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Short Communication

Hypoxylon ochraceotuberosum and *Hypoxylon rickii* (Ascomycota, Hypoxylaceae) in Las Yungas of Salta province, Northwest of Argentina

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Abstract

Hypoxylon ochraceotuberosum and *H. rickii* are reported for the first time for the Argentinean funga based on recently collected specimens from the northwestern montane forest. Until now, *H. ochraceotuberosum* has only been known to occur in the Martinique (French West Indies). In contrast, *H. rickii* was already recorded for the Southern Cone, but based on specimens collected in the Atlantic Rain Forests from Southeast Brazil. Detailed descriptions and photo illustrations are provided for each species.

Key words: Neotropics, nodulisporium-like, Sordariomycetes, virgariella-like, Xylariales.

Resumen

Hypoxylon ochraceotuberosum y *H. rickii* se reportan por primera vez para la funga Argentina con base a especímenes coleccionados recientemente en el bosque montano del Noroeste. Hasta ahora, *H. ocraceotubeosum* solo se conocía de Martinica (French West Indies). En contraste, *H. rickii* ya estaba reportada para el Cono Sur, pero basado en especímenes coleccionados en la Mata Atlántica del Sureste de Brasil. Se aportan descripciones detalladas e ilustraciones fotográficas para cada especie.

Palabras clave: Neotrópico, tipo nodulisporium, Sordariomycetes, tipo virgariella, Xylariales.

The subtropical montane forests (Yungas) in the northwest provinces of Argentina are one of the main areas of plants diversity of the country (Brown *et al.* 2002). Several mycological researches carried out in this area exposed an abundant and rich community of ascomycetous Xylariales related to these flora (Hladki & Romero 2001, 2003, 2010; Kuhnert *et al*. 2017; Sir *et al.* 2012, 2016a, 2018). Among the genera belonging to the family Hypoxylaceae encountered in Las Yungas, *Hypoxylon* Bull., is one of the most common ones according to recent data (Diaz *et al.* 2018). Of the 27 species formally documented for the Argentine funga, 23 have been collected in the forests of the Northwest (Hladki & Romero 2009; Sir *et al.* 2016b; Diaz *et al*. 2018).

Hypoxylon fungi are remarkable inhabitants of decaying wood (as saprotrophs) or in living plants (as endophytes) across all hardwood forests of the world (Sir *et al.* 2019). They exhibit a high morphological diversity, and many of their members have proved to be an exceptional source for biologically active secondary metabolites (Helaly *et al.* 2018).

Species of the genus develop perithecioid ascomata embedded in often colorful effused or pulvinate unipartite stromata

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and nodulisporium-like conidiophores with holoblastic conidiogenesis (Ju and Rogers 1996; Wendt *et al.* 2018). The recently segregation of *Hypoxylon monticulosum* Mont. and its allies in the new genus *Hypomontagnella* Sir, L. Wendt & C. Lambert, restricted the generic concept of *Hypoxylon*. Thus, the members of *Hypoxylon* are currently distinguished from related genera by its homogeneous stromatal context with waxy to woody tissue always releasing pigments in KOH (eventually with fibrous or carbonaceous remnants) and umbilicate or slightly papillate ostioles, without disks (Lambert *et al.* 2019).

This note is providing descriptions of the teleomorphic and anamorphic structures of two new *Hypoxylon* records for the montane forest from the Argentine Northwest. One of them, *H. ochraceotuberosum* J. Fourn. & Lechat, is recorded for the first time in South America, and *H. rickii* Y.M. Ju & J.D. Rogers is recognized for the first time in this region.

The specimens were collected during our continuous mycological expeditions carried out in the montane forest of Argentine northwest (Fig. 1). The occurrence of reproductive stages of *Hypoxylon ochraceotuberosum* and *H. rickii* in this forest appears uncommon as in almost ten years of continuous field work very few stromata of these species have been found. The collections are preserved as reference materials in the LIL herbarium (acronym taken from Index Herbariorum, <http://sweetgum.nybg.org/science/ih/>).

