Crassoascus monocaudatus and *Iodosphaeria podocarpi*, two new species on *Podocarpus parlatorei* from "Las Yungas", Argentina

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During a biodiversity study of micromycetes occurring on bark and decorticated wood of a native gymnosperm, *Podocarpus parlatorei* Pilg., from Argentina, new taxa of *Crassoascus* and *Iodosphaeria* were found. They are described, illustrated and compared with the known species within each genus.

Key words – Amphisphaeriaceae – Ascomycota – Clypeosphaeriaceae – Iodosphaeriaceae

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Introduction

During a biodiversity study of micromycetes occurring on bark and decorticated wood of a native gymnosperm, *Podocarpus parlatorei* Pilg., from Argentina, two undescribed ascomycetes were found. One fits the genus *Crassoascus* Barrasa, Checa & A.T. Martínez and the other the genus *Iodosphaeria* Samuels, E. Müll. & Petrini.

Crassoascus is characterised by subcorticolous, erumpent, black ascomata, separate or gregarious on the host surface; apex papillate, ostiolate. The asci are cylindrical, unitunicate, thick-walled, apical ring enlarged, amyloid and the ascospores are brown, fusoid, asymmetric, septate, gutulate when young, smooth-walled, apiculate at each end with a conspicuous terminal pore and hyaline refractive cap-like appendages. There are no data about its anamorph. Two species have been described (www.indexfungorum.org).

Iodosphaeria is characterised by superficial, black ascomata, with unbranched, brown flexuous hairs radiating from the peridium surface. The asci are unitunicate with an amyloid, discoid apical ring which may be lacking, and ascospores are allantoid to ellipsoidal, aseptate and hyaline, with or without a sheath. Synanamorphs in *Selenosporella* G. Arnaud ex MacGarvie and *Ceratosporium* Schwein. have been found in association with species of *Iodosphaeria* (Samuels et al. 1987). *Iodosphaeria* is a genus comprising seven species (www.indexfungorum.org).

The two genera are reported for the first time on gymnosperms and from Argentina, and in the case of *Crassoascus* this is also the first record for South America. For *Iodosphaeria*, a key is provided for the known species. Both of these fungi are morphologically distinct when compared to the known species.

Methods

Isolates and morphology

The fungi were found on dead branches of *Podocarpus parlatorei*, collected in the forest of Sierra de Medina (Dep. Burruyacu) in Tucumán province, Argentina. The studied area belongs to the District Mountain Forest of the phytogeographic region of "Las Yungas", a subtropical zone of the Amazonic Domain, a Neotropical Region which covers the Andes from Venezuela and Colombia, into the north-west of Argentina as a narrow fringe through Jujuy, Salta, Tucuman and Catamarca Provinces (Cabrera & Willink 1973, Hueck 1978). The material was dried and deposited in the fungal reference collection of Fundación Miguel Lillo (LIL). Observations, photography and measurements of ascomata, asci and ascospores were made on material squash-mounted in distilled water, 5% KOH, phloxine and Melzer's reagent for optical microscopy. The drawings were made using a camera lucida. A cirrus of *Crassoascus* was removed with a sterile needle and transferred to Petri dishes containing PDA (Hawksworth et al. 1995). These were incubated under laboratory conditions at approximately 12 hr fluorescent light per day at 25º C. In addition, holotype specimens of *Crassoascus fusisporus* and *C. canadensis* from MA and DAOM were studied.

Results

Crassoascus monocaudatus Catania & A.I. Romero, **sp. nov**. Figs 1–7; 8–16

Etymology – referring to presence of a single appendage in the ascospore.

A Crassoascus monocaudatus differt ob ascosporas 58.5–82 x 14–16 µm, 5–8 septatas, et appendice singulari basali quasque indutas.

Ascomata immersed, subcortical, erumpent, globose to suglobose, 0.4–1.25 x 0.4–1 mm, ostiolate, with short neck, neck 0.1–0.25 x 0.1 mm, solitary or gregarious. Peridium 26–45 µm wide, outer layer of thick-walled cells, dark brown, irregular to compressed laterally; inner layer of compressed cells, with thin, hyaline walls. Asci cylindrical apically thickened, with 8-spores, biseriate, 255–324 x 24–30 µm, stipitate, pedicel 33–45 µm long, subapical ring conspicuous staining blue in Melzer´s reagent, 2–4 x 5–7 µm. Paraphyses abundant, thin, with numerous guttules, hyaline, 1.5-2.5 µm diam. Ascospores fusiform, slightly curved, (56–)58.5–82(–101) x (13–)14–16(–19) µm, at first hyaline and unicellular with 1-2 large guttules and a basal appendage, later multiseptate (5–)6–8(–9) septa, not constricted at the septa, smooth, bright brown to dark brown with hyaline apical cell and basal appendage, 15.5–27 µm long; the basal appendage collapsed when mature.

