to a gamma distribution shape parameter. Maximum likelihood (ML) analyses were conducted with PhyML (Guindon and Gascuel 2003) as implemented in SeaView 4.1 (Gouy et al. 2010) under these parameters: The above models were implemented, invariable sites and across site variation were optimized and 1000 bootstrap replicates were performed from a BioNJ starting tree employing the best of nearest neighbor interchange (NNI) and subtree pruning and regrafting (SPR) branch swapping. Bayesian analyses employing Markov chain Monte Carlo (MCMC) were performed on each dataset with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) as an additional means of assessing branch support. Constant characters were included, the above models of evolution were implemented and 10 000 000 generations were sampled every 1000th generation resulting in 10 000 total trees. The Markov chain always achieved stationarity after the first 10 000 generations, so the first 1000 trees, which extended well beyond the burn-in phase of each analysis, were discarded. Posterior probabilities were determined from a 95% consensus tree generated in PAUP 4.0b10 (Swofford 2002) using the remaining 9000 trees.

## TAXONOMY

***Acanthostigma patagonica*** R.M. Sánchez, A.N. Mill. et Bianchin., sp. nov. FIGS. 1–2  
MycoBank MB561057

Ascomata superficialia vel immersa, globosa, subglobosa ad conica, atro-brunnea, 100–390  $\mu\text{m}$  diam, 140–490 alta; superficies setosa, setis brunneis, rectis vel curvis, 0–4 septatis, 49–125  $\mu\text{m}$  longis, 5–13  $\mu\text{m}$  in basi latis, 1–5  $\mu\text{m}$  in apice latis. Papillae 30–113  $\times$  50–100  $\mu\text{m}$ . Peridium textura angulari, cellulis brunneis, polyedricis, intra lutescentibus. Pseudoparaphyses tenues, septatae, hyalinae, ramosae, 1–3  $\mu\text{m}$ , reticulum formantes. Periphyses undulatae, hyalinae. Asci bitunicati, cylindrico-clavati, 8-sporei, 85–139  $\times$  15–33  $\mu\text{m}$ . Ascospores fusiformes, ad extremitates decrescentes et dissimiles, hyalinae, laeves, 8–18-septatae, septa constricta, 1–2 cellulis mediis latoribus 62–111  $\times$  5–10  $\mu\text{m}$ .

*Type.* ARGENTINA. NEUQUÉN PROVINCE: Pque. Nacional Lanín, on Provincial Route Number 48, before reaching Playas de Yuco, on bark of *Nothofagus alpina*, 17-V-2007, M.V. Bianchinotti, R.M. Sánchez, MVB573 (HOLOTYPE designated here, 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  $\mu\text{m}$  diam, 140–490  $\mu\text{m}$  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  $\mu\text{m}$  long, 5–13  $\mu\text{m}$  wide at the base, 1–5  $\mu\text{m}$  wide at the apex. Ostiole papillate, 30–113  $\times$  50–100  $\mu\text{m}$ . 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  $\times$  3–8  $\mu\text{m}$ ; outer layer composed of 5–7 rows of thick walled, elongated, dark brown cells, 8–15  $\times$  4–10  $\mu\text{m}$ . Pseudoparaphyses narrow, ramified, septate, forming a strong mesh, hyaline, 1–3  $\mu\text{m}$  thick. Periphyses filling the entire papilla, undulated, hyaline. Asci bitunicate, cylindrical to clavate, short stipitate, eight-spored, 85–139  $\times$  15–33  $\mu\text{m}$  (110  $\times$  20  $\mu\text{m}$ ,  $n = 16$ ). Ascospores 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  $\times$  5–10  $\mu\text{m}$  (83  $\times$  7.5  $\mu\text{m}$ ,  $n = 100$ ). Anamorph not known.