The morphological characterizations and cultures of the specimens follow Sir *et al.* (2015). Colors are assigned based on the color chart of Rayner (1970). The ultrastructure of the perispore were analysed by scanning electron microscopy

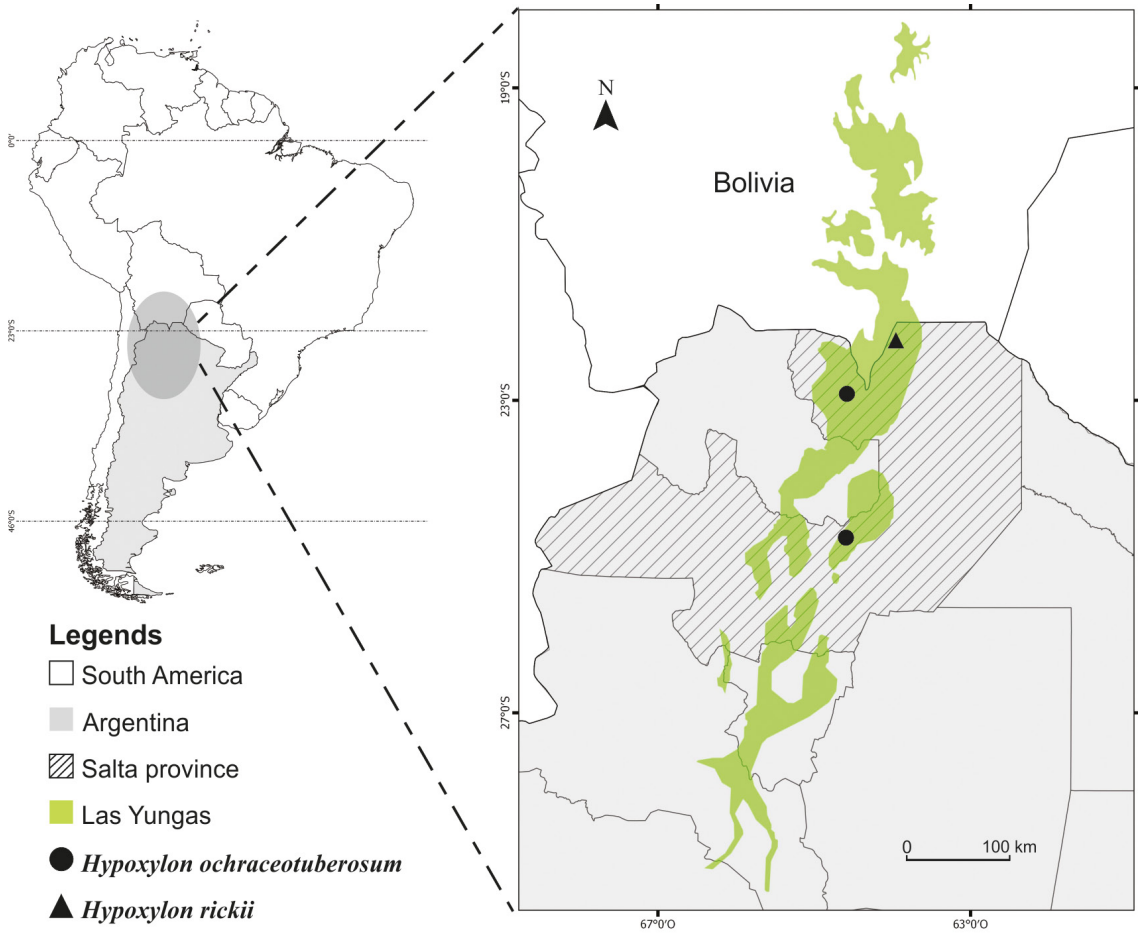

Figure 1 – Sites of collections.

(Zeiss-Supra 55vp) in the Centro Integral de Microscopy Electrónica (CIME, CONICET-UNT).

1. *Hypoxylon ochraceotuberosum* J. Fourn. & Lechat, Ascomycete.org 7(5): 184 (2015).

