

New records of *Hypoxylon* (Ascomycota, Hypoxylaceae) for the Argentine Yungas

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Abstract: *Hypoxylon aeruginosum* var. *aeruginosum*, *H. fendleri* and *H. undulatum* collected in the subtropical montane forest from northwest Argentina are reported for the first time for the Southern Cone. We describe and analyze their teleomorph and anamorph, and provide photographs and drawings of the most relevant structures. Moreover, a key to *Hypoxylon* species so far known from Argentina is given.

Key words: Neotropics, Mycobiota, Xylariales.

Resumen: Nuevos registros de *Hypoxylon* (Ascomycota, Hypoxylaceae) para Las Yungas Argentinas.

Como resultado del estudio de especímenes coleccionados en el bosque montano del Noroeste Argentino, se citan por primera vez para el Cono Sur a *Hypoxylon aeruginosum* var. *aeruginosum*, *H. fendleri* y *H. undulatum*. Se describen y analizan los teleomorfos y anamorfos de esas especies, aportando fotografías y dibujos de las estructuras más relevantes. Además, se proporciona una clave para identificar las especies de *Hypoxylon* hasta ahora conocidas en Argentina.

Palabras clave: Neotrópico, Micobiota, Xylariales.

INTRODUCTION

Hypoxylon Bull. is the largest and most complex genus of the family Hypoxylaceae (Wendt *et al.*, 2018). The genus includes species with unipartite hemispherical to effused-pulvinate stromata with colored surface and homogeneous waxy to fibrous tissue below perithecial layer; and a nodulisporium-like anamorph, but with variations in the branching patterns of the conidiophores (Daranagama *et al.*, 2018; Ju & Rogers, 1996; Wendt *et al.*, 2018). Their stromata release diverse pigments in contact with KOH solution. These pigments are secondary metabolites deposited as colored granules below the stromatal surface and surrounding the perithecia, which frequently can possess species-specific chemical entities useful to discriminate *Hypoxylon* species (Hellwig *et al.*, 2005; Kuhnert *et al.*, 2014ab; Stadler *et al.*, 2008).

Members of the genus are recognized as saprobes or as facultative parasites when found on wood, but can also be isolated as part of the en-

dophytic community in most of the forest trees (Kuhnert *et al.*, 2014a). The majority of species has been reported from warmer climates especially the Tropics. This however could be the result of less extensive sampling in subtropical or temperate areas of the world (Fournier *et al.*, 2015).

Since the beginning of the century, several surveys on the xylariaceous diversity have been carried out in the Argentine Yungas, the southernmost subtropical montane forest of the Neotropics (Hladki, 2007; Kuhnert *et al.*, 2015, 2017; Sir *et al.*, 2012abc, 2013, 2015, 2016ab, 2018; Sir & Hladki, 2014). These mycological trips showed that the genus *Hypoxylon* is a remarkable, yet poorly documented component of the mycobiota of this ecosystem (Sir *et al.*, 2012a). Hladki & Romero (2009ab) and Catania & Romero (2010) published the first reports of *Hypoxylon* species from the Argentine northwest, recording less than ten species. In one of our previous studies, we already increased the knowledge about the diversity of the genus (Sir *et*

al., 2016). In this context we proposed three new species: *H. calileguense* Sir, Kuhnert, Hladki & A.I. Romero, *H. lilloi* Sir, Lamb. & Kuhnert and *H. spegazzinianum* Sir, Kuhnert, Hladki & A.I. Romero; and recognized eight new records for Argentina: *H. carneum* Petch, *H. chionostomum* Speg, *H. flavoargillaceum* J. H. Mill., *H. griseobrunneum* (B.S. Mehrotra) J. Fourn., Kuhnert & M. Stadler, *H. haematostroma* Mont., *H. investiens* (Schwein.) M.A. Curtis, *H. lividipigmentum* F. San Martín, Y. M. Ju & J. D. Rogers, and *H. umbilicatum* Speg.

Here we report, three new species records of *Hypoxylon* for the Southern Cone of South America, collected in the Argentine Yungas. Also, we provide an emended taxonomic key for *Hypoxylon* species known from Argentina.

MATERIALS AND METHODS

Fungal collections were obtained between 2011 and 2015 at the Parque Nacional Calilegua and Reserva Provincial Las Lancitas from Jujuy province, Reserva provincial de Flora y Fauna Acambuco and Parque Nacional el Rey from Salta province, and Parque Sierra de San Javier from Tucuman province.

The materials were studied and isolated according to Sir *et al.* (2016). The color of stromata and extractable pigments are described following the color chart of Rayner (1970). The perispore was analysed under field-emission scanning electron microscope (SEM-Zeiss, Supra 55vp) in the Centro Integral de Microscopía Electrónica (CIME, CONICET-UNT). The reference collections were deposited in the LIL herbarium. Additional specimens examined were obtained from LIP and K herbaria (acronyms are from Index Herbariorum, <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). The author names of the fungal species were taken from Index Fungorum (<http://www.indexfungorum.org/>).