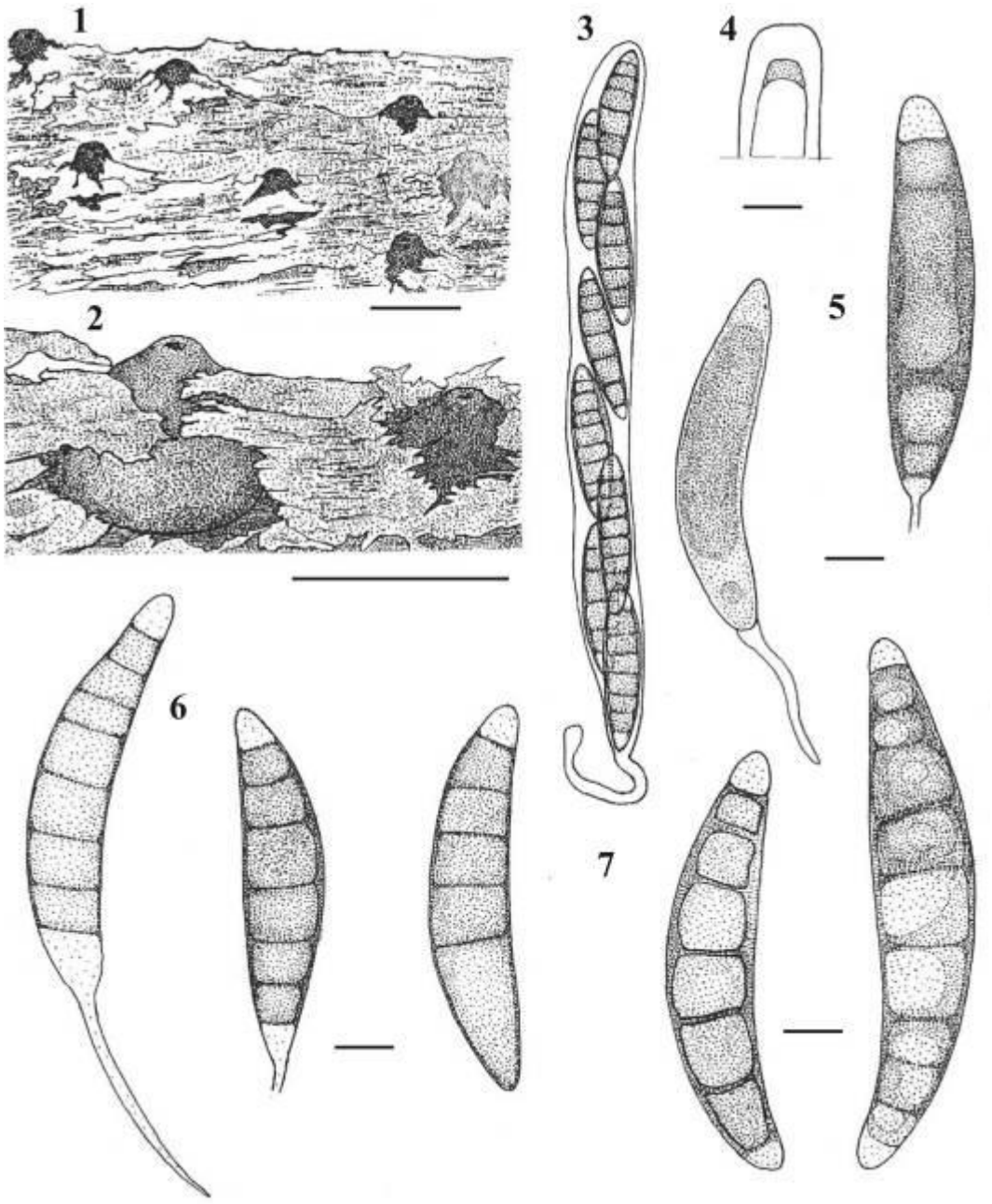
Anamorph – Not seen.

Known distribution – only from type locality.