Figs. 1; 2a-p; 3a-j

Stromata hemispherical to depressed-spherical, the base broadly attached to the substrate, coalescent, 3−13 mm diam × 3−6 thick; with inconspicuous perithecial mounds; surface Ochraceous (44) to Fawn (87), pruinose; orange reddish granules immediately beneath surface, yellow to orange in water; with KOH extractable pigment Orange (7); tissue bellow the perithecial layer very conspicuous, blackish to brown, 2.5−5.5 mm thick. Perithecia spherical to obovoid, 0.25−0.35 mm high × 0.25–0.3 mm diam; ostioles lower than the stromatal surface, umbilicate, surrounded by a fugacious raised disc. Asci 8-spored, cylindrical, 75−125 µm total length, the spore-bearing parts 40−60 µm × 5.5–8.3 µm broad, stipes 35.6−65 µm long; with amyloid, discoid apical apparatus 0.4−0.8 high × 2.1−2.8 µm broad. Ascospores brown to dark brown, ellipsoid-equilateral, with broadly rounded ends, (7.2)7.5−9.0(9.8) × (4.3)4.4−5.6(5.8) µm (N = 40; Me = 8.2 × 5.0 µm) with a faint straight germ slit nearly spore-length; perispore dehiscent in KOH, smooth in LM and SEM; epispore smooth. Conidiogenous structure, produced in artificial culture, mononematose. Conidiogenous structure with virgariella-like branching patterns rarely nodulisporium-like. Conidiophores hyaline to light brown in water (light purple in 3% KOH solution), smooth to finely roughened. Conidiogenous cells hyaline, smooth to finely roughened, (10.6)11.3−17.1(20.3) × (2.5)2.8−3.8(3.9) µm. Conidia ellipsoid, hyaline, smooth, (6.1)6.3−7.7(8.1) × (2.8)3.0−4.1(5.2) µm (N = 20, Me = 6.9 × 3.6 µm). Culture, colonies on Oatmeal Agar medium covering Petri dish in 4 weeks, at first whitish becoming Isabelline (65) to Sepia (63) with Citrine (13) areas of sporulation, velvety to felty, azonate, with entire margins. Reverse Buff (46).

Materials examined: ARGENTINA. SALTA PROVINCE: Anta Department, Parque Nacional El Rey, 24°43’40.6’’S, 64°40’09.1’’W, 973 m alt., on small corticated branches of indeterminate dicotyledonous plant, 6.VI.2019, *Sir & Medina 1231* (LIL). Orán Department, road to Isla de Cañas, 23°03’21.0’’S, 64°33’34.7’’W, 593 m alt., on small corticated branches of indeterminate dicotyledonous plant, 23.V.2015, *Sir & Hladki 941* (LIL).

The material described here as *Hypoxylon ochraceotuberosum* conforms with the concept provided by Fournier *et al.* (2015). The species is distinctive among the other *Hypoxylon* spp. by the following combination of characters: stromata with very thick subperithecial tissue, small ascomata and ellipsoid-equilateral ascospores with dehiscent perispore. The anamorphic structure obtained on cultures from Argentine collections is partially similar to those previously described for the species. Fournier *et al.* (2015) observed remnants of short geniculate conidiophores with vague virgariella-like branching patterns, and hyaline ellipsoid conidia 7.3–8 × 4–4.5 µm associated with young stromata on the natural substrate. Our culture developed conidiophores with virgariella-like to (rarely) nodulisporium-like branching patterns without geniculate conidiogenous cells.

Hypoxylon ochraceotuberosum is a rarely recorded species in the world; it was erected for a single specimens collected in the Martinique (Fournier *et al.* 2015). The specimens from Argentina represent the first collections of this species for the American continent and the second record over all.

2. *Hypoxylon rickii* Y.M. Ju & J.D. Rogers, Mycol. Mem. 20: 174(1996).

