



ANNULOHYPOXYLON (HYPOXYLACEAE) SPECIES FROM ARGENTINA

Esteban B. Sir¹, Eric Kuhnert², Adriana I. Hladki³ & Andrea I. Romero^{4,5}

¹ Fundación Miguel Lillo, CONICET, Laboratorio Criptogámico, Miguel Lillo 251, 4000 San Miguel de Tucumán, Tucumán, Argentina; sirestebanbenjamin@gmail.com (author for correspondence).

² Institute for Organic Chemistry, BMWZ, Leibniz Universität Hannover, Schneiderberg 38, 30167 Hannover, Germany.

³ Fundación Miguel Lillo, Laboratorio Criptogámico, Tucumán, Argentina.

⁴ Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II Piso 4, 1428 Ciudad Universitaria, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

⁵ Instituto de Micología y Botánica (INMIBO), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.

Abstract. Sir, E. B.; E. Kuhnert; A. I. Hladki & A. I. Romero. 2018. *Annulohypoxylon* (Hypoxylaceae) species from Argentina. *Darwiniana*, nueva serie 6(1): 68-83.

Eleven *Annulohypoxylon* species are recognized for Argentina. *Annulohypoxylon purpureonitens* and *A. purpureopigmentum* are recorded for the first time in the Southern Cone, while *A. stygium* and *A. nitens* represent new reports for Northwestern Argentina. Furthermore, the asexual states of *A. purpureopigmentum* and *A. subeffusum* are described for the first time. In addition, a comparative table, a key to the Argentinean species, photographs, and a distributional map of these species in South America are provided.

Keywords. Anamorph; mycogeography; Xylariales; Yungas.

Resumen. Sir, E. B.; E. Kuhnert; A. I. Hladki & A. I. Romero. 2018. Las especies de *Annulohypoxylon* (Hypoxylaceae) de Argentina. *Darwiniana*, nueva serie 6(1): 68-83.

Se reconocen once especies de *Annulohypoxylon* para la Argentina. *A. purpureonitens* y *A. purpureopigmentum* se registran por primera vez en el Cono Sur, mientras que *A. stygium* y *A. nitens* se citan por primera vez para el noroeste de la Argentina. Se describen los estados asexuales de *A. purpureopigmentum* y *A. subeffusum*. Además, se proporcionan una tabla comparativa, una clave para las especies argentinas, fotografías y un mapa con la distribución sudamericana de las especies tratadas aquí.

Palabras clave. Anamorfo; micogeografía; Xylariales; Yungas.

INTRODUCTION

Extensive data about the diversity of xylariaceous fungi in Argentina was generated during the last ten years. This is mainly due to the several mycological expeditions carried out in the subtropical montane forest of the Northwestern region (Hladki & Romero, 2010; Kuhnert et al., 2015, 2017; Medina et al., 2016; Sir et al., 2012a, b, c; 2013; 2015, 2016a, b; Sir & Hladki, 2014).

The recently proposed family Hypoxylaceae (Wendt et al., 2018) is represented by six genera in the country, namely *Annulohypoxylon* Y. M. Ju, J. D. Rogers & H. M. Hsieh, *Daldinia* Ces. & De Not., *Entonaema* Möller, *Hypoxylon* Bull., *Phylacia* Lév., and *Pyrenopolyporus* Lloyd (Hladki & Romero, 2009a, b; Ju & Rogers, 1996; Sir et al., 2015; Kuhnert et al., 2017; Daranagamma et al., 2018; Wendt et al., 2018). Originally, *Annulohypoxylon* was included in

sect. *Annulata* J. H. Miller of *Hypoxyton* (Ju and Rogers, 1996). This section grouped species with (a) carbonized stromata, (b) conical-papillate ostioles surrounded or not by a discoid area, (c) smooth ascospores usually with dehiscent perispore but with a thickening of 1/3 of the length of the ascospores and (d) nodulisporium-like anamorphs. Later, Hsieh et al. (2005) segregated the section *Annulata* as a new genus, based on a β -tubulin and α -actin phylogenetic analysis. Over ten years later, Kuhnert et al. (2017) used a polyphasic approach to improve the resolution of infrageneric relationships. Their work clearly shows the presence of two distinct lineages within the genus in accordance with previous results by Hsieh et al. (2005). Wendt et al. (2018) provided an extensive multigene phylogeny of the Xylariaceae using the internal transcribed spacer region of the ribosomal DNA (ITS), the large subunit of the ribosomal DNA (LSU), the second largest subunit of the RNA polymerase II (RPB2), and beta-tubulin (TUB) as genetic markers. Their data resulted in the segregation of the genus *Jackrogersella* L. Wendt, Kuhnert & M. Stadler from *Annulohypoxyton*. The new genus includes species characterized by: 1) inconspicuous or reduced ostiolar discs and 2) azaphilones of the specific cohaerin type (including minutellins and multiformins) as stromatal pigments (chemotaxonomic markers). Therefore, the generic *Annulohypoxyton* concept is limited only to those species with conspicuous ostiolar discs and stromatal pigments with naphthol derivatives (binaphthalene-tetrol, truncatone A, daldinol A) or truncaquinones (Kuhnert et al., 2017).

According to this new concept, *Annulohypoxyton* currently comprises about 45 species and two varieties, most of them distributed in tropical and subtropical regions (Wendt et al., 2018). In South America, 19 species are recognized (Cruz & Cortez, 2016; Hladki & Romero, 2009a, b; Ju & Rogers, 1996; Fournier & Lechat, 2016; Kuhnert et al., 2017), from which only nine have been formerly cited for Argentina: *A. bovei* (Speg.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *A. leptascum* (Speg.) Y. M. Ju, J.D. Rogers & H.M. Hsieh;

A. moriforme (Henn.) Y. M. Ju, J.D. Rogers & H.M. Hsieh var. *moriforme*, *A. moriforme* var. *macrosporum* Hladki & A.I. Romero, *A. nitens* (Ces.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *A. subeffusum* (Speg.) Hladki & A.I. Romero, *A. stygium* (Lév.) Y.M. Ju, J.D. Rogers & H. M. Hsieh., *A. substygium* (Y. M. Ju, J.D. Rogers & H.M. Hsieh) Sir & Kuhnert and *A. yungensis* Sir, Kuhnert, Hladki & A.I. Romero (Hladki & Romero 2009a, b; Kuhnert et al., 2017; Daranagama et al., 2018).

This work deals with an update for the diversity of the genus *Annulohypoxyton* in Argentina, including two novelties for the Southern Cone. Based on specimens collected during the last six years in the subtropical montane forest (“Las Yungas”) located in the northwest and from previous reports, eleven species are recognized for this country and summarized in a comparative table. We also provide a provisional key to the species, comments, photographs, and a distribution map of these species in South.

MATERIALS AND METHODS

Fresh materials were collected mainly during 2011-2017 in natural reserves of the montane forest of Northwestern Argentina: Baritú, Calilegua, Campo de los Alisos and El Rey national parks; El Nogalar de los Toldos national reserve, Acambuco provincial reserve, and Parque Sierra de San Javier. In addition, several specimens of BPI, LIL, LPS, MFLU, NY, P, and WSP herbaria were examined and compared (acronyms according to Thiers, 2017, <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

The materials were studied, isolated and cultured following the methods described by Ju & Rogers (1996) and Kuhnert et al. (2017). The KOH-extractable pigments were evaluated after 1 min of incubation and compared with the color chart of Rayner (1970).

The author names of the fungi and host species were taken from Index Fungorum (<http://www.indexfungorum.org/>) and Tropicos (<http://www.tropicos.org/Home.aspx>), respectively.