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

*Specimens examined.* ARGENTINA. CHUBUT PROVINCE: Pque. Nacional Los Alerces, on the way to Bagguilt Lake, on bark of *Nothofagus pumilio*, 15-V-2007, M.V. Bianchinotti, R.M. Sánchez, MVB542, BBB; NEUQUÉN PROVINCE: Pque. Nacional Lanín, on Provincial Route

Number 48, before reaching Playas de Yuco, on bark of *N. alpina*, 17-V-2007, M.V. Bianchinotti, R.M. Sánchez, MVB574, BBB; NEUQUÉN PROVINCE: Pque. Nacional Lanín, Paso del Córdoba, 40°35'44"S, 71°08'37"W, 1245 m, on *N. pumilio*, 18-I-2009, M.V. Bianchinotti, R.M. Sánchez, MVB776, BBB.

KEY TO SPECIES OF *ACANTHOSTIGMA*

- |     |  |                            |
|-----|--|----------------------------|
| 1.1 | Ascospores less than 5 $\mu\text{m}$ wide  | 2                          |
| 1.2 | Ascospores $\geq$ 5 $\mu\text{m}$ wide   | 6                          |
| 2.1 | Ascospores up to 4 $\mu\text{m}$ wide  | 3                          |
| 2.2 | Ascospores 4–5 $\mu\text{m}$ wide  | 5                          |
| 3.1 | Ascospores 22–27 $\times$ 3–3.5 $\mu\text{m}$ , 5(–9)-septate  | <i>A. revocatum</i>        |
| 3.2 | Ascospores more than 30 $\mu\text{m}$ long   | 4                          |
| 4.1 | Ascospores (40–)56–78(–95) $\times$ (2–)2.5–3(–3.5) $\mu\text{m}$ , 10–14-septate                          | <i>A. scopulum</i>         |
| 4.2 | Ascospores (75–)85–135(–150) $\times$ 2.5–4 $\mu\text{m}$ , 12–16-septate                                  | <i>A. filiforme</i>        |
| 5.1 | Ascospores (29–)38–51 $\times$ 4–4.5(–5) $\mu\text{m}$ , (4–)6–8-septate                                   | <i>A. longisporum</i>      |
| 5.2 | Ascospores (38–)48–64.5 $\times$ 4–4.5 $\mu\text{m}$ , 6–12-septate  | <i>A. ellisii</i>          |
| 6.1 | Ascospores up to 60 $\mu\text{m}$ long   | 7                          |
| 6.2 | Ascospores > 60 $\mu\text{m}$ long   | 9                          |
| 7.1 | Ascospores with symmetrical ends, not or slightly constricted at septa                                     | 8                          |
| 7.2 | Ascospores with asymmetrical ends, constricted at septa, 40–50 $\times$ 5–6.5 $\mu\text{m}$ , 7–10-septate | <i>A. septoconstrictum</i> |
| 8.1 | Ascospores 30.5–35.5(–42) $\times$ 5–6 $\mu\text{m}$ , (5–)6–7(–8)-septate                                 | <i>A. perpusillum</i>      |
| 8.2 | Ascospores 40–55(–63) $\times$ (5–)6–7(–7.5) $\mu\text{m}$ , 10–14-septate                                 | <i>A. minutum</i>          |
| 9.1 | Ascospores with symmetrical ends, 60–90 $\times$ 5–7.5 $\mu\text{m}$ , 14–18-septate                       | <i>A. multiseptatum</i>    |
| 9.2 | Ascospores with asymmetrical ends, 62–111 $\times$ 5–10 $\mu\text{m}$ , 9–18-septate                       | <i>A. patagonica</i>       |

## 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 ambiguous regions. The ITS data are based on the highly conserved 5.8S