Holotype – Argentina, Tucumán, Burruyacu, Sierra de Medina, provincial route 310, at 31 Km from Villa Padre Monti, Aguas Negras, Finca Mansilla, on dead branch of *Podocarpus parlatorei*, 26º22'06"S, 65º03'46'W, 25 Feb 1999, Catania 1241 (Holotype, LIL).

Additional material examined – *Crassoascus monocaudatus.* Argentina, Tucumán, Burruyacu, Sierra de Medina, provincial route 310, at 31 Km from Villa Padre Monti, Aguas Negras, Finca Mansilla, on dead branch of *Podocarpus parlatorei*, 26º22'06"S, 65º03'46'W, 2 Mar 2000, Catania 1824 (LIL). *Crassoascus canadensis*. Canada, British Columbia, Sidney, beach off Glenelg, 4 October 1991, M.E. Barr 7787 (holotype, DAOM). *Crassoascus fusisporus*. Spain, Cáceres, Natural Park of Monfragüe, Puerto de la Cañadilla, on dead branches of *Erica arborea*, 21 June 1989, Checa, Barrasa & Blanco (holotype, MA-26331).

Notes – *Crassoascus* was established by Barrasa et al. (1993) based on the type species *C. fusisporus* Checa, Barrasa & A.T. Martínez. A second species, *C. canadensis* M.E. Barr, was proposed by Barr (1994). *Crassoascus monocaudatus* is included within the genus principally because it shares with the other two species an ascomatal peridium with "textura globulosa", asci with a prominent amyloid subapical ring and brown, fusiform, septate ascospores. *Crassoascus monocaudatus* is distinguished by ascospore features. They are longer than the ascopores of the other two species, have more septa and a long basal appendage. Holotypes of the other two species were studied. *Crassoascus fusisporus* has



Figs 1–7 – *Crassoascus monocaudatus*. (holotype). **1, 2** Ascomata on substratum. **3** Ascus. **4** Apex of ascus, with amyloid apical ring. **5** Young ascospores with basal appendage. **6** Mature ascospore with appendage. **7** Ascospores without appendages. Bars: 1–2= 0.5 mm; 3–7= 10 µm.

smaller ascospores (40–45 x 8–10 µm), with 3–5 septa, apiculate at both ends with a conspicuous terminal pore and hyaline cap-like appendages and *C. canadensis* has symmetrical ascospores, 26–38 x 8–12 µm, with 3–7 septa and a slight constriction at the median septum.

Originally, *Crassoascus* was placed within Clypeosphaeriaceae by Barrasa et al. (1993), later Barr (1994) transferred it to the Amphisphaeriaceae. Kang et al. (1999) studied the holotype of *C. fusisporus* and suggested to place it within the Clypeosphaeriaceae despite the "textura globulosa" of the peridium and the atypical arrangement of the ascospores. According to Eriksson (2006) the genus is maintained in the Clypeosphaeriaceae. Huhndorf (pers. comm.) has pointed out that the Amphisphaeriaceae has not yet been adequately separated by molecular data from the Clypeosphaeriaceae but if the two families were united, Amphisphaeriaceae is the older name. The placement of *Crassoascus* into the Annulatas-

caceae by Kirk et al. (2008) is not correct.

This is the first time that a species of the genus has been reported from Argentina. It is probably also the first record from South America. It is also the first account on a gymnosperm. *Crassoascus fusisporus* is reported as a saprobe on wood on dead branches of *Erica arborea* L. from Spain and *C. canadensis* on wood of *Salix* (holotype); on wood or periderm of woody plants (*Acer macrophyllum* Pursh; *Cornus occidentalis* Coville) from Canada. These two species from the northern hemisphere were found in areas with a Mediterranean climate, however *C. fusisporus* from Spain was collected at the end of summer-beginning of spring where the summer has high temperatures and the spring is rainy. The Argentine collections were also found in summer although in a subtropical area. The Canadian species was found in autumn but in a place where the daily temperatures seldom climb above 31°C.

Isolations were attempted from ascospores of the Argentine specimen but unfortunately *C. monocaudatus* did not grow in culture. Barrasa et al. (1993) obtained an *Alternaria* anamorph in pure culture, but the relationship with *C. fusiporus* remains uncertain and the chance of a contamination cannot be rejected.

Iodosphaeria podocarpi Catania & A.I. Romero, **sp. nov**. Figs 17-25

Etymology – referring to the associated plant, *Podocarpus*.

A Iodosphaeria phyllophila differt quod longitudinis respectu minores habe ascosporas 19-20 (-22) µm.