RESULTS

Taxonomy

New records of *Annulohyphoxylon* for Argentina and the Southern Cone

Annulohyphoxylon purpureonitens (Y.M. Ju & J.D. Rogers) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 861. 2005. Type: Brazil, Serra Araca, 10/13-III-1984, corticated wood, *G. J. Samuels 808* (holotype NY 01089574!; isotype WSP 69635!). Figs. 1 A-F, 6.

Teleomorph. Stromata effused-pulvinate rarely glomerate; 10-25 mm long × 5.5-12 mm broad × 1-1.5 mm thick, with perithecial mounds 1/2 to 1/4 exposed; surface shiny black; red-brown to black granules immediately beneath surface, orange-brown granules detected by microscopic examination in water; KOH-extractable pigments Vinaceous Purple (101); the tissue below the perithecial layer inconspicuous, black. Perithecia spherical, 0.6-1 mm diam; ostioles conical papillate, encircled with a *bovei*-type disc 0.33-0.4 mm diam. Asci not observed. Ascospores brown, unicellular, ellipsoid-inequilateral, with broadly rounded ends, (7.1)7.9-9.0(9.6) × (3.3)3.5-4.2(4.4) μm (N= 60, Me= 8.5 × 3.8 μm); with straight germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth with a thickening on the convex side; epispore smooth. Anamorph on the natural substrate from isotype (WSP 69635): Conidiogenous structure on tomentose tissue *Sepia* (63). Conidiophores with nodulisporium-like branching pattern, erect, 150-400 μm high, brown-reddish, roughened. Conidiogenous cells hyaline to pale brown, roughened, 13.3-20.6(27.1) × (1.9)2.2-3.3 μm. Conidia hyaline, smooth to slightly roughened, ellipsoid, 3.5-4.7(5.6) × 2-2.7 μm.

Culture. Not obtained.

Distribution. Argentina, Brazil, and Mexico (Ju & Rogers, 1996).

Observations. *Annulohyphoxylon purpureonitens* was so far only known from northern Brazil and Mexico (Ju & Rogers, 1996). The presence of this taxon in the Southern Cone is evidenced by a

unique collection from the north of Salta province. The species is very closely related to *A. nitens* but can be easily differentiated by its pigments (purple vs green) (Fig. 1A). The isotype (WSP) contained an anamorph attached to the stromata, which was not described in the original publication. Fournier & Lechat (2016) observed this structure, and presented a picture that showed the ramification with nodulisporium-like branching pattern. Here we provide, for the first time, a full description and illustration of this anamorph (Fig. 1E, F).

Kuhnert et al. (2017) reported binaphthalene-tetrol (BNT) as sole chemotaxonomic marker detectable for this species.

Specimens examined

ARGENTINA. Salta. Dpto. Santa Victoria, Parque Nacional Baritú, 28-XII-2011, *Sir & Hladki 154* (LIL).

Annulohyphoxylon purpureopigmentum Jad.

Pereira, J.D. Rogers & J.L. Bezerra, *Mycologia* 102(1): 250. 2010. Type: Brazil, Bahia, Una, Una Ecopark, 15° 10' 02"W 39° 03' 16"S, 44 m, on indeterminated branch of dicotyledonous tree, 17-IV-2008, *Jad. Pereira s.n.* (holotype WSP 71615!). Figs. 1G-M, 2, 6.

Teleomorph. Stromata glomerate with few perithecia or effused-pulvinate; 2-23 mm long × 3-10 mm broad × 1-1.5 mm thick, coalescent, with inconspicuous perithecial mounds up to 1/2 exposed; surface Umber (9), becoming *Sepia* (63) to blackish when old, pruinose; orange brown granules immediately beneath surface, red-brown granules detected by microscopic examination in water; KOH-extractable pigments Vinaceous Purple (101); the tissue below the perithecial layer inconspicuous, 0.4-0.7 mm thick, black. Perithecia spherical to subspherical, sometimes compressed, 0.45-0.5 mm high × 0.34-0.5 mm diam.; ostioles conical papillate, encircled with a *truncatum*-type disc 0.17-0.25 mm diam. Asci 8-spored, cylindrical, 99-106.5 μm total length, spore-bearing part 43-52 × 3.9-5.5 μm, stipe 29-60 μm long; with amyloid, discoid apical apparatus, 0.5-0.7 × 1.2-1.4 μm. Paraphyses filiform 2-3 μm, wide at the base, tapering above asci. Ascospores brown, unicellular,



Fig. 1. A-F, *Annulohypoxylon purpureonitens*. **A**, stromatal habit and KOH-extractable pigments. **B**, detail of stromatal surface showing ostiolar discs. **C**, ascospores showing germ slit (arrows). **D**, ascospores and dehiscent perispore showing thickening (arrow). **E**, details of nodulisporium-like branching pattern (arrow). **F**, anamorph from substrate. G-M, *Annulohypoxylon purpureopigmentum*. **G**, mature and young stromata (arrows) on substrate. **H**, stromata surface showing dehiscence of ostiolar disc (arrow). **I**, detail of ostiolar discs (arrows). **J**, amyloid apical apparatus (arrow). **K**, ascospores in water showing germ slit (arrows). **L**, ascospores and perispore showing thickening (arrow) under polarized light. **M**, asci in water. A-D from *Sir & Hladki 154*; E-F from WSP and G-M from *Sir & Hladki 1067*. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/777/757>

ellipsoid-inequilateral, with broadly rounded ends, $(6.3)6.6-7.8(8) \times (2.8)3.1-3.5(3.7) \mu\text{m}$ ($N=40$; $Me=7.1 \times 3.3 \mu\text{m}$); with straight germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth with a thickening on the convex side; epispore smooth. Anamorph on the natural substrate: Conidiogenous structure on young stromata or around of mature stromata on tomentose tissue, Umer (9). Conidiophores with virgariella-like (Fig. 2B) to nodulisporium-like (Fig. 2C) branching patterns, erect, 150-400 μm high, brown-reddish, roughened. Conidiogenous cells hyaline to pale brown, roughened, $13.3-20.6(27.1) \times (1.9)2.2-3.3 \mu\text{m}$. Conidia hyaline, smooth to slightly roughened, ellipsoid, $3.5-4.7(5.6) \times 2-2.7 \mu\text{m}$.

Culture. Colonies on OA covering a 90 mm Petri dish in 4 weeks, at first whitish, becoming Greyish Sepia (105) with reddish tones, velvety to felty, zonate, with entire margin, Brick (59); reverse Brown Vinaceous (84). Conidiogenous structures developing on white to Salmon (41) areas; with virgariella-like (Fig. 2H) to nodulisporium-like (Fig. 2I-J) branching patterns, up to 400 μm high, hyaline, smooth to roughened. Conidiogenous cells $10.8-20.2 \times 2-3.3 \mu\text{m}$, hyaline, smooth to roughened. Conidia hyaline, smooth, ellipsoid. $3.4-4.9 \times 1.7-2.5 \mu\text{m}$.

Distribution. Argentina, Brazil, French Guiana and Martinique (Pereira et al., 2010; Fournier & Lechat, 2016).

Observations. *Annulohypoxyylon purpureopigmentum* is a species with doubtful affinities to the genus (Kuhnert et al., 2017; Wendt et al., 2018). It has been recorded from tropical regions of South America and the Caribbean. Pereira et al. (2010) erected this species based on a collection from Bahia, Brazil. Fournier & Lechat (2016) and Fournier et al. (2016) cited it for French Guiana, Guadalupe Island, and Martinique Island. The Argentinean material is the first recorded from a southernmost tropical region of the South America, indicating the species may have a widespread distribution in the Neotropics.