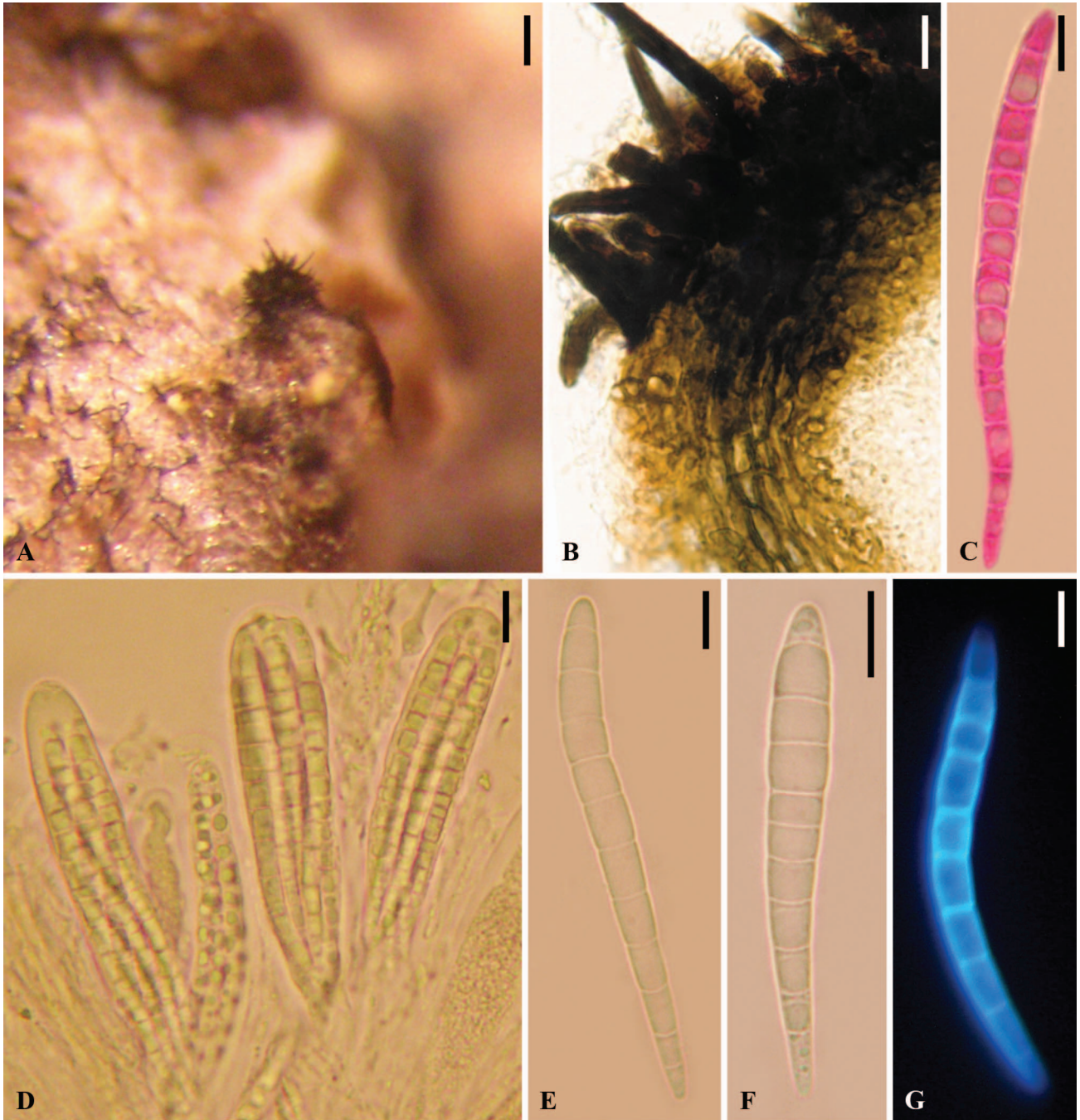


FIG. 1. *Acanthostigma patagonica* (all from Holotype). A. Ascoma on substrate. B. Longitudinal section through ascomatal wall. C. Ascospore 16-septate (in phloxine). D. Asci. E–F. Ascospores (in water). G. Ascospore viewed with fluorescence microscopy. Bars: A = 200  $\mu\text{m}$ , B = 20  $\mu\text{m}$ , C–G = 10  $\mu\text{m}$ .

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. multi-septatum* Promputtha & A.N. Mill. in that both species possess long ascospores with the greatest number of