RESULTS

Taxonomic part

Hypoxylon aeruginosum J.H. Mill., var.
aeruginosum Mycologia, 25 (4): 321(1933).
Figs. 1 and 4a

Stromata effused-pulvinate; 10–25 mm long × 5–15 mm broad × 0.4–6 mm thick; with inconspicuous perithecial mounds to plane; surface

Verdigris (74) to Cyan Blue (26) with Brick (59) margin, pruinose; black granules immediately beneath the surface and between perithecia, pale blue and pale green in water; with KOH-extractable pigments Umber (9) with Pale Vinaceous (85) tones; tissue below the perithecial layer inconspicuous, black. Perithecia obovoid to spherical 0.25–0.4 mm high × 0.1–0.2 mm diam., ostioles umbilicate. Asci cylindrical, 8-spored, 118–154 μm total length, the spore-bearing parts 68–75 × 6.8–8 μm and stipes 49–80 μm long, with amyloid apical apparatus, discoid, 0.5–0.7 μm high × 2–2.5 μm broad. Ascospores light brown to brown, unicellular, ellipsoid, nearly equilateral, with broadly to less frequently narrowly rounded ends, 8–11.3(11.5) × 4.4–6 μm (N= 60, Me= 10.1 × 5.5 μm), with straight germ slit less than to nearly spore-length; perispore indehiscent in 10% KOH; epispore smooth under LM and SEM. Conidiogenous structure with virgariella-like branching patterns. Conidiophores hyaline, smooth. Conidiogenous cells hyaline, smooth, 15–27(38.5) × 2.3–2.9 μm. Conidia ellipsoid, hyaline, smooth, 4–6.4 × 2.9–3.8 μm.

Culture: not obtained.

Secondary metabolites: derivatives of lepranic acid (Læssøe *et al.*, 2010).

Specimens examined: ARGENTINA. Tucumán, depto. Tafi Viejo, Parque Sierra de San Javier, la Cascada, 6/5/2013, Sir & Hladki 410 (LIL). FRENCH GUIANA: Sinnamary, Paracou, edge of parking area of CIRAD field station, on a corticated branch, possibly on old remnants of a Corticiaceae, 26/6/2012, leg. J. Fournier, GYJF 12236 (LIP, epitype).

Discussion: *Hypoxylon aeruginosum* is a species that was unknown from subtropical regions of the American continent, being previously recorded only from sites of the American tropical zone, i.e. Ecuador, French Guiana, Guadeloupe, Guyana, Martinique and Mexico (Fournier *et al.*, 2015; Fournier & Lechat, 2015). This taxon is distinct from other *Hypoxylon* by its blue effused-pulvinate stromata and nearly equilateral ascospores which measure less than 11.5 μm (Ju & Rogers, 1996). The anamorph of this species is characterized and illustrated for first time herein.

In our collection a part of the specimen was found on top of another *Hypoxylon* sp. This phenomenon was also observed by Læssøe *et al.* (2010), who suggested a fungicolous life style.