The Argentinean collection was found on dead branches of an undetermined Myrtaceae species, coexisting with an undescribed species of *Annulohypoxyylon* (having green KOH-extractable

pigments). The observed stromata, ascospores size, and extractable pigments are typical of the species. Fournier & Lechat (2016) determined a *truncatum*-type ostiolar disc for this taxon, contrary to what was interpreted by Pereira et al. (2010) (who characterized it as *bovei*-type ostiolar disc). This is in agreement with our observations for the holotype and the Argentinean material.

Kuhnert et al. (2017) reported this species contains an unknown stromatal main compound related to hinnulin A.

Specimens examined

ARGENTINA. **Jujuy.** Dpto. Ledesma, Parque Nacional Calilegua, near to El Alejo trail, on dead branch of "guayabil", 7-VI-2017, *Sir & Hladki 1067* (LIL).

FRENCH GUIANA. Sinnamary, Paracou, CIRAD field station, Guyaflux plot, lowland rainforest, dead corticated branch, 24-VI-2012, *J. Fournier, GYJF 12178* (LIP).

Species formerly cited for Argentina

Annulohypoxyylon bovei (Speg.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 857. 2005. Type: Argentina, Tierra del Fuego, Isla de los Estados, on *Fagus* sp. 1882, *Spegazzini s.n.* (holotype LPS 1707!). Figs. 3A-D, 6.

For teleomorph and anamorph descriptions, see Ju & Rogers (1996: 207, as *Hypoxyylon bovei*).

Distribution. Argentina, Australia, Chile, Indonesia, and New Zealand (Ju & Rogers, 1996).

Observations. *Annulohypoxyylon bovei* is probably host-specific for *Nothofagus* Blume (Ju & Rogers, 1996). Its stromata are always pulvinate with only a few perithecia (Fig. 3A) and ostiolar discs with *bovei*-type dehiscence (Fig. 3B, C). The ascospores are ellipsoid-inequilateral with a central and short germ slit on the convex side (Fig. 3D).

The young stromata release Vinaceous Purple (101) pigments in contact with 10% KOH, but in mature stromata they are Fawn (87) or lacking. Kuhnert et al. (2017) reported the presence of daldinone A as chemotaxonomic marker for this species.

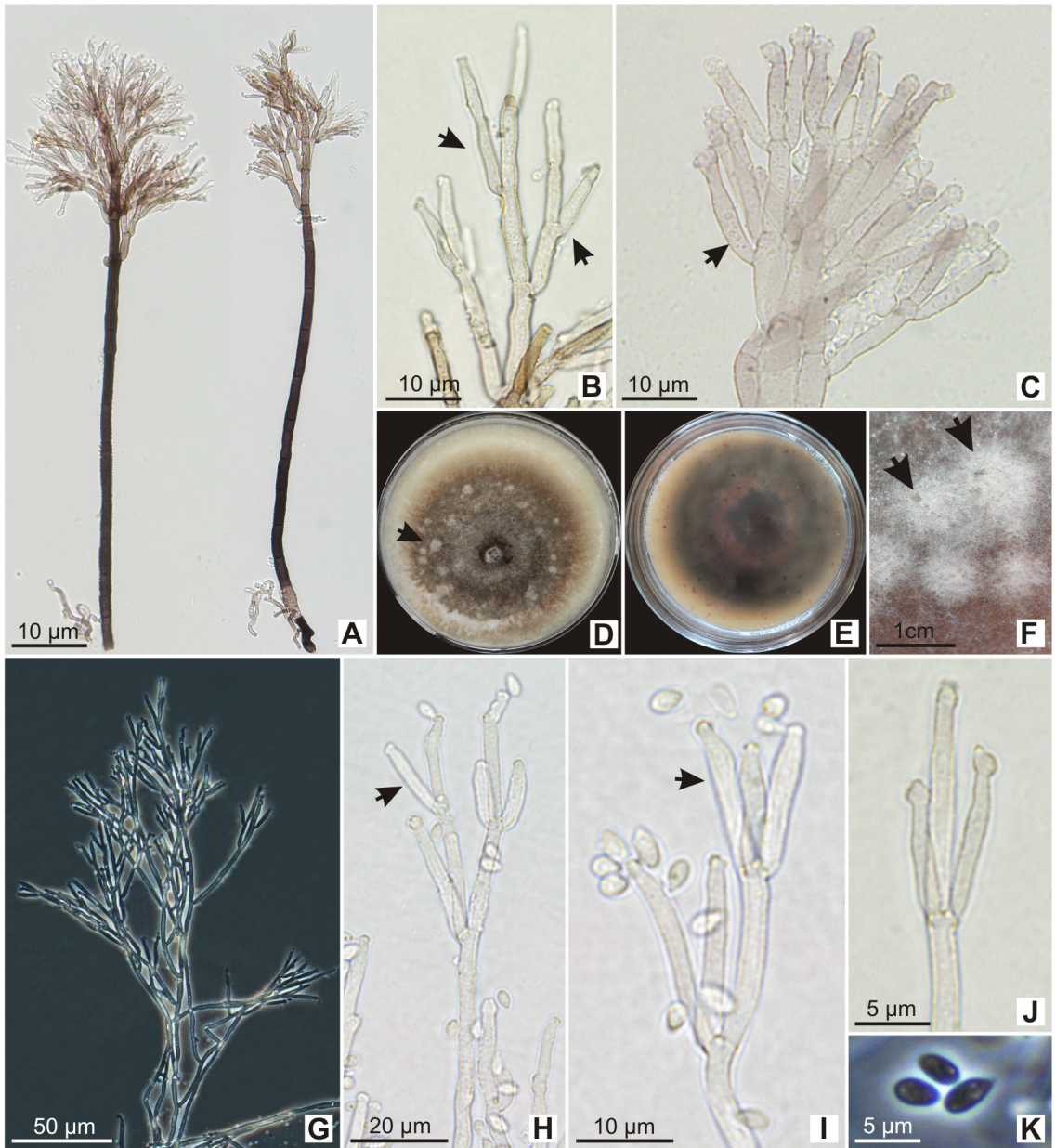


Fig. 2. *Annulohypoxyton purpureopigmentum*. A-C. Anamorph from natural substrate. D-K. Cultural characteristics on oatmeal agar after 3 weeks on 9 cm Petri dish and anamorph in vitro. **A**, conidiogenous structure. **B**, details of virgariella-like branching pattern (arrows). **C**, details of nodulisporium-like branching pattern (arrow). **D**, colony surface showing sporulation area (arrow). **E**, reverse of culture. **F**, close-up view of sporulation (arrows). **G**, conidiogenous structure from culture under polarized light. **H**, details of virgariella-like branching pattern (arrow). **I**, **J**, details of nodulisporium-like branching pattern (arrow). **K**, conidia under polarized light. A-C from *Sir & Hladki 1067* and D-K from culture *EBS 1067*. Color version at <http://www.ojs.darwin.edu.au/index.php/darwiniana/article/view/777/757>

Specimens examined

ARGENTINA. **Tierra del Fuego**, 1985, *M. M Schiavone s/n* (LIL). CHILE. **Punta Arenas**. Patagonia, 27-XI-1995, *Dusen P. 2938*, as *H.*

bovei (BPI 588062); *ibid*, 16-XII-1895, as *H. bovei* (BPI 587541).

INDONESIA. **Java**. Auth. Spec. Lév., ex Herb. Paris, corticated wood, as *H. annulatum* (BPI 738474).

NEW ZEALAND. Granville Forest, Totara Flat, Buller District, 22-V-1983, *Rossmann Amy, Samuels G., Matsushima T.*, as *H. bovei*, (BPI 1105424).