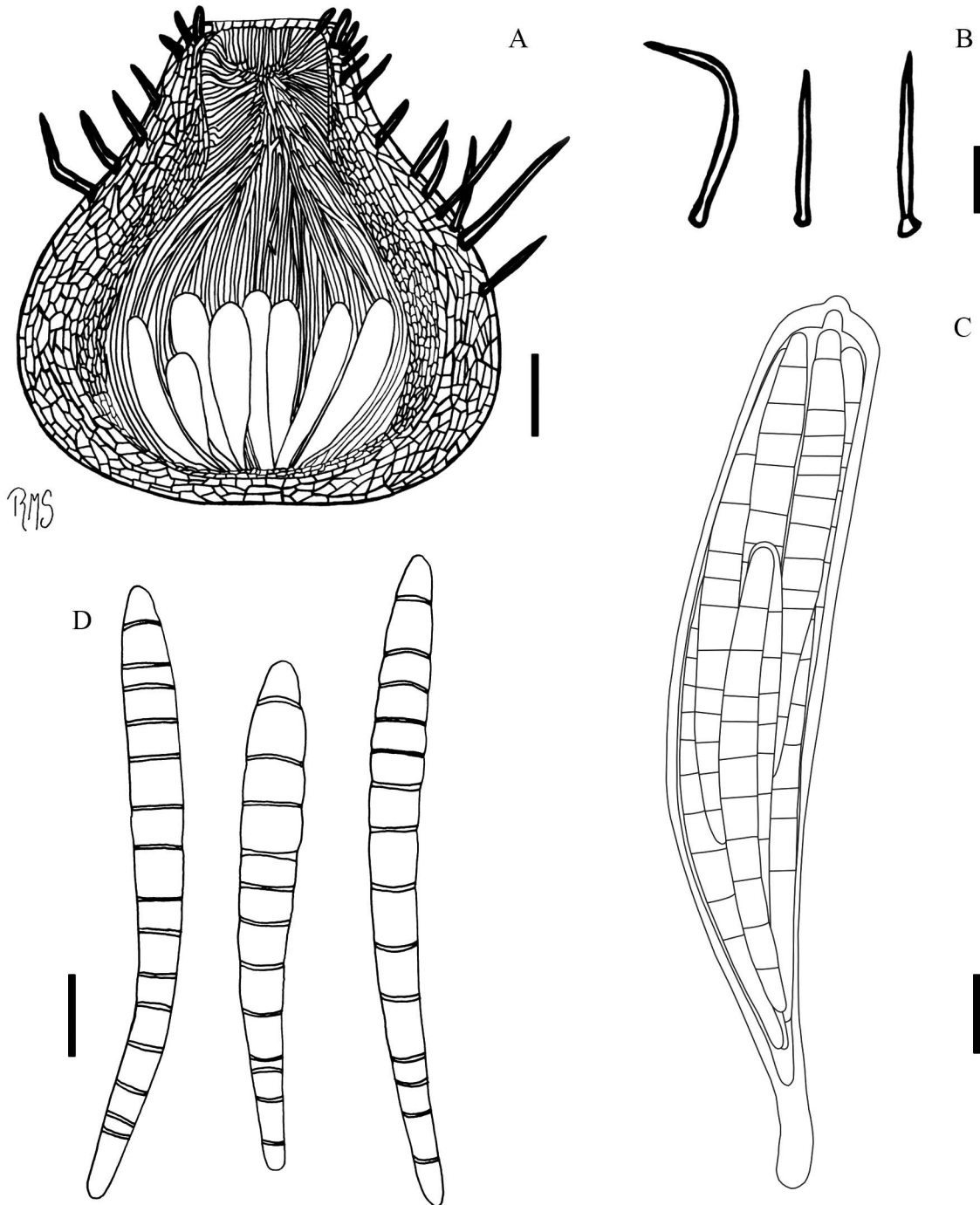


FIG. 2. *Acanthostigma patagonica*. A. Longitudinal section of ascoma. B. Setae. C. Ascus. D. Ascospores. Bars: A = 50  $\mu\text{m}$ , B = 22  $\mu\text{m}$ , C–D = 10  $\mu\text{m}$ .