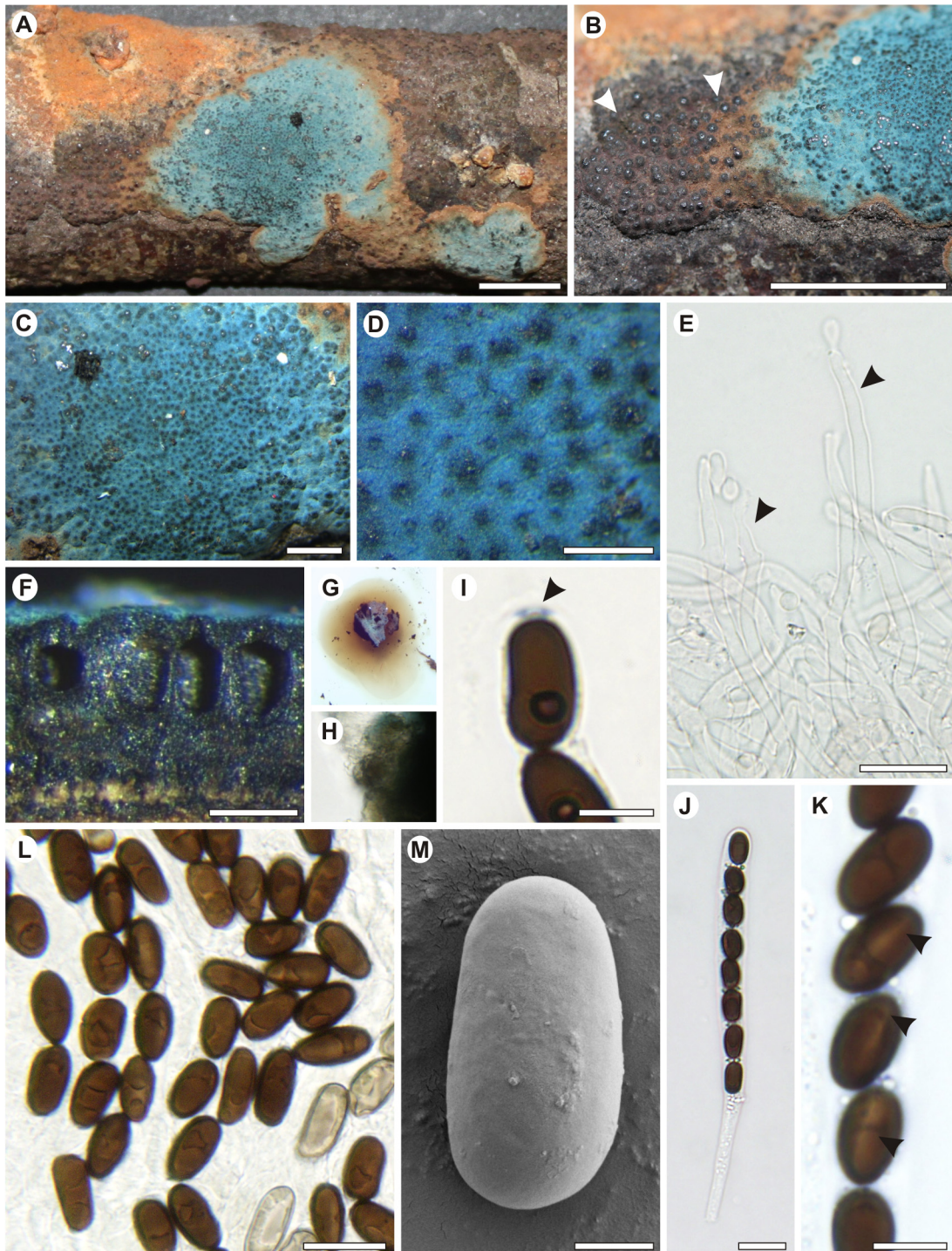


Fig. 1. *Hypoxylon aeruginosum* (Sir & Hladki 410-LIL). A: stromata on substrate, B: detail of stroma on top of *Hypoxylon* sp. (arrows). C and D: details of stromatal surface showing ostioles. E: portion of anamorph showing conidiogenous cells (arrows). F: stroma in section showing the perithecia. G: KOH-extractable pigments, H: stromatal granules in water. I: ascus apical apparatus in Melzer's reagent. J: mature ascus. K: ascospores in KOH solution showing germ slit (arrows). L: ascospores in water. M: ascospores view under SEM showing smooth episore. Scale bars. A, B: 5 mm. C: 1 mm. D, F: 0.3 mm. E, J, L: 10 μ m. I, K: 5 μ m. M: 2 μ m.

Hypoxyylon fendleri Berk. ex Cooke, Grevillea
11: 132. 1883. Figs. 2 and 4b.

Stromata effused-pulvinate; 5–40 mm long × 3–20 mm broad × 0.7–1 mm thick; with conspicuous to inconspicuous perithecial mounds, in some case with wrinkled surface; surface Brown Vinaceous (84) with orange tones, pruinose; orange granules immediately beneath the surface and between perithecia, orange in water; with KOH-extractable pigments Orange (7); tissue below the perithecial layer inconspicuous, black. Perithecia obovoid 0.4–0.6 mm high × 0.3–0.5 mm diam., ostioles umbilicate. Asci cylindrical, 8-spored, 85–160 μm total length, the spore-bearing parts 70–80 × 6–7.88 μm and stipes 45–80 μm long, with amyloid apical apparatus, discoid, 0.7–1.4 μm high × 2–2.7 μm broad. Ascospores brown to dark brown, ellipsoid-inequilateral, with narrow rounded ends, (8.4)9.3–11.7(12.4) × (4.6)4.8–5.9(6.6) μm (N= 60, Me= 10.7 × 5.4 μm), with sigmoid germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth to faintly striated under LM, striated under SEM; epispore smooth. Conidiogenous structure with virgariella-like and nodulisporium-like branching patterns. Conidiogenous cells hyaline, smooth to slightly roughened, 15–25 × 2.1–3.4 μm. Conidia ellipsoid, hyaline, smooth, 5.0–5.9 × 3.1–3.9 μm.

Culture: for description see Ju & Rogers (1996).

Secondary metabolites: mitorubrin, mitorubrinol, mitorubrinol acetate, orsellinic acid and BNT (Stadler *et al.*, 2008).

Specimens examined: ARGENTINA, Jujuy, dpto. Santa Bárbara, Reserva Provincial Las Lancitas, 13/5/2012, Sir & Hladki 248 (LIL). Salta, La Candelaria, El Jardín, 3/5/2013, Sir & Hladki 387 (LIL). Dpto. Gral. José de San Martín, Reserva de Flora y Fauna Acambuco, 22/4/2014, Sir & Hladki 505 (LIL); road to Reserva de Flora y Fauna Acambuco, 23/4/2014, Sir & Hladki 574 (LIL); 21/5/2015, Sir & Hladki 950 (LIL). Dpto. Anta, Parque Nacional El Rey, 29/4/2014, Sir & Hladki 712, 720 (LIL); 28/5/2015, Sir & Hladki 887 (LIL). VENEZUELA: 261, corticated wood (K, holotype).