Figs. Figs. 1; 4a-n; 5a-f

Stromata effused-pulvinate, plane or with inconspicuous to conspicuous perithecial mounds, 15−30 mm long × 10−20 mm broad, 0.8−1 mm thick; with inconspicuous perithecial mounds; surface Rust (39), Fulvous (43) or Sienna (8), pruinose; orange red granules immediately beneath surface, orange in water; with KOH extractable pigment Orange (7); tissue bellow the perithecial layer inconspicuous, black. Perithecia obovoid to tubular, 0.55−0.7 mm high × 0.25−0.3 mm diam; ostioles lower than the stromatal surface, umbilicate. Asci 8-spored, cylindrical, 98−139 µm total length, the spore-bearing parts 46−49.5 µm × 4.5−5.7 µm broad, stipes 50−100 µm long; with amyloid, discoid apical apparatus 0.3−0.6 high × 1.1−1.7 µm broad. Ascospores brown, ellipsoid-inequilateral, with narrowly rounded ends, 6.5−7.9 × 3.2−3.8(4.0) µm (N = 40, Me = 7.3 × 3.6 µm) with slightly

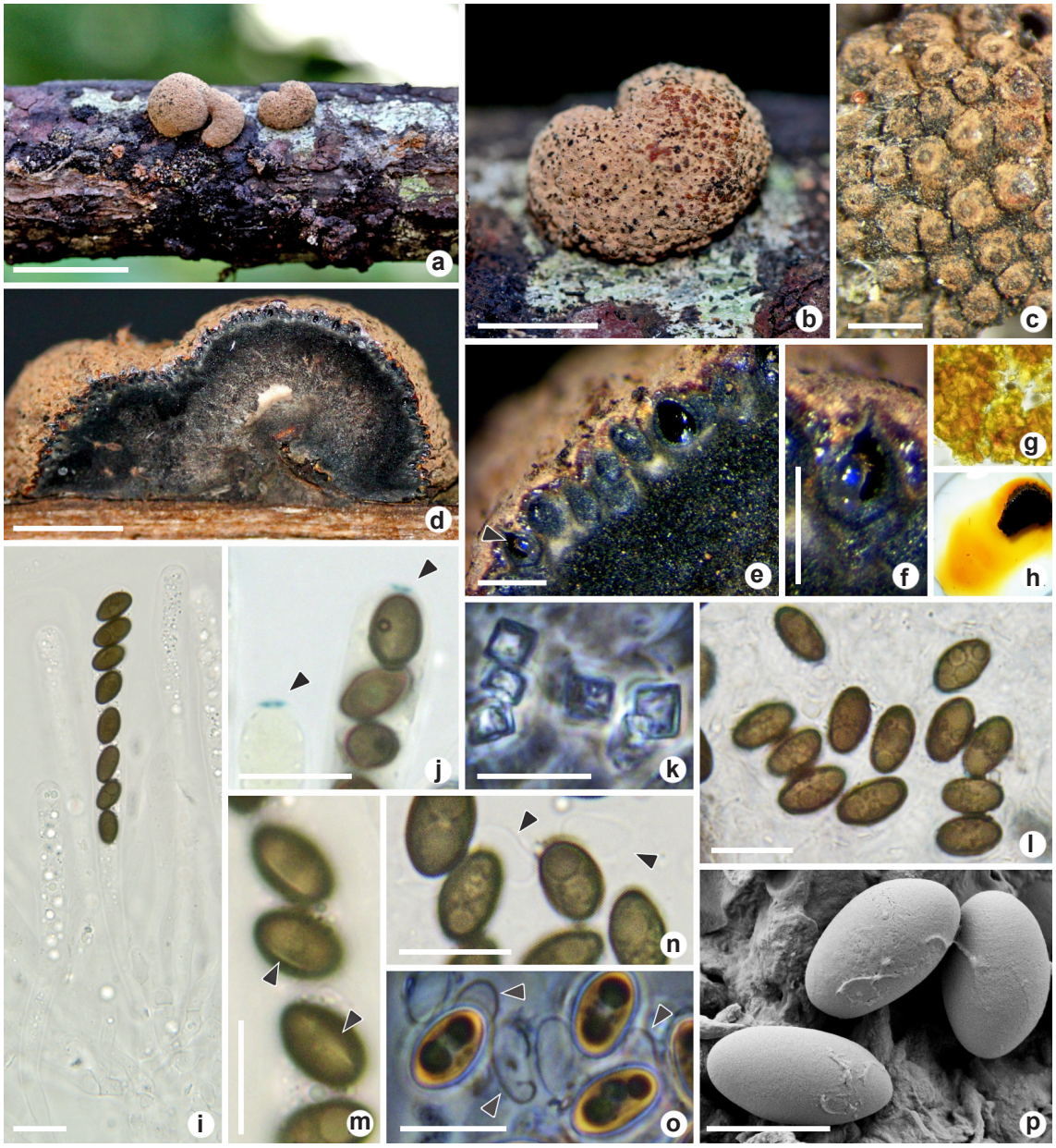

Figure 2 – a-p. Teleomorphic structures of *Hypoxylon ochraceotuberosum* – a,b. stromatal habit; c. stromatal surface showing ostioles with raised disc; d. stromata in section, showing the conspicuous internal tissue; e. perithecial layer, arrow show insect larva inside perithecia; f. detail of perithecia with insect larva; g. stromatal granules in water; h. KOH-extractable pigments; i. mature and immature asci; j. asci in Melzer's reagent, revealing amyloid apical apparati (arrows); k. subhymenial crystals visualized under polarized light; l. ascospores in water; m. ascospores in KOH solution showing straight germ slit (arrows); n,o. ascospores in KOH solution (o view under polarized light), showing dehiscing perispore (arrows); p. ascospores under SEM. (a-p. *Sir & Medina 1231*). Scale bars: a = 20 mm; b,c = 5 mm; c,e,f = 0.5 mm; i-o = 10 µm; p = 5 µm.

