

## Two new species of *Stilbohypoxylon* and the taxonomic positions of *Hypoxylon cyclopicum*, *H. chionostomum* and *Anthostoma chionostoma*

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Two new species of Xylariaceae, *Stilbohypoxylon macrosporum* and *Stilbohypoxylon minor*, are described, illustrated and compared with the other three species of the genus. A key to these taxa is provided. *Hypoxylon cyclopicum* is considered a later synonym of *Kretzschmaria micropus*. *Hypoxylon chionostomum* and *Anthostoma chionostoma*, which have been treated as synonyms by some authors, are in fact not closely related. *Hypoxylon chionostomum* is a good *Hypoxylon* species, whereas *A. chionostoma* is a later synonym of *Leptomassaria simplex*.

Keywords: *Stilbohypoxylon*, *Hypoxylon*, *Anthostoma*, Argentina.

The genus *Stilbohypoxylon* Henn. was recently clarified and resurrected by Rogers & Ju (1997); a lectotype (from Brazil) and an epitype (from British West Indies) of the type species *S. moelleri* Henn. were designated. They accepted three species in the genus, viz. *S. quisquiliarum* (Mont.) J. D. Rogers & Y. M. Ju, *S. samuelsii* J. D. Rogers & Y. M. Ju and *S. moelleri*, and provided a key to them. In the same paper, *Hypoxylon cyclopicum* Speng. was mentioned as possibly being a *Stilbohypoxylon*. In the revision of the genus *Hypoxylon* Bull., Ju & Rogers (1996) listed *H. cyclopicum* as having an uncertain taxonomic position and they excluded it from this genus. Later, in their monograph of the genus *Kretzschmaria* Fr., Rogers & Ju (1993) stated that “It is probable that *H. cyclopicum* and *K. micropus* represent variants of the same species”.

Two taxa originally described by Spegazzini were involved with one of the *Stilbohypoxylon* species by Miller (1961). He considered

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*Hypoxylon chionostomum* Speg. and *Anthostoma chionostoma* Speg. to be synonyms of *Hypoxylon quisquiliarum* Mont. [= *S. quisquiliarum* (Mont.) J. D. Rogers & Y. M. Ju]. While studying *Xylariaceae* from Argentina, we collected some *Stilbohypoxylon* in the northwest of the country. In this study, we describe two new *Stilbohypoxylon* species. In addition, we clarify the taxonomic position of *H. cyclopicum* and its relation to *Stilbohypoxylon*, and clarify the taxonomic problem involving *H. chionostomum*, *A. chionostoma* and *S. quisquiliarum*.

### Material and methods

Fresh collections of *Stilbohypoxylon* species and their holotypes and other related taxa were studied. The fresh collections were made in the montane forest of Tucumán and Salta, specifically in “The Yungas Phytogeographic Province” or “Tucumano-Bolivian Forest” (Cabrera, 1971; Hueck, 1978). The specimens were air-dried, cultured, and deposited in BAF, LIL, K and BPI. Type materials from BPI, GZU, K, LPS, NY were also studied (herbarium abbreviations follow Holmgren & al., 1990).

Observations and measurements were taken from fresh material squash-mounted in distilled water, 5% KOH and phloxine for optical microscopy and in Melzer’s reagent to test for the amyloid reaction (IK). Drawings were made with a camera lucida.

Cultures were initiated from fresh anamorphic stromata and ascospores of the Argentinean specimens. The upper part of a conidium-bearing stroma was removed with a sterilized razor blade. The conidial masses were transferred to a 9 cm diam Petri plate containing 2% Oatmeal agar. Petri plates were incubated under laboratory conditions at approximately 12 hr fluorescent light per day at 20 °C. Alternatively, a perithecium was first placed in a wet chamber, a group of ascospores was removed from it and transferred to a Petri plate containing the same medium.

### Results and discussion

#### Taxonomy

The holotypes of the *Stilbohypoxylon quisquiliarum* and *S. samuelsii* and material of *S. moelleri* were studied. None of these are conspecific with the two Argentinean *Stilbohypoxylon* species. Two new species, *S. macrosporum* and *S. minor*, are thus proposed. Several attempts to culture them were unsuccessful.