Ascomata globose to subglobose, 220–250 x 220–240 µm, superficial black, surrounded by abundant, dark brown, septate, flexuous, unbranched hairs, 7–8 µm wide, to 500 µm long; ostiole apical, periphysate. Peridium 40–80 µm wide, comprising outer layers of dark brown cells forming a *"textura angularis"* and inner layers of flattened light brown to hyaline cells. Paraphyses up to 4 µm wide, hyaline, filamentous, septate, flexuous, deliquescing and disappearing when mature. Asci, cylindrical, unitunicate, 97.5–120 x 8–12 µm 8-spored, uniseriate or partially biseriate, short pedicellate, apex rounded, with an amyloid apical ring. Ascospores inequilaterally ellipsoid to navicular, slightly allantoid, often acute at both ends, unicellular, hyaline, smooth, 19–20(–22) x 4.5–5(–6.5) µm.

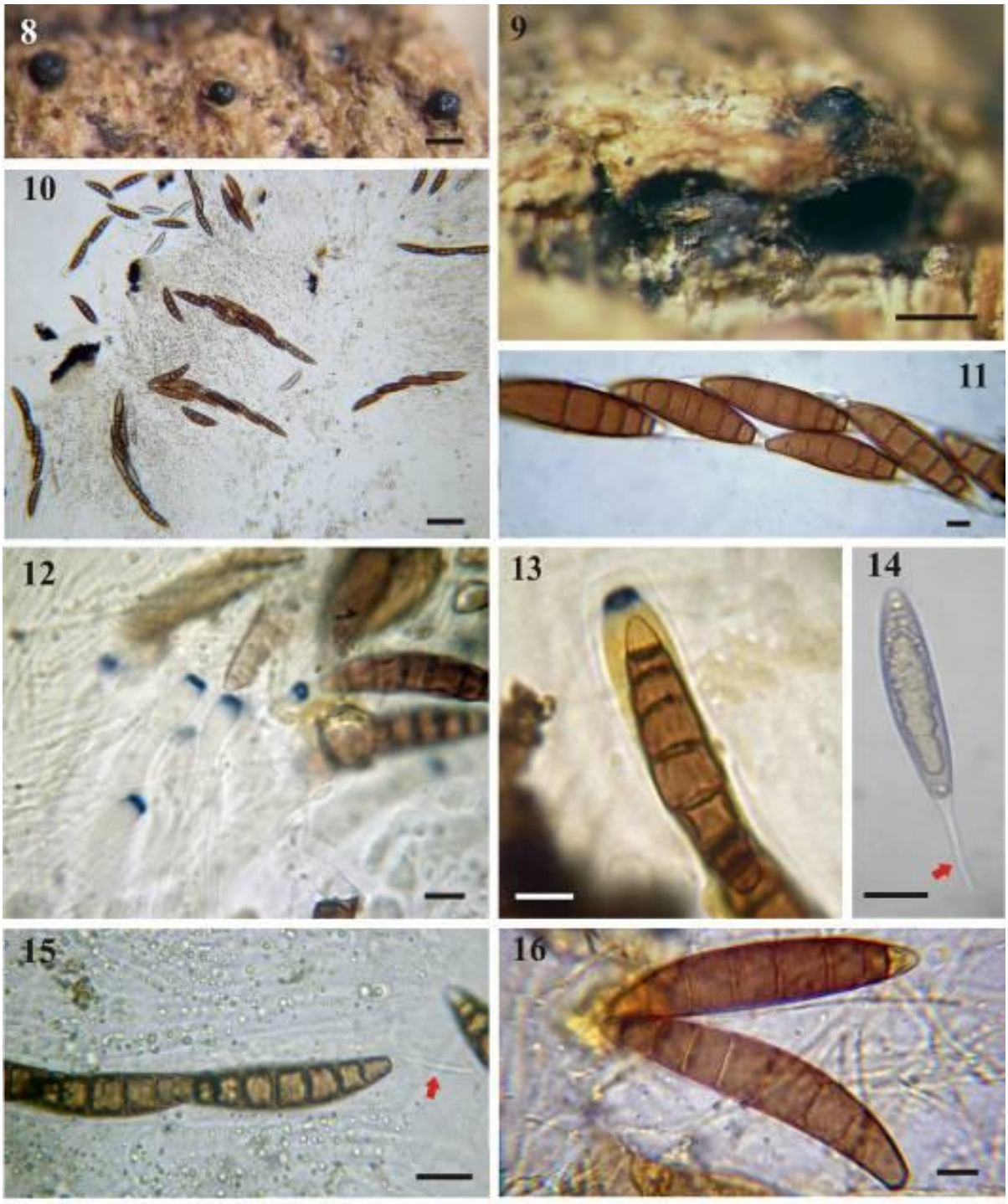
Anamorph: *Selenosporella*-like, found near or among the ascomata in nature. Conidiophores 100–140(–250) x 4–5 µm, arising from repent, coarse, brown hyphae; erect, straight or flexuous, branched, gregarious, septate, brown, smooth. Conidiogenous cells polyblastic, 13–18 x 4 µm, integrated and terminal, tapering slighly from base to tip, with minute denticles on the tip of each conidiogenous cell. Conidia straight or slightly curved, aseptate, hyaline, 14–17 x 1 µm.