sigmoid germ slit spore-length on convex side; perispore dehiscent in KOH, with inconspicuous ornamentation; in LM and SEM; epispore smooth. Conidiogenous structure, in orange area on natural substrate, mononematose with nodulisporium-like branching patterns. Conidiophores hyaline to yellowish smooth to finely roughened. Conidiogenous cells hyaline, smooth to finely roughened, (10.7)11.6−17.6(18.0) × (2.8)3.1−4.1 µm. Conidia ellipsoid, hyaline, smooth, 4.5−5.6 × 2.4−3.8 µm (N = 20, Me = 4.9 × 3.1 µm). Culture not obtained.

Materials examined: ARGENTINA. SALTA PROVINCE: Gral. José de San Martín Department, on a way to Reserva Provincial de Flora y Fauna Acambuco, 22°20'44.4"S, 63°49'04.3"W, 838 m alt., on corticated and decorticated indeterminate wood, 21.V.2015, *Sir & Hladki 846, 859* (LIL).

Hypoxylon rickii has been previously reported in the tropical and subtropical areas of America (Fournier *et al.* 2015, Ju & Rogers 1996). Their southernmost distribution was recorded for the southern portion of the Atlantic Forest in the Brazilian states of Paraná and Rio Grande do Sul (Cruz & Cortez 2015). Therefore, this is the first record of the species in the subtropical montane Andean forest from the east of South America.

Hypoxylon rickii can be distinguished from similar species that occur in Las Yungas, *e.g.*, *H. haematostroma* Mont. and *H. crocopeplum*