***Stilbohypoxylon macrosporum* A. I. Hladki & A. I. Romero, sp. nov. – Fig. 1A–K; Fig. 2A–K.**

A *Stilbohypoxylon quisquiliarum* differt in ascosporis  $30\text{--}40 \times 12\text{--}13 \mu\text{m}$  et in conidiis  $6\text{--}7(-9) \times 2\text{--}2.5 \mu\text{m}$ .

Holotypus. – Argentina: Tucumán: Depto: Taff del Valle, La Heladera, ad viam prov. 307, via ad Taff del Valle, 14.6.1997, Romero 507, BAFC 50915.

Stromata perithecioid, globose or semiglobose, 0.6–0.9 mm diam, solitary or slightly fusionate, gregarious, generally with 1–2 synnemata borne on mature stromata, 0.4–0.5 mm long; surface black, verrucose to rugulose; interior black with small amount of white tissue around the lower 2/3 of the perithecium, wider below it, becoming hollow and fragile when mature. – Perithecia spherical to ovoid, 0.5–0.8 mm diam., 1 per stroma. – Ostioles papillate, conical, dark brown to black, encircled with yellow or light brown disc at early stage. – Asci 8-spored, cylindrical, stipitate, 200–260 (total length)  $\times$  9–12  $\mu\text{m}$ , the spore-bearing part 135–195  $\mu\text{m}$  long, the stipe 50–70  $\mu\text{m}$  long, with an apical ring IK+, dark blue, rectangular, 8–10.5  $\times$  6.5  $\mu\text{m}$ . – Paraphyses abundant, filiform, unbranched. – Ascospores dark brown, unicellular, ellipsoid to navicular, inequilateral, with narrowly rounded ends, usually pinched, with hyaline appendages on both ends of young ascospores, 30–40  $\times$  12–13  $\mu\text{m}$ , with a spiral germ slit nearly spore-length.

Synnemata borne laterally on mature stromata or directly on wood, acicular to conical to somewhat cylindrical; fertile region greyish white, 0.4–0.5  $\times$  0.1 mm, the stipe almost black, ca 1 mm long. – Conidiophores of a few cells. – Conidiogenous cells terminal, cylindrical, 10–14  $\times$  2–3.5(–4)  $\mu\text{m}$ , hyaline, denticulate at the apical region. – Conidia produced holoblastically in sympodial sequence, hyaline, smooth, lageniform, with a truncate base, 6–7(–9)  $\times$  2–2.5  $\mu\text{m}$ .

**Etymology.** – Referring to the large ascospores.

**Material examined.** – Holotype, BAFC 50915. ARGENTINA: Tucumán, Depto. Chichigasta, Parque Provincial El Cochuna, ad viam prov. 331, Camping Samay, 1300 m asl, 14. 6. 1997, Catania & Hladki 486, LIL and BPI 840822; *ibid.* on dead branch of bush, 12. 11. 1999, Hladki 2355, LIL. Depto. Taff del Valle, La Heladera, ad viam prov. 307, via ad Taff del Valle, 14. 6. 1997, Romero, BAFC 50916.

**Additional material examined.** – *Stilbohypoxylon quisquiliarum*: FRENCH GUIANA: Cayenne, on bark, Leprieur 366, K(M) 79220, isotype; *ibid.*, Leprieur 460, K(M) 79219.

*Stilbohypoxylon macrosporum* and *S. quisquiliarum* (Mont.) J. D. Rogers & Y. M. Ju are the two species in the genus which have a