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.*

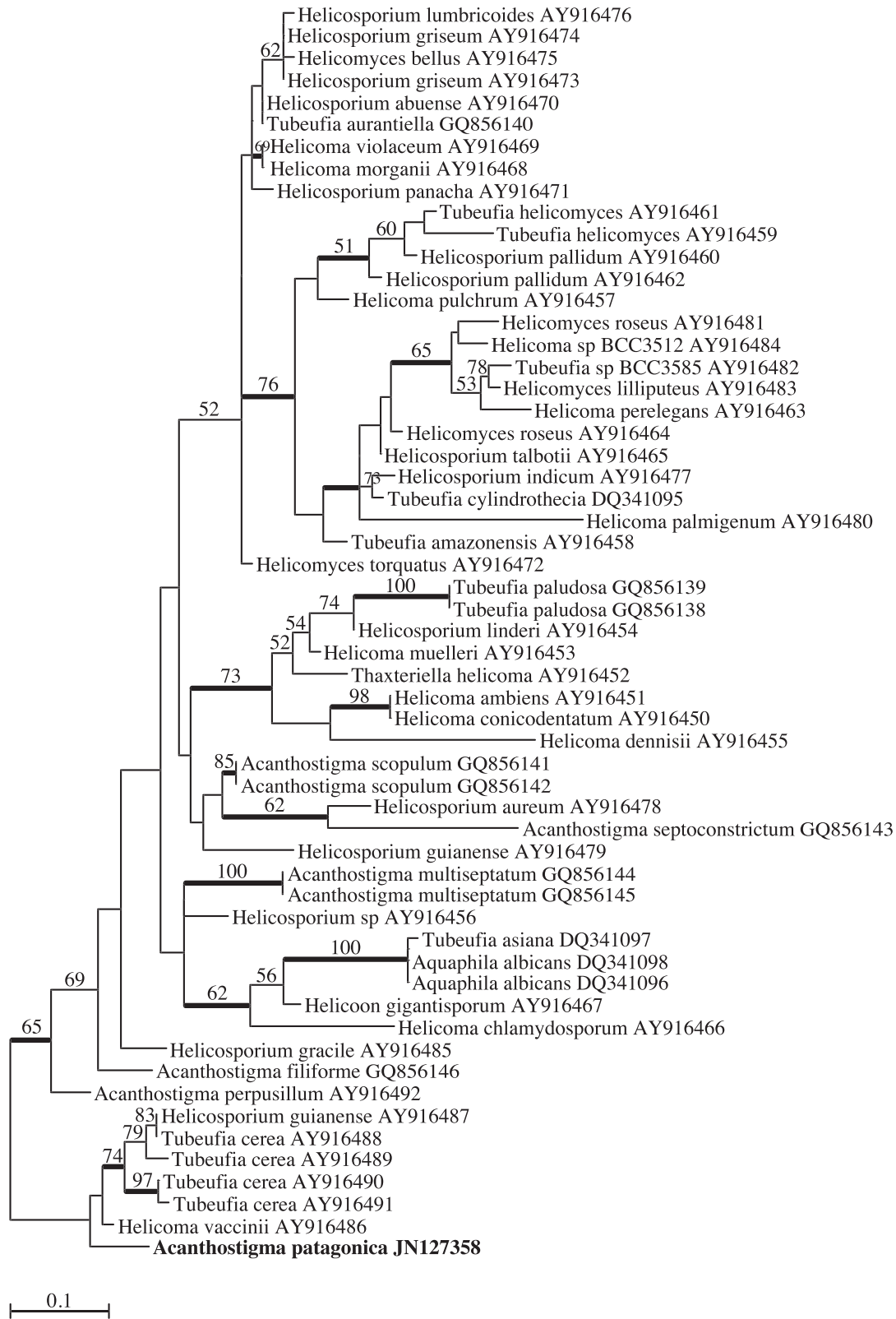


FIG. 3. Tubeufiaceae phylogeny (ln L = -1883.7) generated from a PhyML analysis of 57 ITS sequences showing the phylogenetic placement of *A. patagonica* (in boldface). Thickened branches indicate Bayesian posterior probabilities  $\geq 95\%$ , while numbers above or below branches refer to PhyML bootstrap values  $\geq 50\%$ . The tree is rooted with taxa in the *Tubeufia cerea* clade based on the LSU analysis.