Annulohypoxylon leptascum (Speg.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 859. 2005. Type: Brazil, São Paulo, Apiyahy, on bark, 1888, *Puiggari 2769* (holotype LPS1951!). Figs. 3E-G, 6.

For teleomorph and anamorph descriptions, see Ju & Rogers (1996: 213, as *Hypoxylon leptascum*).

Distribution. Argentina, Brazil, Paraguay, Thailand, USA, and Venezuela (Ju & Rogers, 1996).

Observations. This species was reported for first time in Argentina by Daranagama et al. (2018). It can be easily distinguished from other species occurring in Argentina by its fusoid ascospores (Fig. 3G). The shape of ascospores resembles that of other *Annulohypoxylon* species, such as *A. macrosporum* (Y.M. Ju & J.D. Rogers) Sir & Kuhnert; *A. thailandicum* Daranag. & K.D. Hyde, and *A. urceolatum* (Rehm) Y.M. Ju, J.D. Rogers & H.M. Hsieh (Ju & Rogers, 1996; Liu et al., 2015). Its smaller ascospores are useful to distinguish it from *A. macrosporum* ($18-20.7 \times 4.2-5.7 \mu\text{m}$ vs $10-16.8 \times 3.5-5.2 \mu\text{m}$). Moreover, *A. thailandicum* has smaller ostiolar discs (1-2 mm vs 0.28-0.35 mm), whereas *A. urceolatum* has pigments of different color (purple vs green). *Annulohypoxylon leptascum* is also characterized by containing truncatones A & C and BNT as chemotaxonomic markers (Kuhnert et al., 2017).

Specimens examined

ARGENTINA. **Salta.** Dpto. Anta, Parque Nacional El Rey, 14-V-2012, *Sir & Hladki 186* (LIL). **Tucumán.** Dpto. Yerba, Parque Sierra de San Javier, El Balcón, 24-V-199, *Hladki 2506* (LIL); *ibid*, Horco Molle, Cuesta vieja trail, 27-XI-2017, *Sir & Hladki 1095* (LIL).

THAILAND. **Chiang Rai.** Mea Fah Luang district, 16-VIII-2013, *EK 13008* (MFLU 13-0350); *ibid*, 12-VIII-2014, *EK* (MFLU).

USA. **Florida.** Miami, 07-II-1922, *Shear C. L.*, as *H. truncatum* (BPI 593697).

Annulohypoxylon moriforme (Henn.) Y.M. Ju, J.D. Rogers & H.M. Hsieh var. **moriforme**, *Mycologia* 97(4): 859 (2005). Type: SAMOA. Upolu, corticated wood, *Reinecke s.n.* (lectotype PAD! designated by Ju & Rogers, *Mycol. Mem.* 20: 215. 1996). Figs. 3H-K, 6.

For teleomorph and anamorph descriptions, see Ju & Rogers (1996: 215-216, as *Hypoxylon moriforme*).

Distribution. Argentina, Brazil, Colombia, Guiana, Mexico, New Zealand, Samoa, Singapur, Taiwan, Trinidad, USA, and Vietnam (Ju & Rogers, 1996; Kuhnert et al., 2017).

Observations. *Annulohypoxylon moriforme* var. *moriforme* is distinguished by having glomerate or hemispherical stromata, ellipsoid-inequilateral ascospores ($6-9 \times 2.5-4 \mu\text{m}$) and green extractable pigments (Fig. 3H). Occasionally, the stromata are effused-applanate (Fig. 3I); this tendency was observed when the species grows on wood, while the stromata are glomerate to effused-pulvinate on bark.

Kuhnert et al. (2017) included the specimens cited here in their polyphasic analyses, confirming the identity of this fungus. The Argentinean specimen was found to contain truncatone A & C and hypoxylonol C & F as stromatal constituents (Kuhnert et al., 2017).

Specimens examined

ARGENTINA. **Salta.** Dpto. Gral. José de San Martín, road to Reserva Provincial de Flora y Fauna Acambuco, 27-XI-2012, *Sir & Hladki 322, 346* (LIL); *ibid*, 21-V-2015, *Sir & Hladki 813* (LIL); *ibid*, 22-V-2015, *Sir & Hladki 815, 823, 946* (LIL).

Annulohypoxylon moriforme var. **macrosporum** Hladki & A.I. Romero, *Darwiniana* 47(2): 279. 2009. Type: Argentina, Misiones, P. N. Iguazú, Macuco trail, 28-X-2003, *M. M. Schiavone s.n.* (holotipo LIL 2922!). Figs. 3L, 6.

For teleomorph description, see Hladki & Romero (2009: 279).

Distribution. Argentina (Misiones).