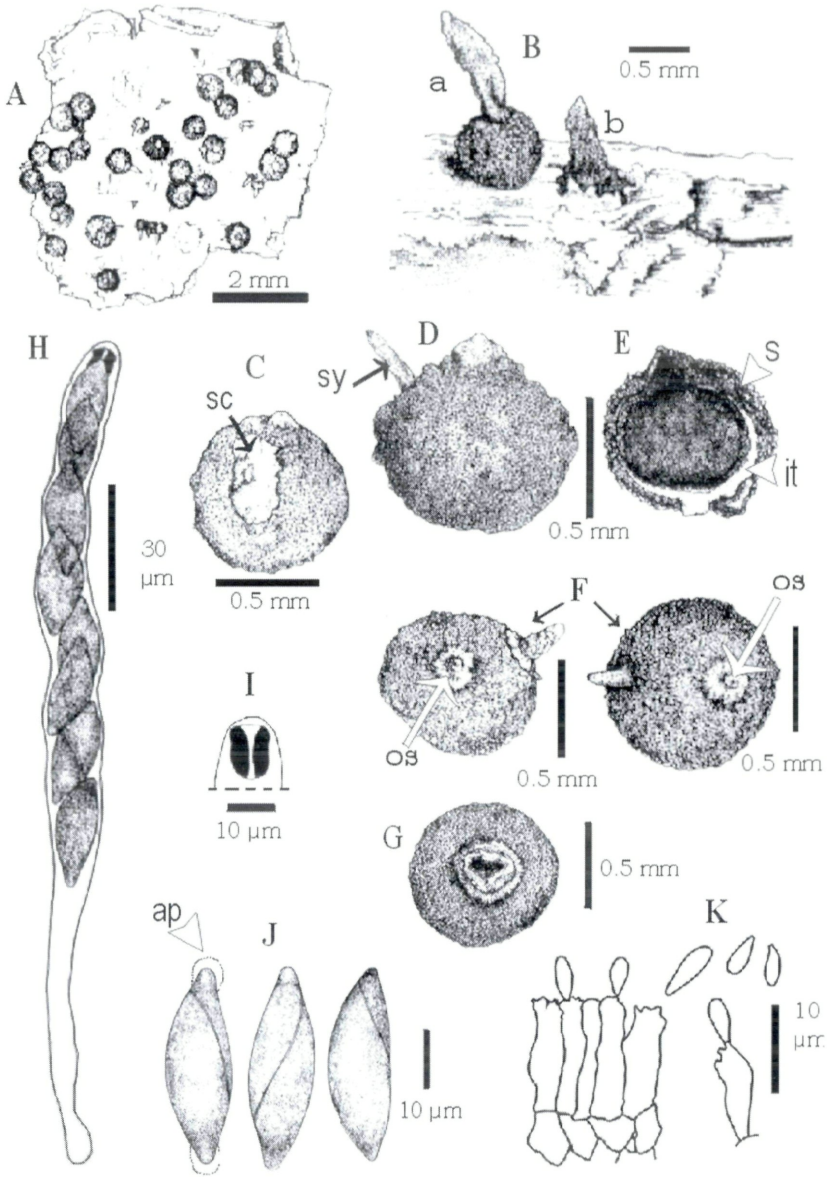


Fig. 1A-K. - *Stilbohypoxylon macrosporum*. - A. General aspect. - B. Two developmental stages of the synnemata (a = on a young stroma; b = directly on the substratum). - C. Young stroma with scales (sc). - D. Mature stroma with a lateral synnema (sy). - E. Stroma in longitudinal section (s = stroma; it = internal tissue). - F. Two stromata showing papillate ostiole and synnemata (arrows OS = ostiole). - G. Stroma in ventral view. - H. Ascus with apical ring, I\*. - I. Detail of apical ring. - J. Ascospores, one of them with appendages (ap) when young. - K. Conidiogenous cells and conidia.