Discussion: *Hypoxyylon fendleri* is a very common fungus in the tropical regions of the world (Fournier *et al.*, 2015); this is the first record of the species for a subtropical area of South America. The taxon can be identified by its vinaceous stromatal surface, orange granules, orange extractable pigments, ascospores with sigmoid

germ slit and nodulisporium-like anamorph (Ju & Rogers, 1996). The characteristics of the cultures obtained from Argentinean material were in accordance with previous descriptions for species (Ju & Rogers, 1996).

Hypoxyylon undulatum Y.M. Ju, J.D. Rogers & Læssøe, Mycol. Mem. 20: 199 (1996).
Figs. 3 and 4c.

Stromata effused-pulvinate; 10–25 mm long × 5–15 mm broad, 0.4–1 mm thick; with conspicuous to very conspicuous perithecial mounds, in some case approaching to rosellinoid; surface Brown Vinaceous (84) to Fuscous (103), pruinose; dull rusty brown granules immediately beneath the surface and between perithecia, reddish brown in water; without apparent KOH-extractable pigments; tissue below the perithecial layer inconspicuous, black. Perithecia spherical to spherical compressed 0.2–0.3 mm diam, ostioles umbilicate, opening at the centre of a raised disc, 0.1–0.13 mm diam. Asci cylindrical, 8-spored, 89–117 μm total length, the spore-bearing parts 64–77 × 5–7.7 μm and stipes 20–40 μm long, with amyloid apical apparatus, discoid, 1 μm high × 1.5–1.8 μm broad. Ascospores brown to dark brown, ellipsoid-inequilateral, with narrow rounded ends, (9.1)9.6–11.0(11.6) × (3.5)3.9–4.8(5.1) μm (N= 60, Me= 10.3 × 4.2 μm), with straight germ slit spore-length on the convex side; perispore dehiscent in 10% KOH, smooth to faintly striated under LM, faintly striated under SEM; epispore smooth. Conidiogenous structure with virgariella-like and nodulisporium-like branching patterns. Conidiophores hyaline, smooth to slightly roughened, Conidiogenous cells hyaline, smooth to slightly roughened, 10.4–23 × 2–2.8 μm. Conidia ellipsoid, hyaline, smooth, 3.5–5 (6.5) × 2.2–3.5 μm.

Culture: for description see Ju & Rogers (1996).

Secondary metabolites: unknown.

Specimens examined: Argentina. Jujuy, Ledesma, Parque Nacional Calilegua, 12/12/2015, Sir & Hladki 997 (LIL). Gral. José de San Martín, road to Reserva de Flora y Fauna Acambuco, 21/5/2015, Sir & Hladki 849 (LIL).

Discussion: *Hypoxyylon undulatum* was only known by its type material from Saint John (U. S. Virgins Island). This species is characterized by having effused-pulvinate stromata, fuscous surface with strongly exposed perithecial mounds, ostioles with a raised disc and by lacking apparent