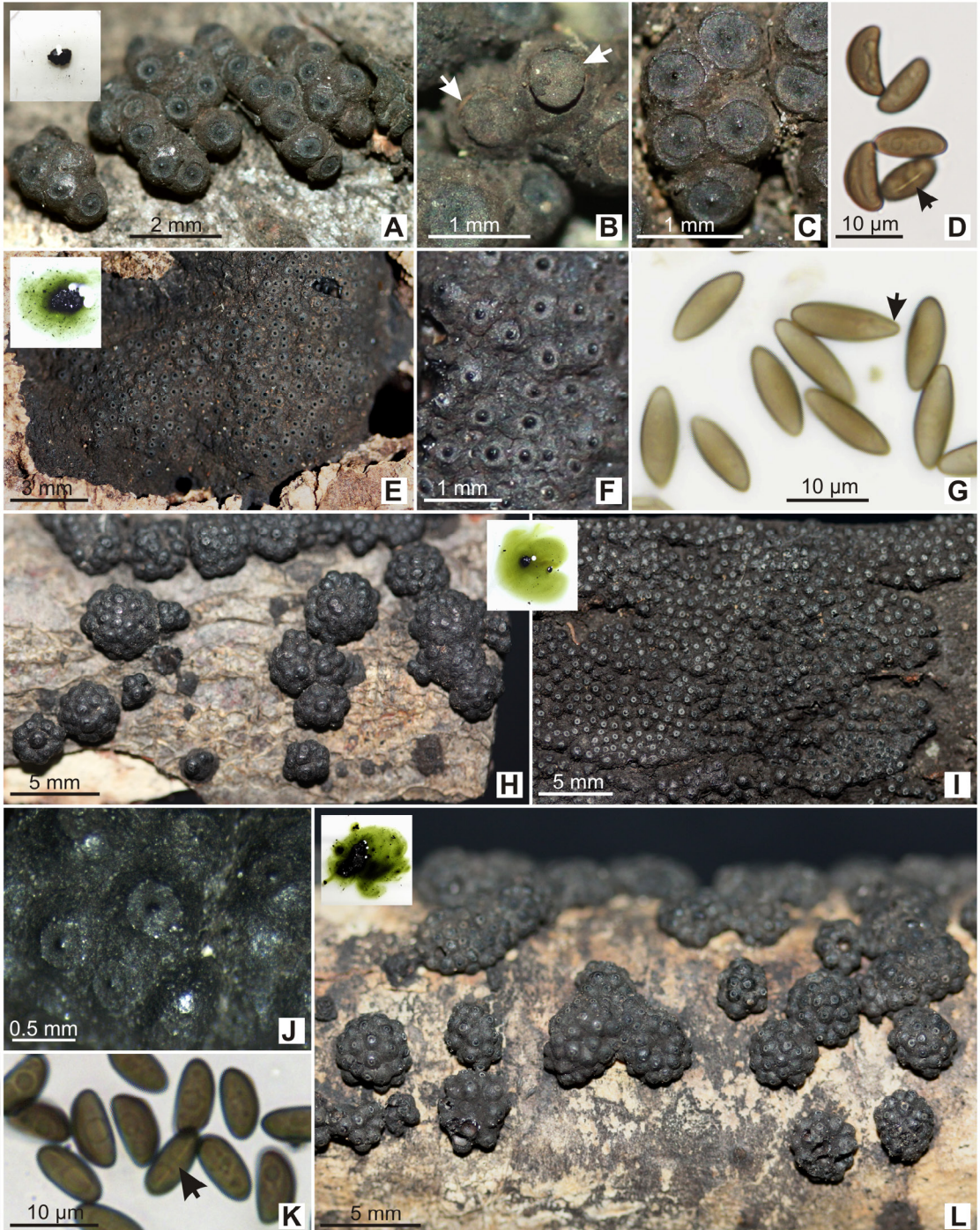


Fig. 3. A-D, *Annulohypoxyton bovei*. **A**, stromata on substrate and detail of a fragment in 10% KOH solution (inset). **B**, dehiscence of ostiolar discs (arrows). **C**, ostiolar discs. **D**, ascospores showing germ slit (arrow). E-G, *Annulohypoxyton leptascum*. **E**, stromata on substrate and extractable pigments. **F**, details of stromatal surface showing papillate ostioles and ostiolar discs. **G**, ascospores showing inconspicuous short germ slit (arrow). H-K, *Annulohypoxyton moriforme* var. *moriforme*. **H**, glomerate stromata on bark and extractable pigments. **I**, effused-applanate stromata on wood. **J**, ostiolar discs. **K**, ascospores showing germ slit on convex side (arrow). **L**, glomerate stromata and extractable pigments of *Annulohypoxyton moriforme* var. *macrosporum*. A-D from *M. M. Schiavone s/n*; E-G from *Hladki 2506*; H-K from *Sir & Hladki 322* and *L* from *LIL 2922*. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/777/757>

Table 1. Characteristics of *Annulohyphoxylon* species known from Argentina. (Abbreviations. a: effused-applanate, ep: effused-pulvinate, g: glomerate, h: hemispherical, p: pulvinate, r: rosellinoid, in: inconspicuous, c: on convex side, f: on flattened side, l: spore-length, s: short, n: nodulisporium-like branching pattern, p: periconiella-like branching pattern, v: virgariella-like branching pattern, d: unknown).

	Stroma shape	Perithecial mounds exposition	Pigments mature stroma	Ostiole discs (type and diam.)	Ascospores (µm)	Germ slit	Anamorph
<i>A. bovei</i>	p	in. to 1/3	s/p ^a	<i>bovei</i> , 5-7 mm	10.5-14 × 5-6.5	s, c	d
<i>A. leptascum</i>	ep	in.	green (90, 70, 21)	<i>truncatum</i> , 0.2-0.3 mm	7.5-13 × 3-4	s, f	p
<i>A. moriforme</i> var. <i>moriforme</i>	g, h, a, ep	in. to 1/3	green (90, 70)	<i>truncatum</i> , 0.2-0.45 mm	6-9 × 2.5-4	l, c	n
<i>A. moriforme</i> var. <i>macrosporum</i>	g, h	in. to 1/3	green (90, 70)	<i>truncatum</i> , 0.3-0.5 mm	9-10.5 × 4-5.5	l, c	d
<i>A. nitens</i>	ep, g	1/4 to 1/2	green (90)	<i>bovei</i> , 0.2-0.5 mm	6.5-11 × 3-4.5	l, c	n
<i>A. purpureonitens</i>	ep	1/4 to 1/2	Vinaceous Purple (101)	<i>bovei</i> , 0.33-0.4 mm	7.1-9.6 × 3.3-4.2	l, c	n
<i>A. purpureopigmentum</i>	ep, g	1/4 to 1/2	Vinaceous Purple (101)	<i>truncatum</i> , 0.2-0.25 mm	6.3-8 × 2.9-3.7	l, c	vn
<i>A. stygium</i>	a, ep	in.	green (90, 70)	<i>bovei</i> , 0.1-0.2 mm	5-7 × 2-3	l, f	p
<i>A. subeffusum</i>	a, ep	in.	dilute Olivaceous grey ^a (121)	<i>truncatum</i> , 0.3-0.35 mm	7-9.8 × 3-33.8	l, c	n
<i>A. substygium</i>	a, ep	in.	Fuscos Black (70), Fawn (87) or green (60)	<i>bovei</i> , 0.5-0.35 mm	6.5-8.5 × 2.8-3.5	l, f	p
<i>A. yungensis</i>	g, ep, r	in. to 2/3	Olivaceous Grey (121) to Dark Brick (60)	<i>truncatum</i> , 0.2-0.3 mm	7.6-9.9 × 3.2-4.5	l, c	p

^a See observations in each species comments.

Observations. This variety is only known from the holotype found in Misiones, Argentina. It can be separated from the type variety by its larger ascospores (9.4-10.9 × 4.5-5.4 µm; Me= 10.2 × 4.9 µm vs 7-10 × 3.3-4.6 µm; Me= 8.3 × 3.8 µm) (Table 1).

***Annulohyphoxylon nitens* (Ces.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, Mycologia 97(4): 861. 2005.** Type: Malasia: Borneo, Sarawak, corticated wood, *O. Beccari 10* (holotype RO, no visto). Figs. 4A-C, 6.

For teleomorph and anamorph descriptions, see Ju & Rogers (1996: 220, as *Hypoxyton nitens*).

Distribution. Pantropical (Fournier et al., 2016).

Observations. This is the first report of *A. nitens* for northwestern Argentina. Previously, this species had been reported for the northeastern (Entre Ríos province) and central (Buenos Aires province) regions of the country (Hladki & Romero 2009a).

The shiny black stromata, *bovei*-type ostiolar discs, and green pigments (Fig. 4A-C) with truncatone A & C, are diagnostic characters useful for the identification of this species (Ju & Rogers, 1996; Kuhnert et al., 2017).

Specimens examined

ARGENTINA. **Entre Ríos**, Parque Nacional Predelta, 32° 03'43" S 60° 38' 39" W, 11-XI-2005, *A. B. Biasuso 2946, 2947* (LIL). **Salta**. Dpto. Gral. José de San Martín, road to Reserva Provincial de Flora y Fauna Acambuco, 23-IV-2014, *Sir & Hladki 558* (LIL). Dpto. Santa Victoria, Reserva Nacional El Nogalar de los Toldos, 27-XII-2011, *Sir & Hladki 098* (LIL).

CHINA. 18-III-1934, corticated wood, *Teng, S.C. 1398*, as *H. bovei* var. *microspora* (BPI 588067).

THE PHILIPPINES. 1920, wood, *Reinking, O. 9596*, as *H. bovei* var. *microspora* (BPI 588066).

Annulohypoxyton stygium (Lév.) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *Mycologia* 97(4): 861. 2005. Type: Dominican Republic: St. Domingo, wood, *Poiteau s.n.* (holotype PC 723916!). Figs. 4D-H, 6.

For teleomorph and anamorph descriptions, see Ju & Rogers (1996: 225, as *Hypoxyton stygium*).

Distribution. Pantropical (Fournier & Lechat, 2016).

Observations. *Annulohypoxyton stygium* is cited for the first time for the north of Argentina; it was previously collected in Buenos Aires on *Eucalyptus viminalis* Labill. (Romero, 1998).

The species is characterized by having a vinaceous stromatal surface when young, becoming blackish at maturity. Its perithecial mounds are inconspicuous. In comparison with other Argentinean species, it has smaller ostiolar discs (≤ 2 mm) and ascospores, these have a germ slit on the flattened side (Table 1, Fig. 4F).