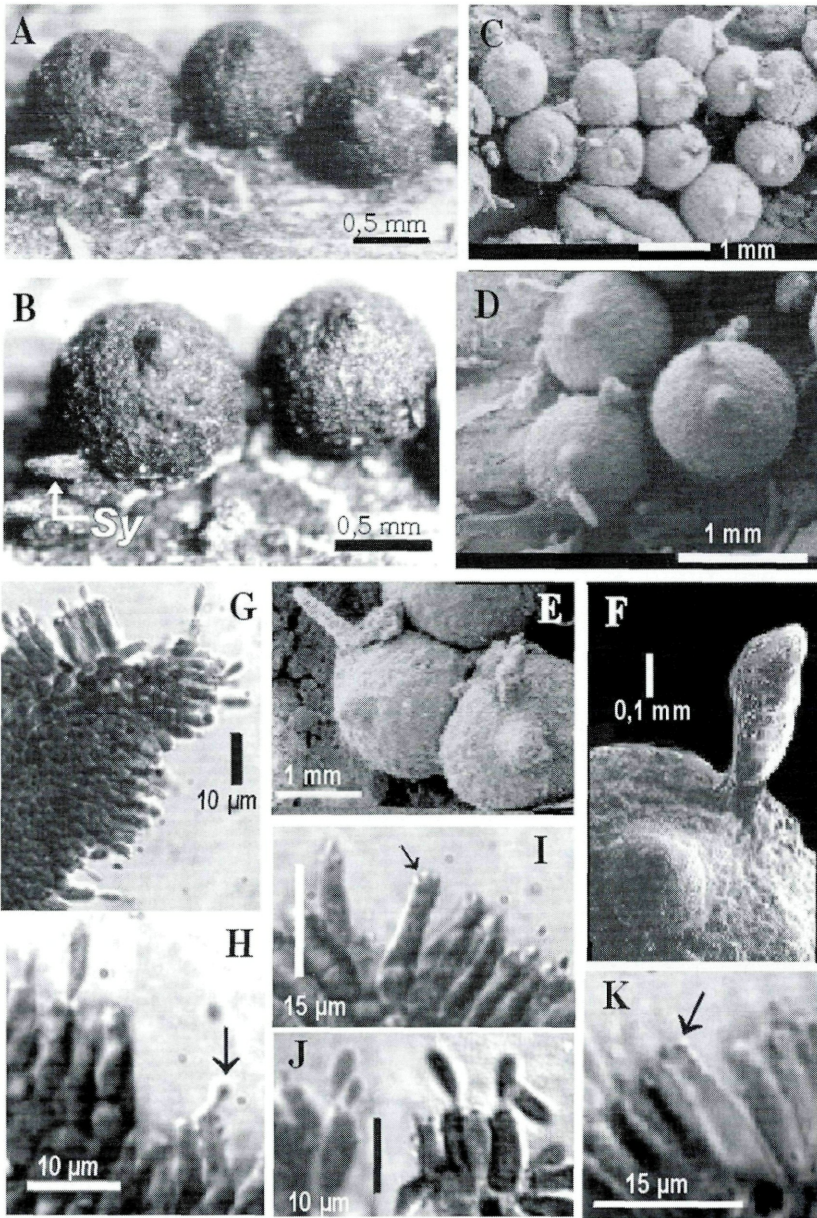


Fig. 2A-K. - *Stilbohypoxylon macrosporum*. - A-B. General aspect (sy = synnemata). - C-F. Different views of stromata by SEM. - G-J. Anamorph. - G. Apical region of a synnema; note the palisade of conidiogenous cells. H. Note a conidiogenous cell with 4 scars and a young conidium (arrow). - I-J. Several conidiogenous cells with scars and some with attached conidia.

spiral germ slit on the ascospores. The ascospores of the Argentinean species, however, are longer ( $30\text{--}40 \times 12\text{--}13 \mu\text{m}$ ) and, although the width is in the range of that of *S. quisquiliarum* ( $25\text{--}32.5 \times 11\text{--}15 \mu\text{m}$ ), no ascospores with  $14\text{--}15 \mu\text{m}$  width were seen. The apical rings of *S. macrosporum* are notably larger. Another difference is the coalescence of several single peritheciate stromata in *S. quisquiliarum* (at least in the isotype studied), a feature not seen in *S. macrosporum*. On the other hand, Rogers & Ju (1997) observed synnemata of *S. quisquiliarum* only in culture apparently because they seem to be very fragile and friable in nature. In *S. macrosporum* the synnemata persist on some perithecia or directly on the substratum. Moreover, the conidiogenous cells and conidia are smaller than those of *S. quisquiliarum*. Finally, the colors are different: in *S. macrosporum* the fertile area is grey and the stalk is black whereas Rogers & Ju (1997) describe the fertile portion of *S. quisquiliarum* as being dark olivaceous to blackish and the stalk being yellow.