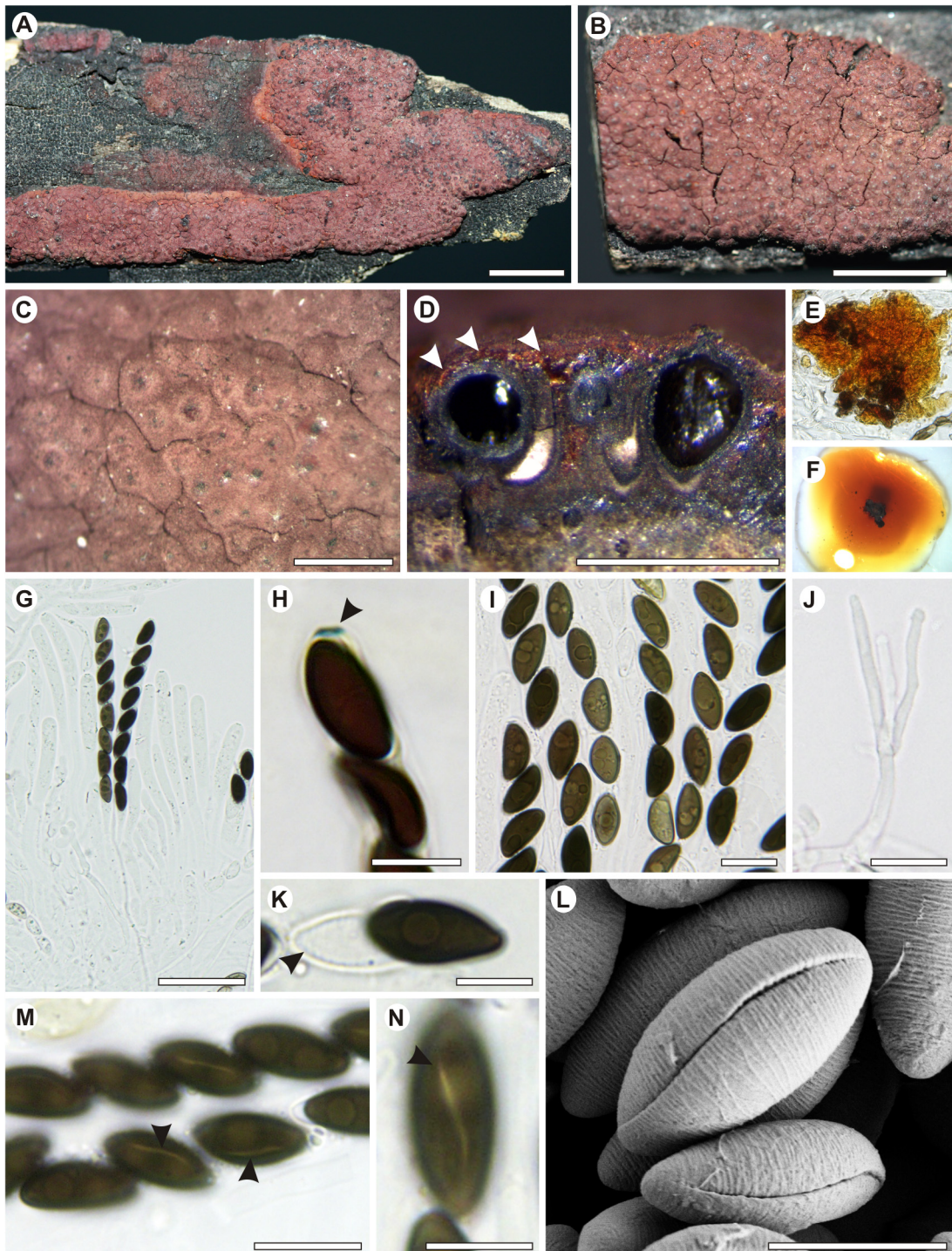


Fig. 2. *Hypoxylon fendleri* (Sir & Hladki 574-LIL). A and B: stromatal habit. C: stromatal surface. D: stroma in section showing perithecia and granules (arrows). E: stromatal granules in water. F: extractable pigments. G: immature and mature asci. H: ascial apical apparatus in Melzer's reagent. I: immature and mature ascospores in water. J: branching of anamorph. K: ascospores in KOH solution showing dehiscent perispore. L: ascospores view under SEM showing striated perispore. M and N: ascospores in KOH solution showing germ slit (arrows). Scale bars. A, B: 5 mm. C, D: 1 mm. G: 20 μ m. H, I, J, M: 10 μ m. K, L, N: 5 μ m.