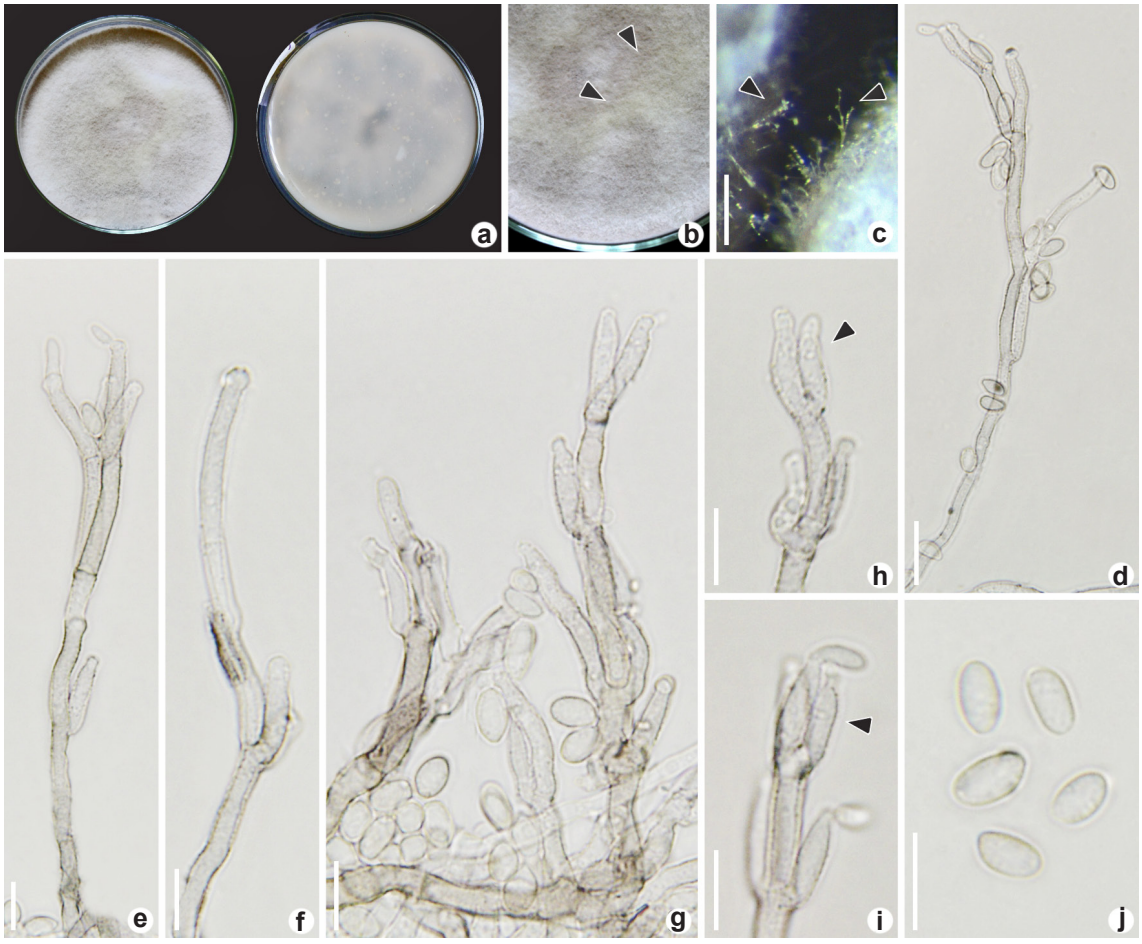

Figure 3 – a-j. Culture and anamorph of *Hypoxylon ochraceotuberosum* – a. surface and reverse of colony after 4 weeks on OA; b. details of colony surface showing sporulation area; c. conidiogenous structure on colony surface (arrows); d-g. conidiogenous structures in water; h. details of a virgariella-like branching pattern (arrow); i. details of a nodulisporium-like branching pattern (arrow); j. conidia. (a-j. *Sir & Medina 1231*). Scale bars: c = 300 µm; d = 20 µm; e-j = 10 µm.