***Stilbohypoxylon minor*** A. I. Hladki & A. I. Romero, **sp. nov.** – Fig. 3A–H.

A *Stilbohypoxylon moelleri* differt in stromatibus peritheciiorum specie  $0.4\text{--}0.6 \text{ mm}$ , in ascosporis  $23.5\text{--}26(\text{--}27.5) \times 12\text{--}13 \mu\text{m}$  et in conidiis  $4\text{--}6 \times 1.5\text{--}2.5 \mu\text{m}$ .

Holotypus. – Argentina: Salta: Depto: Santa Victoria, Los Toldos, finca El Nogalar,  $22^{\circ}16'670''\text{S}$ ,  $74^{\circ}42'735''\text{O}$ , 1600 m asl, 2. 8. 2001, Hladki 2370, LIL. Iso-typi BAFC 51145 et K(M) 102261.

Stromata perithecioid, globose,  $0.4\text{--}0.6 \text{ mm}$  diam, rarely fusionate, gregarious, generally with 1 small synnema borne on immature stromata; surface black, shining, furfuraceous, interior with small amount of white tissue around the perithecium. – Perithecia spherical,  $0.4\text{--}0.5 \text{ mm}$  diam., 1 per stroma. – Ostioles diminute, papillate to conical, black. – Asci 8-spored, cylindrical, stipitate,  $165\text{--}185$  (total length)  $\times 13\text{--}14.5 \mu\text{m}$ , the spore-bearing part  $150\text{--}162 \mu\text{m}$  long, the stipe  $14\text{--}21 \mu\text{m}$  long, with an apical ring IK+, light blue, rectangular,  $5\text{--}6.5 \times 6.5\text{--}7.8 \mu\text{m}$ . – Paraphyses abundant, filiform, unbranched. – Ascospores brown, unicellular, ellipsoid to navicular, inequilateral, with narrowly rounded ends,  $23.5\text{--}26(27.5) \times 12\text{--}13 \mu\text{m}$ , with a straight germ slit nearly spore-length.

Synnemata borne laterally on immature stromata or directly on wood, cylindrical to conical,  $0.1\text{--}0.2 \text{ mm}$  long; fertile region yellowish white. – Conidiophores of few cells. – Conidiogenous cells terminal, cylindrical,  $6.5\text{--}9 \times 2.5\text{--}4 \mu\text{m}$ , light brown, denticulate at the apical region. – Conidia produced holoblastically in sympodial sequence, light brown, smooth, lageniform, with a truncate base,  $4\text{--}6 \times 1.5\text{--}2.5 \mu\text{m}$ .