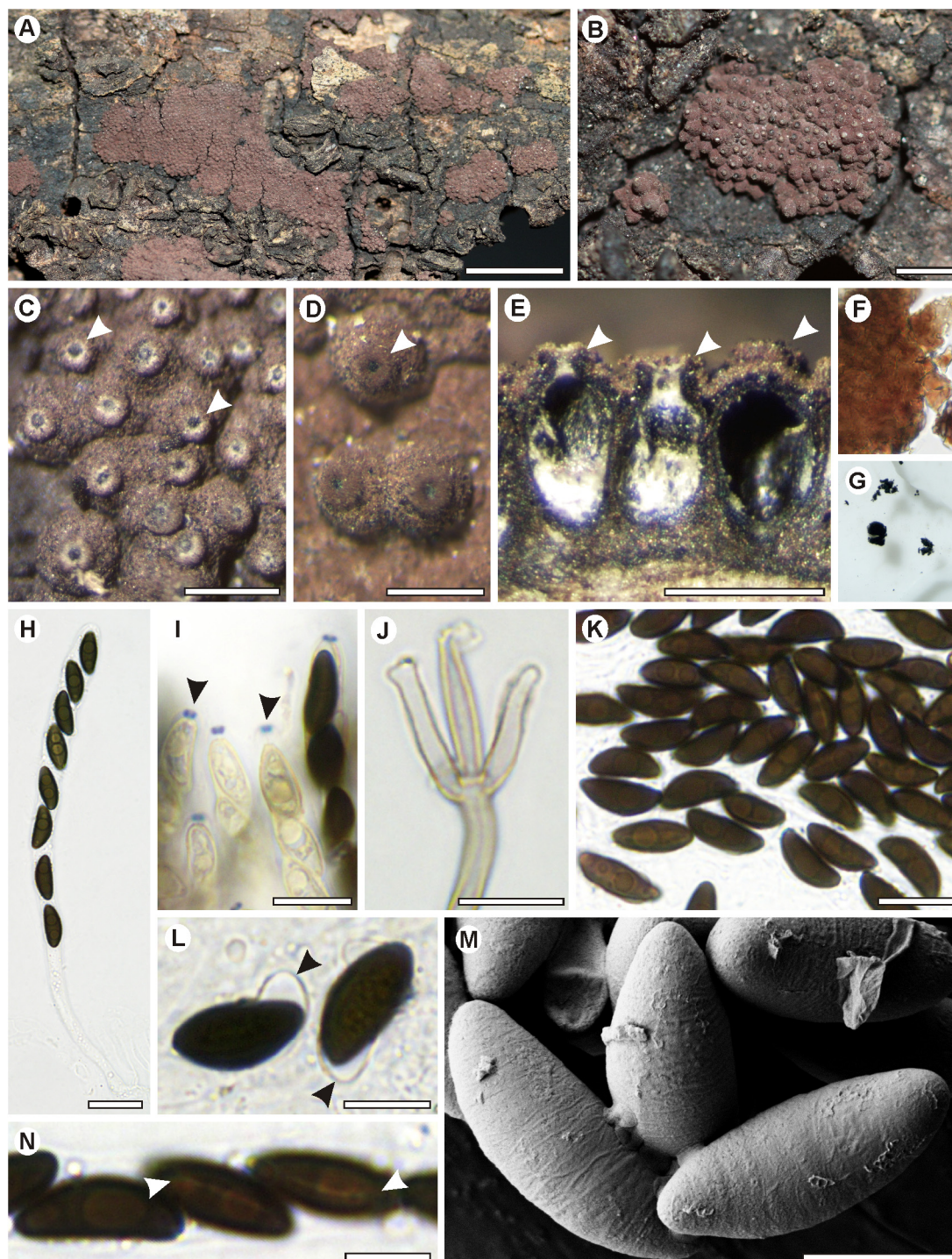


Fig. 3. *Hypoxylon undulatum* (Sir & Hladki 997-LIL). A and B: stromata on bark. C and D: stromatal surface showing ostiolar discs (arrows). E: stromata in vertical section showing perithecia and ostiolar disc (arrows). F: stromatal granules in water. G: portion of stroma in 10% KOH. H: ascus, I: ascus in Melzer's reagent (arrows). J: branching of anamorph. K: ascospores. L: ascospores in KOH solution showing dehiscent perispore. M: ascospores view under SEM showing faintly striated perispore. N: ascospores with germ slit (arrows). Scale bars. A: 5mm, B: 1 mm. C-E: 0.3 mm, H-K: 10 μ m. L-N: 5 μ m.