Known distribution – only from type locality.

Holotype – Argentina, Tucumán, Burruyacu, Sierra de Medina, provincial route 310, at 31 Km from Villa Padre Monti, Aguas Negras, Finca Mansilla, on branch of *Podocarpus parlatorei*, 26º22'06"S, 65º03'46'W, 1 April 1998, Catania 773 (Holotype, LIL).

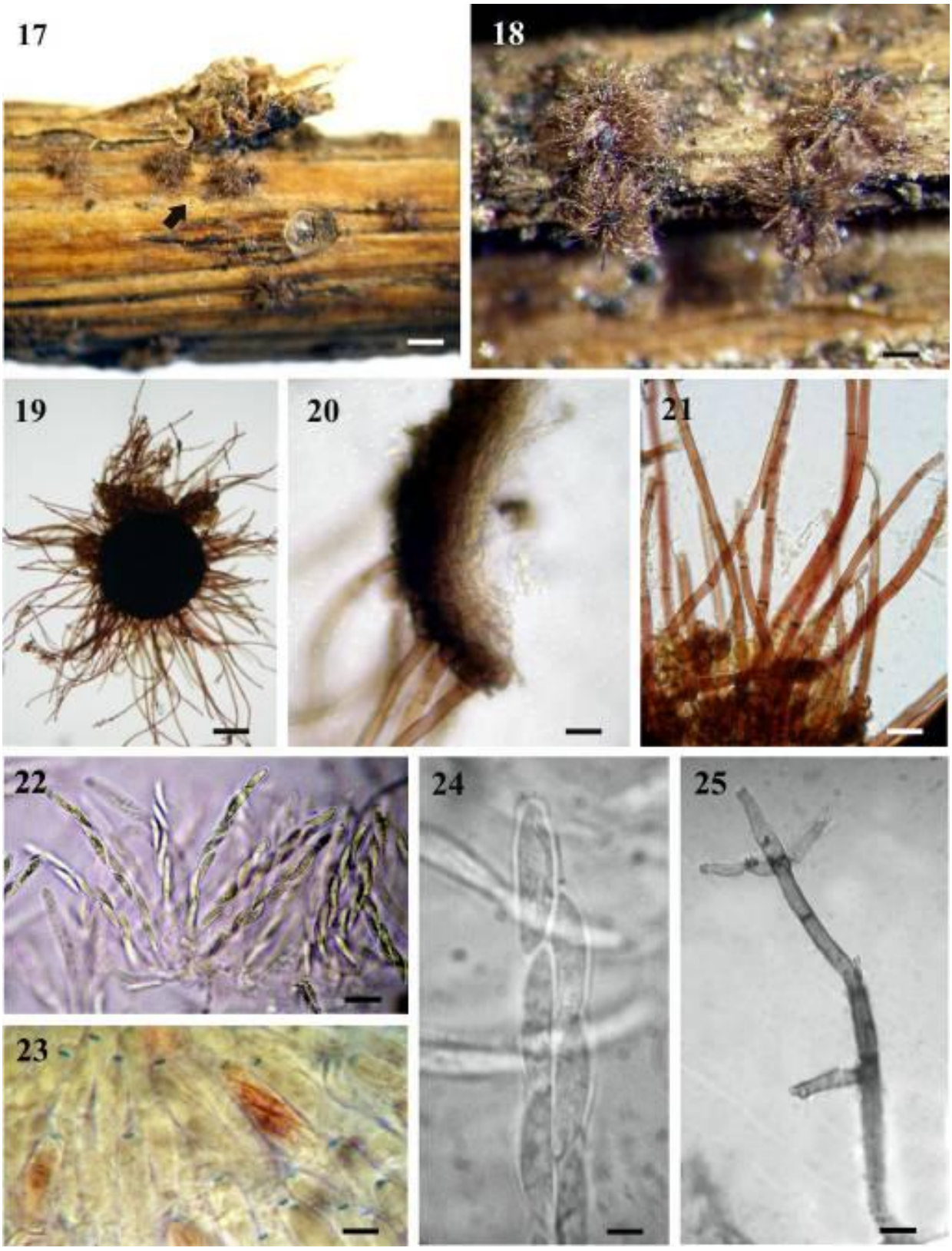
Notes – *Iodosphaeria podocarpi* resembles *I. polygoni* W.H. Hsieh, C.Y. Chen & Sivan. and *I. phyllophila* (Mouton) Samuels, E. Müll. & Petrini because of the positive reaction in Melzer´s reagent of the apical ring. *I. polygoni* has ascospores of similar length (18–23 µm long) but *I. polygoni* has ellipsoidal ascospores which are wider (5.5–8 µm) and longer asci (up to 150 µm long) (Hsieh et al. 1997). The Argentine collection differs from *I. phyllophila*, which has longer ascospores (16–31 µm long) with both ends less acute than those of *I. podocarpi* (Samuels et al. 1987).
This is the first report of the genus *Iodosphaeria* on a gymnosperm. Previously recorded substrata were arborescent ferns and dicots for *I. phyllophila* and various monocots for other species. Species of the genus *Iodosphaeria* have been reported from tropical and temperate regions of the world: China (Taiwan, Hong Kong); USA (Louisiana); Europe (Belgium, Germany, Great Britain); New Zealand and South America (Brazil, French Guiana) (Samuels et al. 1987, Barr 1993, Hyde 1995, Candoussau et al. 1996, Hsieh et al. 1997, Taylor & Hyde 1999).