The anamorph of *A. stygium* was observed on young stromata or surrounding the mature stromata with periconiella-like branching patterns (as defined by Ju & Rogers, 1996) (Fig. 4G, H). The species usually contains BNT, truncatone A & C and an unknown major compound in its stromata (Kuhnert et al., 2017).

Specimens examined

ARGENTINA. **Jujuy**. Dpto. Ledesma, Parque Nacional Calilegua, Guaraní trail, 6-VI-2017, *Sir & Hladki 1069* (LIL). **Misiones**. Dpto. Oberá, Municipio Campo Ramón, "Centro de Investigaciones Antonia Ramos", 25-II-2015, *Sir 968* (LIL). **Salta**. Dpto. Orán, road to "Isla de Cañas", on dead trunk, 23-V-2015, *Sir & Hladki 800* (LIL).

THAILAND. **Chiang Rai**. Mea Fah Luang district, 26-VIII-2013, *E. Kuhnert 13020* (MFLU); *ibid*, Highway 1095 at 22 km market (Tapha Village) 12-VIII-2014, *E. Kuhnert 14013* (MFLU).

Annulohypoxyton subeffusum (Speg.)

Hladki & A.I. Romero, *Mycologia* 101(5): 739. 2009. Type: Paraguay, Santo Tomás, on *Eugenia* sp., 15-XII-1882, *Balansa 3766*, (holotype LPS 1939!). Figs. 4I-M, 6.

Teleomorph. Stromata effused, appanate to pulvinate; 0.7-80 mm long \times 0.5-40 mm broad \times 0.8-1 mm thick, with inconspicuous perithecial mounds up to 1/4 exposed; surface Umber (9) with grey tones when young, becoming black to blackish when old; brown to black granules immediately beneath surface, brown granules detected by microscopic examination in water; KOH-extractable pigments pale Olivaceous Grey (121); the tissue below the perithecial layer inconspicuous, black. Perithecia spherical 0.55-0.75 mm diam; ostioles conical papillate,

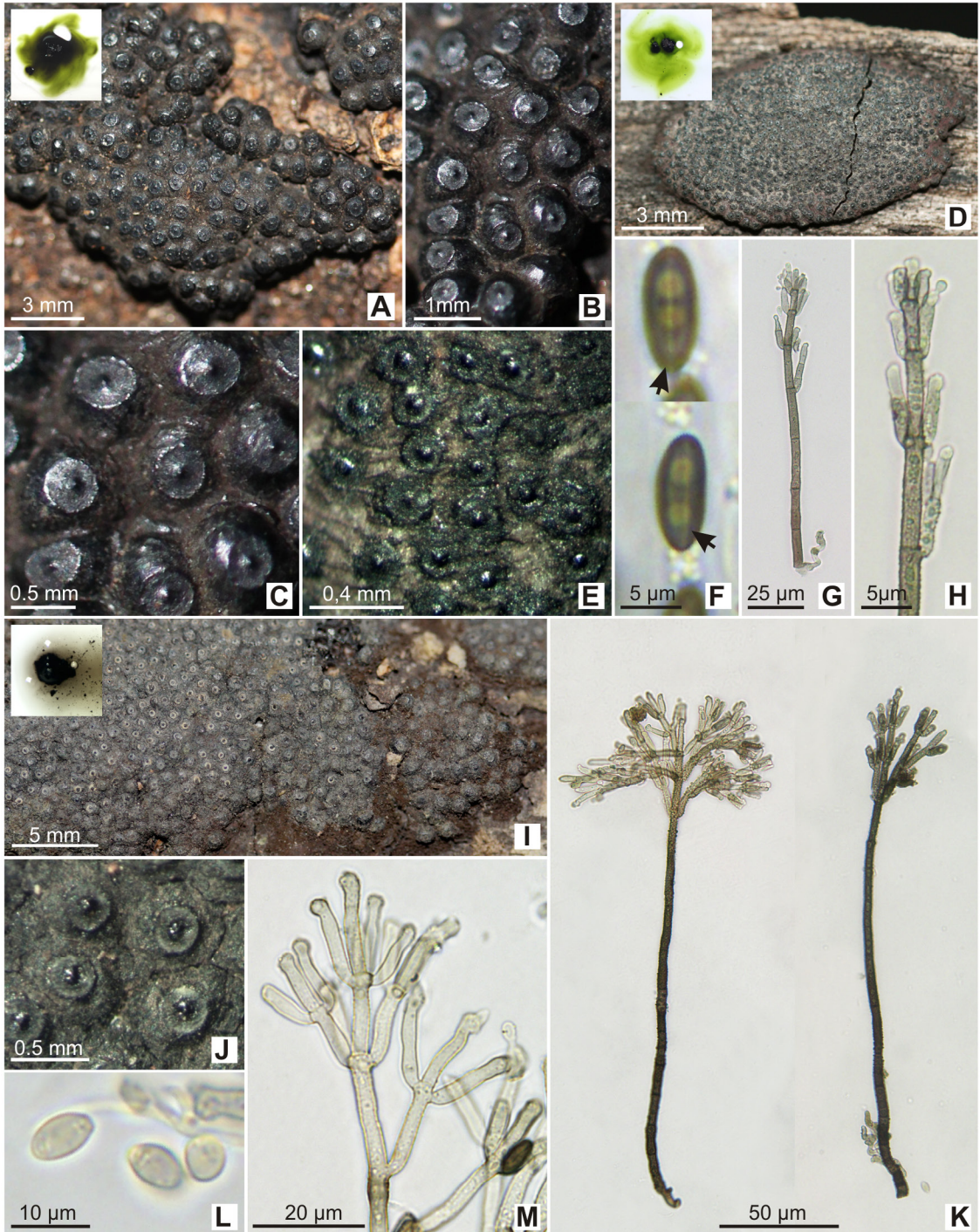


Fig. 4. A-C, *Annulohyphoxylon nitens*. **A**, stromata on bark and extractable pigments. **B**, details of stromatal surface. **C**, ostiolar discs. D-H, *Annulohyphoxylon stygium*. **D**, stromatal habit on wood and extractable pigments. **E**, stromatal surface showing papillate ostioles and ostiolar discs. **F**, ascospores showing germ slit (arrows). **G**, conidiophore from substrate. **H**, details of periconiella-like branching pattern. I-M, *Annulohyphoxylon subeffusum*. **I**, stromatal habit and extractable pigments. **J**, ostiolar discs. **K**, conidiophores from substrate. **L**, conidia. **M**, details of nodulisporium-like branching pattern. A-C from *Sir & Hladki 098*; D-H from *Sir & Hladki 800* and I-M from *Sir & Hladki 805*. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/777/757>

encircled with a *truncatum*-type disc 0.3-0.35 mm diam. Asci 8-spored, cylindrical, 109-169 μm total length, spore-bearing part 42-57 \times 4-7 μm , stipe 59-120 μm long; with amyloid, discoid apical apparatus, 0.65-0.95 \times 1.2-1.7 μm . Paraphyses filiform 2-3.7 μm , wide at the base, tapering above asci. Ascospores brown, unicellular, ellipsoid-inequilateral, with broadly rounded ends, (7)8-9(9.8) \times 3-3.8 μm (N= 60; Me= 8.5 \times 3.4 μm); with straight germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth with a thickening on the convex side; episporium smooth. Anamorph (from Sir & Hladki 805): Conidiogenous structure on tomentose tissue *Sepia* (63) color. Conidiophores with nodulisporium-like branching pattern, 120-400 μm high, brown, roughened. Conidiogenous cell hyaline to pale brown, roughened, 11-20 \times 2.5-3 μm . Conidia hyaline to pale brown, smooth to slightly roughened, ellipsoid, 4.8-6 \times 2.6-3.2 μm .