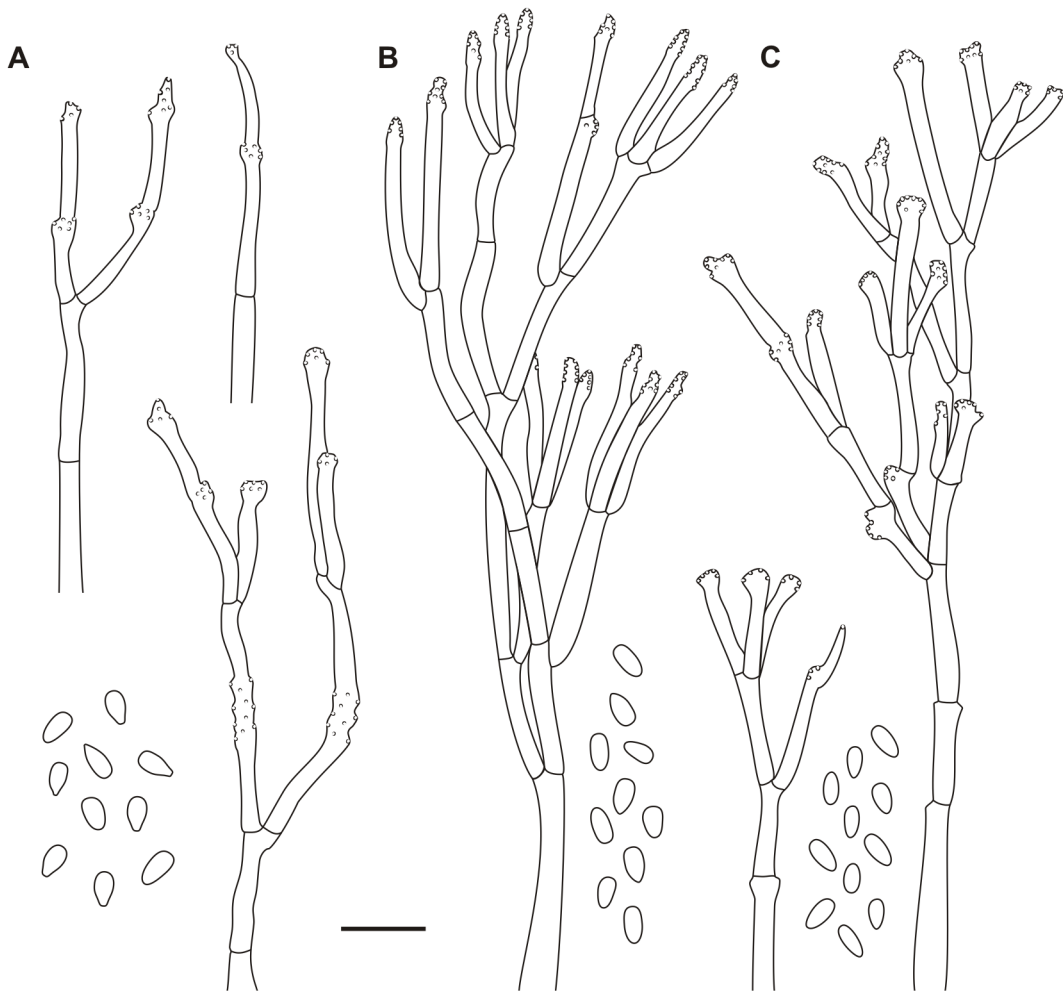


Fig. 4. Conidiophores and conidia. A: *H. aeruginosum* var. *aeruginosum* (from substrate, Sir & Hladki 410-LIL), B: *H. fendleri* (from substrate, Sir & Hladki 574-LIL), C: *H. undulatum* (from culture, EBS 849-LIL). Scale bar 10 μ m.

Key to species of *Hypoxylon* in Argentina

1. Ascospores averaging more than 25 μ m in length 2
- 1'. Ascospores averaging less than 25 μ m in length 4
2. Extractable pigments isabelline, ascospores subglobose, ellipsoid to pyriform, 28-41.5 \times 17-22 μ m..... *H. megalosporum*
- 2'. Without apparent pigments or with dilute vinaceous grey or brown vinaceous pigments, ascospores ellipsoid 3
3. Perithecia obovoid 0.5-0.7 mm diam.; ascospores 29.9-48.2 \times 16-29 μ m *H. umbilicatum*
- 3'. Perithecia spherical, 1-1.4 mm diam.; ascospores 27.5-33 \times 15.5-21 μ m*H. chionostomum*
4. Stromatal surface color blue *H. aeruginosum* var. *aeruginosum*

- 4'. Stromatal surface color other than above 5
5. KOH-extractable pigments dark livid, vinaceous purple or without apparent pigments 6
- 5'. KOH-extractable pigments with other than above colors 8
6. Stromatal surface chestnut or sepia, KOH-extractable pigments dark livid, ascospores $7.9-14.5 \times 5-7 \mu\text{m}$ *H. lividipigmentum*
- 6'. Stromatal surface purplish gray or vinaceous gray, KOH-extractable pigments livid purple or absent, ascospores less than $11 \mu\text{m}$ long 7
7. Ascospores with straight germ slit at the center of a dotted band, perispore dehiscent, smooth under SEM; conidiophores with nodulisporium-like branching patterns *H. carneum*
- 7'. Ascospores with straight germ slit not at the center of a dotted band, perispore dehiscent with inconspicuous striated ornamentation under light microscope, very conspicuous striated under SEM, conidiophores with virgariella-like branching patterns *H. lilloi*
8. Ascospores nearly equilateral to slightly inequilateral, with germ slit on flattened side, perispore indehiscent in 10 % KOH 9
- 8'. Ascospores conspicuously inequilateral, with germ slit on convex side, perispore dehiscent in 10% KOH 11
9. Stromata pulvinate with inconspicuous to conspicuous perithecial mounds, surface sulfur yellow, KOH-extractable pigments red livid or vinaceous livid, ascospores $11-12 \text{ mm} \times 5-6 \mu\text{m}$ *H. kermesii*
- 9'. Stromata effused-pulvinate, plane or with inconspicuous perithecial mounds, surface dark vinaceous to brown vinaceous, KOH-extractable pigments dull green or dark green 10
10. Ascospores $9-11 \times 4-5 \mu\text{m}$ *H. investiens* var. *magnisporum*
- 10'. Ascospores $7-10 \times 3.3-4.5 \mu\text{m}$ *H. investiens* var. *investiens*
- 11'. Stromata hemispherical, pulvinate, effused, effused pulvinate; plane or with inconspicuous to conspicuous perithecial mounds 12
- 11'. Stromata glomerate to effused-pulvinate with conspicuous to very conspicuous perithecial mounds, sometimes with the tendency to be perithecioid (approaching rosellinioid) 24
12. Extractable pigments orange, scarlet or rust 13
- 12'. Extractable pigments olivaceous, gray olivaceous, greenish olivaceous, dull green, dark green, amber, isabelline or fawn 20
13. Stromata with dull-colored granules, yellowish brown or brown 14
- 13'. Stromata with bright-colored granules, orange or orange red 15
14. Ascospores $8-12 \times 4-5.5 \mu\text{m}$ *H. rubiginosum* var. *rubiginosum*
- 14'. Ascospores $7.5-9 \times 3.5-5 \mu\text{m}$ *H. rubiginosum* var. *microsporium*
15. Ascospores greater than $15 \mu\text{m}$ long 16
- 15'. Ascospores less than $15 \mu\text{m}$ long 17
16. Perithecia long tubular, more than 1.5 mm high, ascospores $15.1-23.5 \times 6.0-8.9 \mu\text{m}$, with smooth perispore, straight germ slit; conidiophores with periconiella-like branching patterns *H. haematostroma*
- 16'. Perithecia spherical, ovoid, obovoid or tubular, less than 1.5 mm high, ascospores $14.5-17 \times 6.5-7 \mu\text{m}$ with faintly striated to striated perispore, straight or slightly sigmoid germ slit; conidiophores with virgariella-like branching patterns *H. crocopeplum*
17. Ascospores less than $12 \mu\text{m}$ long and $5.5 \mu\text{m}$ wide 18
- 17'. Ascospores greater than $12 \mu\text{m}$ long and $5.5 \mu\text{m}$ wide 19
18. Stromatal surface brown vinaceous, dark vinaceous, dark brick, or sepia, ascospores $8-12 \times 4-5.5 \mu\text{m}$, sigmoid germ slit, conidia $4-6 \times 2.5-4 \mu\text{m}$ *H. fendleri*
- 18'. Stromatal surface umber, sepia, rust, sienna, dark brick, or bay; ascospores $7-11 \times 3.5-5.5 \mu\text{m}$, straight to slightly sigmoid germ slit, conidia $3-4.5 \times 1.5-2 \mu\text{m}$ *H. subgilvum*