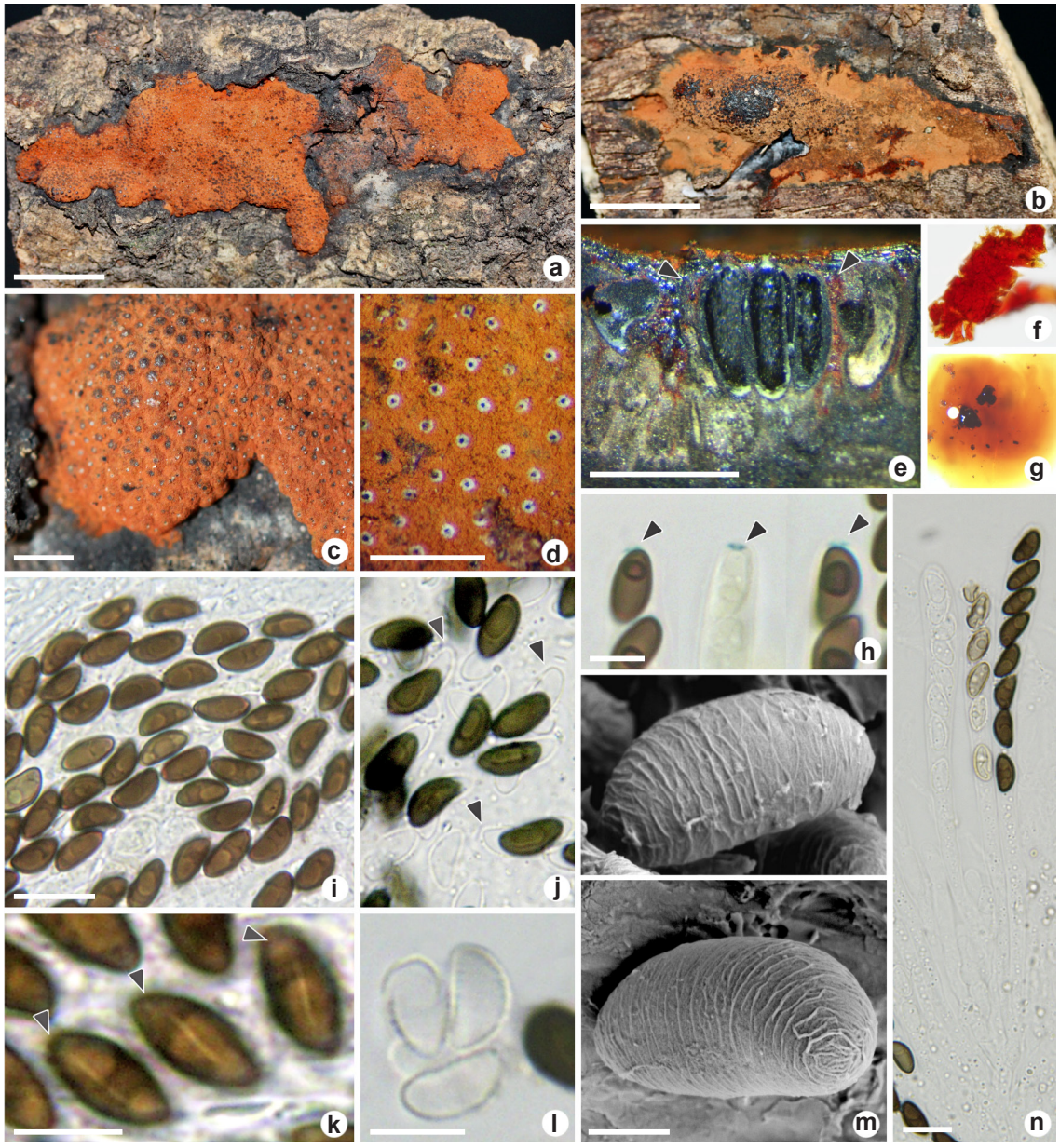

Figure 4 – a-n. Teleomorphic structures of *Hypoxylon rickii* – a. mature stroma on corticated wood; b. immature stroma on decorticated wood; c. stromatal surface; d. stromatal surface showing ostioles; e. section of stroma showing perithecia and orange-red granules (arrows); f. granules in water; g. KOH-extractable pigments; h. ascus tips in Melzer's reagent (arrows); i. ascospores in water; j. ascospores in KOH solution, showing dehiscing perispore (arrows); k. ascospores in KOH solution, showing sigmoid germ slit (arrows); l. details of perispore in KOH solution; m. ascospores under SEM; n. immature and mature asci in water. (a-n. *Sir & Hladki 846*). Scale bars: a,b = 10 mm; c-e = 1 mm; h,k,l = 5 µm; i,j,n = 10 µm; m = 2 µm.

Berk. & M.A. Curtis, by its smaller ascospores (*vs* 15.1−23.5 × 6.0−8.9 and 14.5−17 × 6.5−7 µm, respectively) and conidiogenous structure (*vs* periconiella-like and *vs* virgariella-like, respectively) (Ju & Rogers 1996; Kuhnert *et al.* 2014; Fournier *et al.* 2015; Sir *et al.* 2016b).