Culture. Not obtained.

Distribution. Argentina and Paraguay (Hladki & Romero, 2009b; Agüero et al., 2010).

Observations. *Annulohypoxyton subeffusum* was erected by Spegazzini (1884) and is only known from the Southern Cone. It is characterized by effused, applanate to pulvinate blackish stromata with brown tones when young, inconspicuous up to 1/4 exposed perithecial mounds; ostiolar *truncatum*-type discs; amyloid apical rings and brown ellipsoid-inequilateral ascospores with straight germ slits spore-length and dehiscent perispore in KOH. The most useful character for delimiting this species is their diluted Olivaceous Grey (107) extractable pigments composed of BNT and truncatones A & C as chemotaxonomic markers (Kuhnert et al., 2017).

Specimens examined

ARGENTINA. **Salta.** Dpto. Gral. José de San Martín, road to Reserva Provincial de Flora y Fauna Acambuco, 21-V-2015, on dead trunk of Myrtaceae, *Sir & Hladki* 805, 810, 818 (LIL).

Annulohypoxyton substygium Sir & Kuhnert, *Fungal Diversity* 85: 20 (2017). Type: Georgia, Batum?, Cehis-Dzisi Caucasiae?, in horto Penkov?, corticated wood of *Carpinus*, 20-II-1912, G. Newodowski 77 (holotype S F10761!). Fig. 5A-G, 6.

For teleomorph and anamorph descriptions, see Kuhnert et al. (2017: 20-21).

Distribution. Argentina, Georgia, and Iran (Kuhnert et al., 2017).

Observations. This species was erected by elevating the former variety *A. stygium* var. *annulatum* to species rank, based on morphological, molecular, and chemotaxonomic data (Kuhnert et al., 2017). Its ascospores are similar to *A. stygium* but can be easily differentiated by the size of the perithecia and ostiolar discs (Table 1). Typical stromatal metabolites are daldinone A and BNT (Kuhnert et al., 2017).

The species has a particular distribution and a variety of hosts. It was found on *Carpinus* L. in Georgia and on *Acer* L. in Iran; while the Argentinean materials were found on *Junglans australis* Griseb. (“nogal criollo”), on *Celtis ehrenbergiana* (Klotzsch) Liebm. (“tala”) and on remains of others unidentified native trees.

Specimen examined

ARGENTINA. **Salta.** Dpto. Santa Victoria, Reserva Nacional El Nogalar de los Toldos, on trunk of *Junglans australis* Griseb. (“nogal criollo”), 27-XII-2011, *Sir & Hladki* 083, 093, 112 (LIL); *ibid*, 26-VI-2013, *Sir & Hladki* 468 (LIL, BAFC). **Tucumán.** Dpto. Chicligasta, road to Parque Nacional Campo de los Alisos, on dead branch of “tala”, 18-V-2015, *Sir & Hladki* 799 (LIL); *ibid*, Parque Nacional Campo de los Alisos, on dead trunk of “nogal criollo”, 15-V-2017, *Sir & Hladki* 1032 (LIL).

Annulohypoxyton yungensis Sir, Kuhnert, Hladki & A. I. Romero, *Fungal Diversity* 85: 13. 2017. Type: Argentina, Prov. Tucumán, Dpto. Yerba Buena, Parque Sierras de San Javier, La Cascada, 7-V-2013, *Sir & Hladki* 412 (holotype LIL!). Figs. 5H-L, 6.

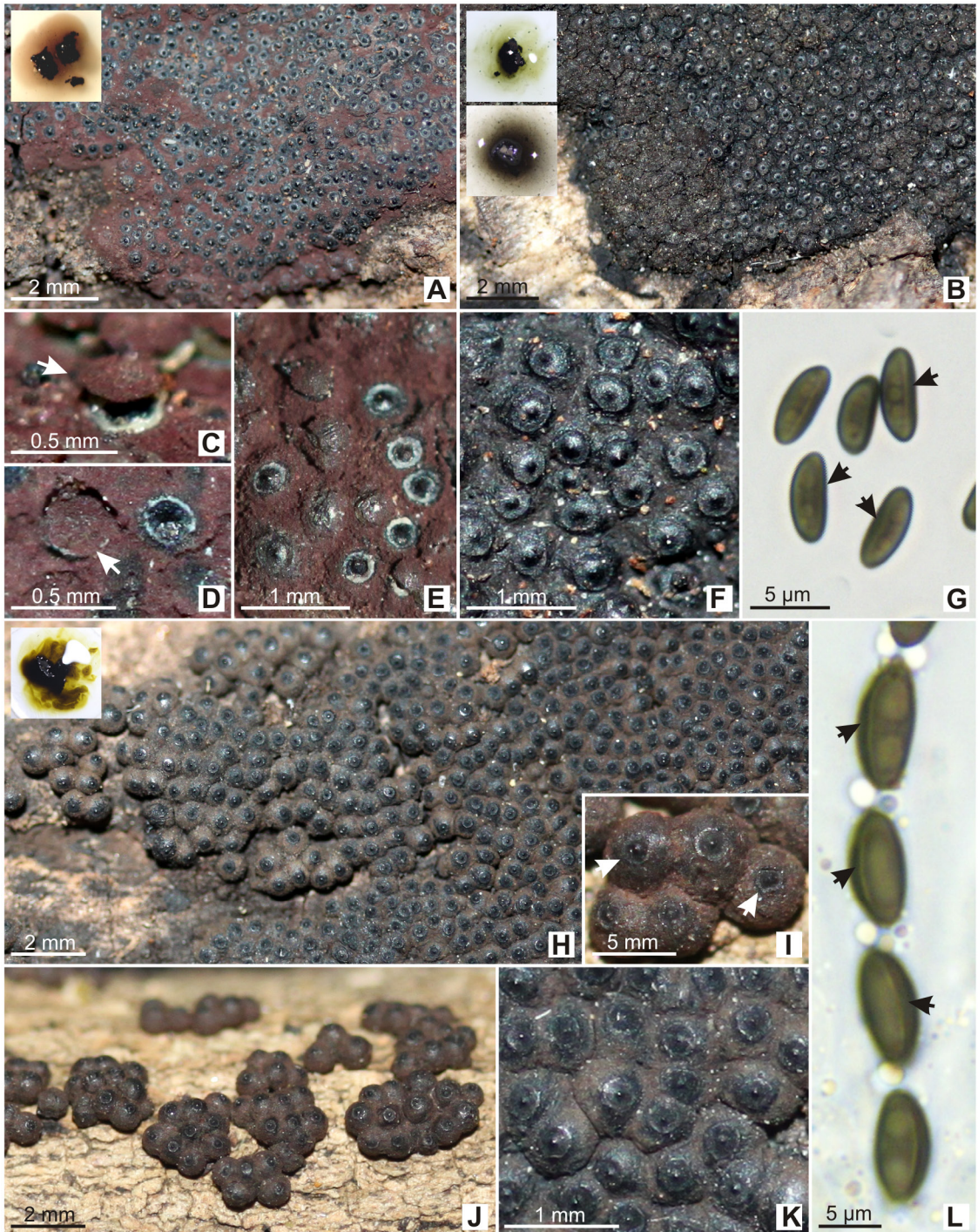


Fig. 5. A-G, *Annulohypoxyton substygium*. A, young stromata and extractable pigments. B, mature stromata and extractable pigments. C, D, details of the formation of *bovei*-type discs (arrows). E, stromatal surface of young stromata. F, stromatal surface of mature stromata showing ostiolar discs. G, ascospores showing germ slit (arrows). H-L, *Annulohypoxyton yungensis*. H, stromatal habit on wood of trunk. I, details of the formation of *truncatum*-type discs (arrows). J, stromatal habit on bark of small branch. K, details of ostiolar discs. L, ascospores showing germ slit (arrows). A-G from Sir & Hladki 468; H, I, K, L from Sir & Hladki 412 and J from Sir & Hladki 455. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/777/757>

For teleomorph and anamorph descriptions, see Kuhnert et al. (2017: 13).