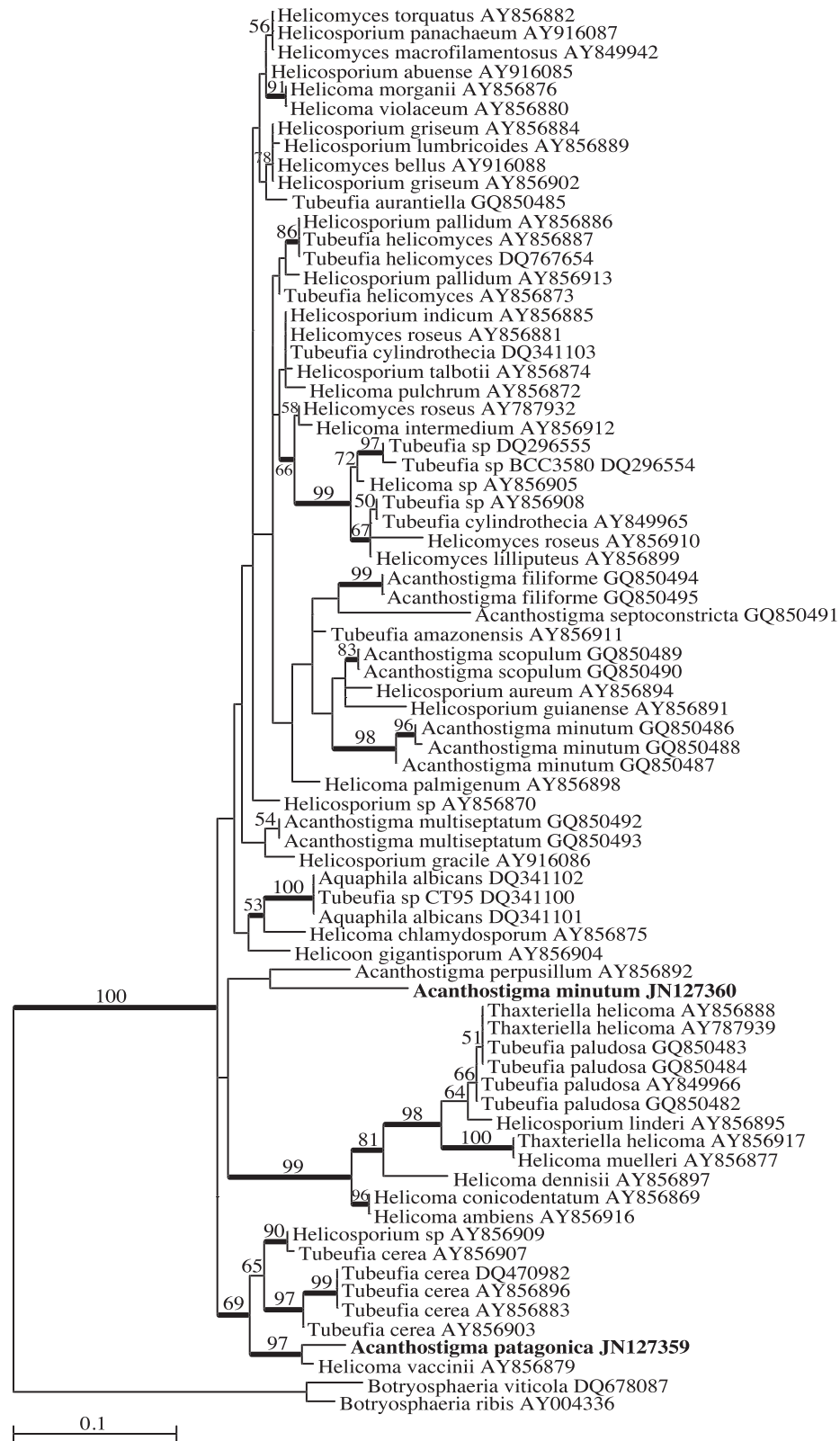


FIG. 4. Tubeufiaceae phylogeny (ln L = -2619.3) generated from a PhyML analysis of 75 LSU sequences showing the phylogenetic placement of the newly sequenced taxa (in boldface) in this study. Thickened branches indicate Bayesian posterior probabilities  $\geq 95\%$ , while numbers above or below branches refer to PhyML bootstrap values  $\geq 50\%$ . 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 dubia, differs from *A. patagonica* in having smaller, 5–6-septate ascospores ( $35 \times 6\text{--}6.5 \mu\text{m}$ ).

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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