The taxonomic position of this genus is still unclear and it has been placed in various



Figs 8–16 – *Crassoascus monocaudatus* (holotype). **8, 9** Ascomata on substratum. **10** Asci and ascospores. **11** Ascus with ascospores. **12, 13** Amyloid apical ring. **14** Young ascospore with appendage (arrow). **15** Mature ascospore with appendage (arrow). **16** Matures ascospores. Bars: 8–9 = 0.5 mm; 10 = 50 µm; 11–16 = 10 µm.

families by different authors. Samuels et al. (1987)placed *Iodosphaeria* in the Amphisphaeriaceae, Barr (1990, 1994) suggested that the genus *Iodosphaeria* is better placed in the Lasiosphaeriaceae (Sordariales), Réblová (1999) placed it in the Trichosphaeriaceae and Eriksson et al. (2001) placed it in the Amphisphaeriaceae. It was placed in its own family, Iodosphaeriaceae by Hilber & Hilber (2002).



Figs. 17–25 – *Iodosphaeria podocarpi*. **17**, **18** Ascomata on substrata. **19** Ascoma. **20** Peridium. **21** Unbranched hairs arising from peridium. **22** Asci. **23** Apex of ascus, with an amyloid apical ring. **24** Ascus with apical ring and ascospores. **25** *Selenosporella* conidiophores. Bars: 17 = 750 µm; 18 = 250 µm; 19 = 75 µm; 20–21= 25 µm; 22–23 = 10 µm; 24 = 5 µm; 25 = 10 µm.

Key to *Iodosphaeria* species known to date

1. Asci with apical apparatus 2
1. Asci lacking apical apparatus 6
2. Apical apparatus J+ 3
2. Apical apparatus J- 5
3. Asci longer than 150 µm, ascospore ellipsoidal, 18–23 x 5,5–8 µm ..... *I. polygoni*
3. Asci less than 150 µm in length 4
4. Ascospores allantoid, commonly on debris of tree ferns, Cyperaceae and on fallen, dead twigs of *Acer*, *Salix* and *Populus*..... *I. phyllophila*
4. Ascospores inequilaterally ellipsoid to navicular, on fallen, dead twigs of Podocarpaceae..... *I. podocarpi*
5. Ascospore wall verruculose ..... *I. arundinariae*
5. Ascospore wall smooth ..... *I. tarda*
6. Ascospores ellipsoidal, with a mucilaginous sheath, 19–29 x 7–9 µm ..... *I. ripogoni*
6. Ascospores ellipsoidal to fusiform, lacking a mucilaginous sheath, 14–22 x 4–6 µm ..... *I. hongkongensis*

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