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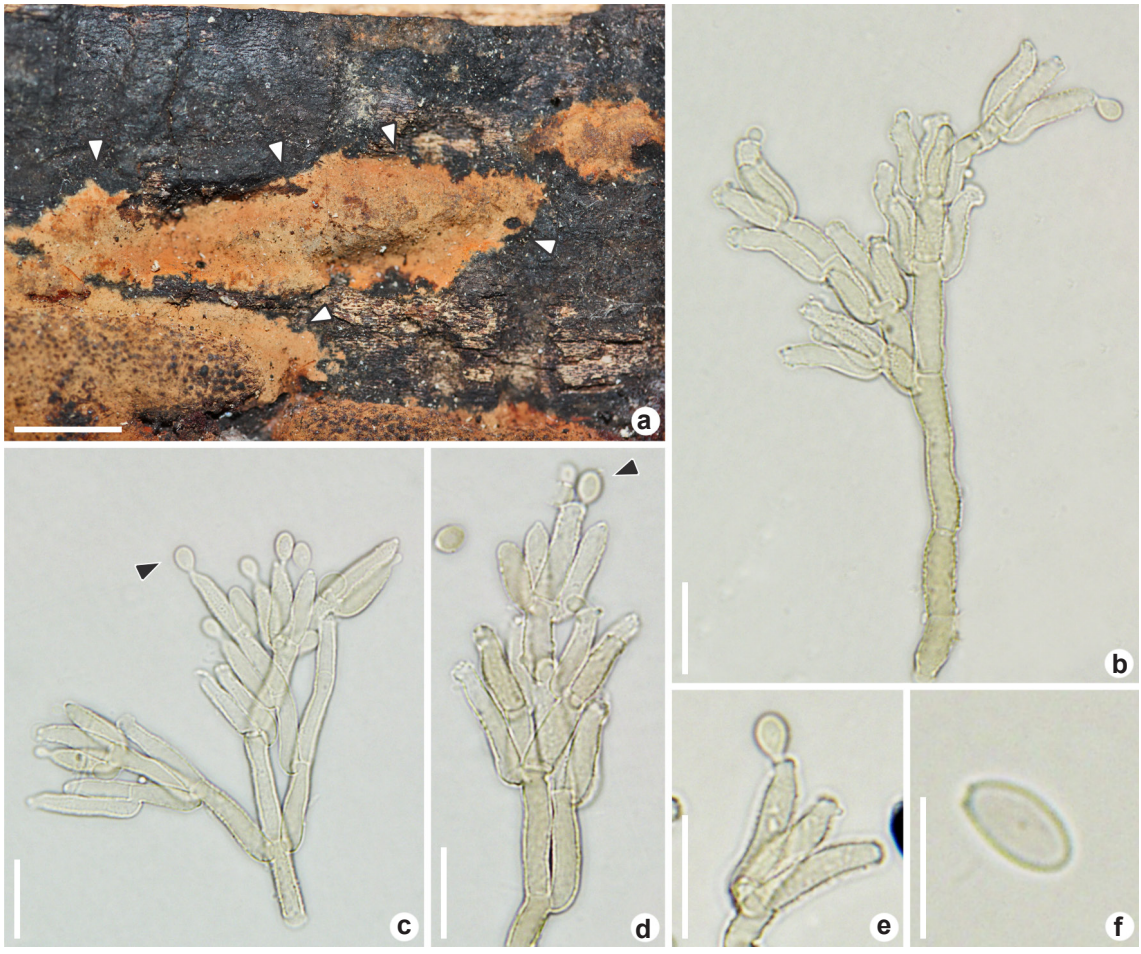

Figure 5 – a-f. Anamorphic structures of *Hypoxylon rickii* from natural substrate – a. immature stroma, showing conidiogenic areas (arrows); b-d. conidiophores with nodulisporium-like branching patterns, showing immature conidia (arrows); e. detail of conidiogenous cells and immature conidia; f. conidium. (a-f. *Sir & Hladki 846*). Scale bars: a = 5 mm; b-e = 10 µm; f = 5 µm.

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