Distribution. Argentina (Kuhnert et al., 2017).

Observations. *Annulohypoxyton yungensis* is very common in the northwest of Argentina but it was not found in the montane forest of Jujuy province. The diagnostic characters are exposed in table 1.

Specimens examined

ARGENTINA. **Salta.** Dpto. Anta, Parque Nacional El Rey, 29-IV-2014, *Sir & Hladki* 727, 728, 734 (LIL). Dpto. Santa Victoria, Reserva Nacional El Nogalar de los Toldos, 27-XII-2011, *Sir & Hladki* 291 (LIL, BAFC). **Tucumán,** Dpto. Yerba Buena, Parque Sierra de San Javier, La Cascada, 7-V-2013, *Sir & Hladki* 414 (LIL); *ibid*, Horco Molle, 21-V-2013, *Sir & Hladki* 448 (LIL, BAFC). Dpto. Chicligasta, Parque Provincial El Cochuna, La Virgen, 13-V-2013, *Sir & Hladki* 454, 455, 463 (LIL, BAFC).

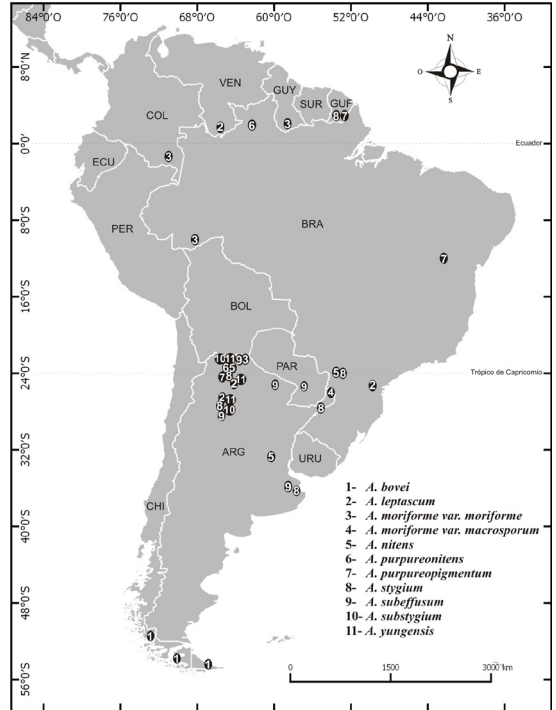


Fig. 6. South American distribution of the *Annulohypoxyton* species treated in this work.

Provisional key to *Annulohypoxyton* in Argentina

- 1. KOH-extractable pigments vinaceous to purple 2
- 1. KOH-extractable pigments otherwise 3
- 2(1). Mature stromata shiny black, ostiolar discs 0.33-0.4 mm diam. *A. purpureonitens*
- 2. Mature stromata umber, sepia to blackish; ostiolar discs 0.2-0.25 mm diam. *A. purpureopigmentum*
- 3(1). Ostiolar discs 0.1-0.2 mm diam. *A. stygium*
- 3. Ostiolar discs ≥ 2 mm diam. 4
- 4(3). Ascospores ellipsoid-fusoid slightly inequilateral with inconspicuous short germ slit in one end and indehiscent perispore *A. leptascum*
- 4. Ascospores not fusoid, ellipsoid-inequilateral with germ slit spore-length or central short and dehiscent perispore 5
- 5(4). Stromata pulvinate always with few perithecia, usually without extractable pigments, ostiolar discs 5-7 mm diam. and ascospores with central and short germ slit *A. bovei*
- 5. Stromata glomerate, effused-pulvinate, hemispherical, usually with many perithecia, always with extractable pigments, ostiolar discs ≤ 5 mm diam. and ascospores with spore-length germ slit 6
- 6(5). Ascospores with germ slit on the flattened side *A. substygium*
- 6. Ascospores with germ slit on the convex side 7
- 7(6). KOH-extractable pigments dilute olivaceous grey *A. subeffusum*
- 7. KOH-extractable pigments concentrate, usually green 8
- 8(7). Ostiolar discs up to 0.3 mm diam., extractable pigments olivaceous grey becoming dark brick *A. yungensis*
- 8. Ostiolar discs up to 0.5 mm diam., extractable pigments green, dark green or olivaceous 9
- 9(9). Stromata effused-pulvinate, rarely glomerate to hemispherical, with reddish-brown tones, becoming shiny black at maturity *A. nitens*
- 9. Stromata glomerate, hemispherical, or rarely effused-applanate, surface with olivaceous tones, becoming dull blackish at maturity 10
- 10(9). Ascospores $7-10 \times 3.3-4.6 \mu\text{m}$ (Me= $8.3 \times 3.8 \mu\text{m}$) *A. moriforme* var. *moriforme*
- 10. Ascospores $9.4-10.9 \times 4.5-5.4 \mu\text{m}$ (Me= $10.2 \times 4.9 \mu\text{m}$) *A. moriforme* var. *macrosporum*

DISCUSSION AND CONCLUSIONS

The research of the diversity of xylariaceous fungi carried out in the last fifteen years in the northwest of Argentina can be considered as one of the most extensive and intensive made in the country. As a consequence, almost a hundred of xylariaceous species have been recorded, from which 52% belong to the Xylariaceae, 45% to the Hypoxylaceae, 2 % to the Graphostromataceae and 1 % to the Lopadostomataceae (Daranagama et al., 2018; Hladki & Romero, 2010; Kuhnert et al., 2015, 2017; Medina et al., 2016; Sir et al., 2012a, b, c; 2013; 2015, 2016a, b, 2017; Sir & Hladki, 2014). Additionally, we are aware of the existence of more than 130 species (unpublished data).

Previously, nine *Annulohypoxyton* species had been cited for Argentina. In the present work, we report eleven species, including two new records (*A. stygium* and *A. nitens*) for the Argentinean mycobiota. Particularly, we must highlight that prior to our surveys, only *A. subeffusum* was known for the northwest of Argentina (Agüero et al., 2010). Since then, we reported additional species for this region, i.e. *A. moriforme* var. *moriforme*, *A. leptascum*, *A. substygium* and *A. yungensis* (Kuhnert et al., 2017; Daranagama et al., 2018). Thus we recognize eight species of the genus for the northwest of Argentina, which is the highest percentage of species for the country. This figure is due to the greater sampling effort carried out in the subtropical montane forest of Argentina. We expect at least ten more species of *Annulohypoxyton* to be present in “Las Yungas” area reflecting the high diversity of the mycobiota of Argentina.

ACKNOWLEDGEMENTS

The authors express their appreciation to the authorities of Fundación Miguel Lillo Tucumán, DAAD (German Academic Exchange Service) and to the Ministerio de Ciencia, Tecnología e Innovación Productiva of Argentina, for financial support. We would also like to thank the Administración de Parques Nacionales of Argentina, Ministerio de Medio Ambiente of Salta Province and Dirección Provincial de Biodiversidad of Jujuy Province for authorization of

collection. We gratefully acknowledge support from the curators of various international herbaria, who provided important specimens for the present study.

We are thankful to Dr. Marc Stadler for revising the manuscript and EBS thanks Rodrigo Delgado and Jorge Salas from the FML for the support in the field trips.

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