19. Ascospores $10.7-15.0 \times 4.9-7.7 \mu\text{m}$, sigmoid germ slit; conidiophores with virgariella-like branching patterns, conidia $4.5-5 \times 2-2.5 \mu\text{m}$ *H. spegazzinianum*
- 19'. Ascospores $9.5-14.3 \times 4.7-6.5 \mu\text{m}$, straight germ slit; conidiophores with nodulisporium-like branching patterns, conidia $5-8 \times 3-4 \mu\text{m}$ *H. calileguense*
20. Ostioles umbilicate with white tissue surrounding the ostioles; conidiophores with virgariella-like branching patterns..... *H. perforatum*
- 20'. Ostioles umbilicate without white tissue surrounding the ostioles; conidiophores with nodulisporium-like branching patterns 21
21. Stromata pulvinate to effused-pulvinate (rare glomerate) with inconspicuous to conspicuous perithecial mounds, surface sepia, chestnut, perithecia spherical $0.2-0.5 \text{ mm diam.}$
.....*H. subrutilum*
- 21'. Stromata effused-pulvinate, plane or with inconspicuous perithecial mounds, surface brown vinaceous or dark vinaceous, perithecia obovoid to tubular $0.2-0.3 \text{ mm diam.}$ 22
22. Stromatal granules dull olivaceous yellow; ascospores $9.7-13.0 \times 4.0-5.7 \mu\text{m}$, with straight germ slit spore-length *H. griseobrunneum*
- 22'. Stromatal granules dull reddish brown or blackish; ascospores with straight to slightly sigmoid germ slit spore-length 23
23. Ascospores $10-12.5 \times 4.2-5.6 \mu\text{m}$, conidia $5-7 \times 3-4 \mu\text{m}$ *H. anthochroum*
- 23'. Ascospores $12.3-13.5 \times 5-6 \mu\text{m}$, conidia $3-4.5 \times 1.5-2 \mu\text{m}$ *H. duranii*
24. Ostioles slightly higher than the stromatal surface or with small papilla, ascospores $11-17 \times 4-6 \mu\text{m}$ with sigmoid germ slit; conidiophores with nodulisporium-like branching patterns.....
..... *H. lenormandii*
- 24'. Ostioles umbilicate most often opening at the centre of a raised disc, ascospores with straight or straight to slightly sigmoid germ slit, conidiophores with virgariella-like or nodulisporium-like branching patterns..... 25
25. Without apparent extractable pigments; ascospores shorter than $12 \mu\text{m}$ and narrower than $6 \mu\text{m}$; conidiophores with nodulisporium-like and virgariella-like branching patterns
..... *H. undulatum*
- 25'. Extractable pigments amber, orange or pure yellow; ascospores longer than $13 \mu\text{m}$ and wider than $6 \mu\text{m}$; conidiophores with virgariella-like branching patterns 26
26. Apical apparatus bluing in Melzer's iodine reagent, discoid, ascospores $13.9-21 \times 6.8-10.2 \mu\text{m}$, conidia $5-6.5(-7) \times 2-3 \mu\text{m}$ *H. flavoargillaceum*
- 26'. Apical apparatus highly reduced or lacking, not bluing in Melzer's iodine reagent, ascospores $14.5-15.5 \times 6.5-8 \mu\text{m}$, conidia $4.5-5.5 \times 3-3.5 \mu\text{m}$ *H. notatum*

KOH-extractable pigments (Ju & Rogers, 1996). The cultures obtained from Argentine material show the same features described for the ex-type culture of this species.

Medina et al. (2017) reported one specimen for Argentina found on *Ocotea porphyria* (Griseb.) van der Werff (Lauraceae) as *H. cf. undulatum*. It differs principally from the specimens here described by having orange extractable pigments.

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