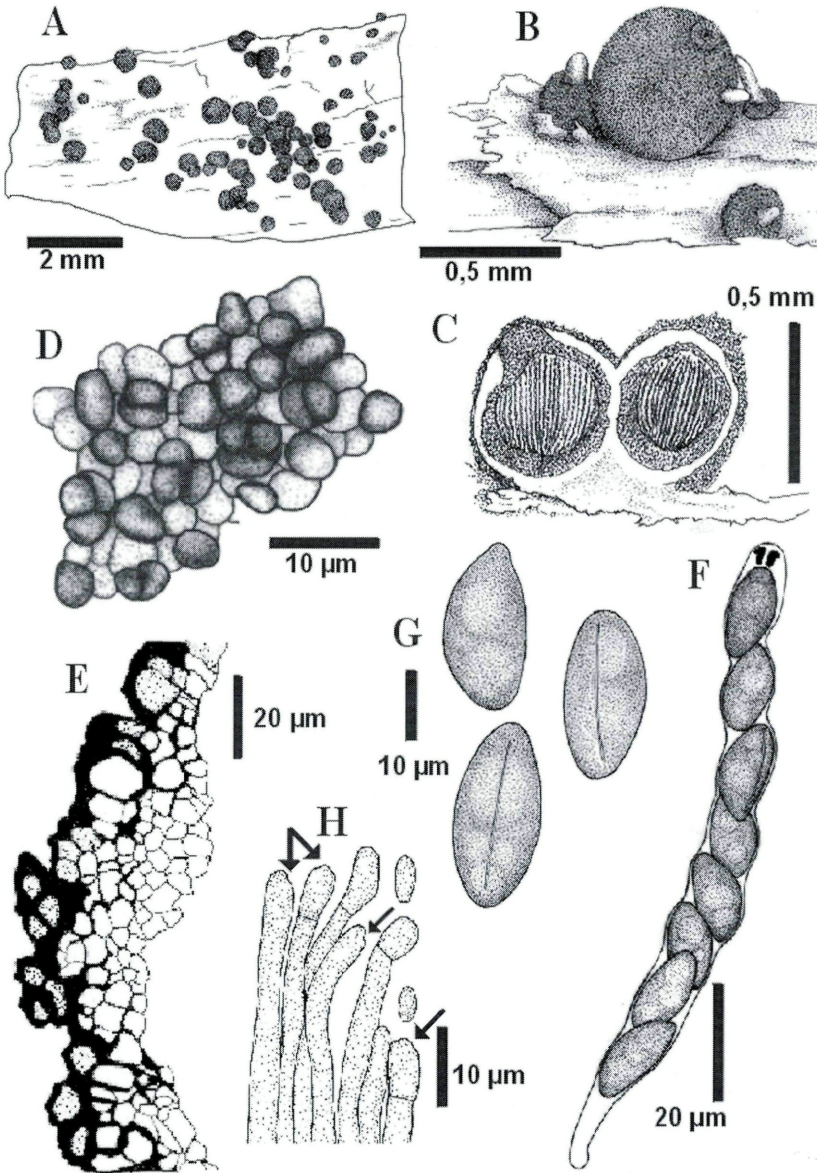


Fig. 3A-H. - *Stilbohypoxylon minor*. - A. General aspect. - B. Ascomata with two developmental stages of the synnemata. - C. Stroma in longitudinal section. - D. Apical view of the stromatal wall showing groups of cells in different planes. - E. Longitudinal section of the stromatal wall. - F. Ascus with apical ring, I<sup>+</sup>. - G. Ascospores. - H. Conidiogenous cells with inconspicuous scars (arrows) and conidia



Etymology. – Referring to the small stromata.

Material examined. – Holotype LIL and isotypes K, BAFC.

Additional material examined. – *Stilbohypoxyton samuelsii*: FRENCH GUIANA: Upper Marouni R, ca 3 h walk W of river toward Roche Koutou, 1 km E of Rouche Koutou, corticated wood, elev. 150–350 m, 15. 8. 1987, G. J. Samuels, & al. 5797, NY, type. *Stilbohypoxyton moelleri*: FRENCH GUIANA: Upper Marouni R, between Roche Koutou and an unnamed granitic 350 m high inselberg, on palm, elev. 200–300 m, 19. 8. 1987, G. J. Samuels & al. 5941, NY; Upper Marouni R, 2 km N of Oumanfou-Langa Soula, on palm, elev. 150 m, 12. 8. 1987, G. J. Samuels & al. 5974, NY.

Among the *Stilbohypoxyton* species with straight ascospore germ slits, the length of *S. minor* ascospores range between *S. moelleri* and *S. samuelsii*. Besides, a shiny and rugose stromatal surface was observed in *S. minor*. The appearance is apparently due to the thick and dark stromatal cells, especially those most external.

#### Key to the *Stilbohypoxyton* species known so far

1. Ascospores with straight germ slits . . . . . 2
- 1.\* Ascospores with spiral germ slits . . . . . 4
2. Ascospores  $14.5\text{--}17 \times 6\text{--}8 \mu\text{m}$  . . . . . *S. moelleri*
- 2.\* Ascospores larger . . . . . 3
3. Ascospores  $30\text{--}36 \times 8.5\text{--}11 \mu\text{m}$  . . . . . *S. samuelsii*
- 3.\* Ascospores  $23.5\text{--}26 \times 12\text{--}13 \mu\text{m}$  . . . . . *S. minor*
4. Ascospores  $25\text{--}32.5 \times 11\text{--}15 \mu\text{m}$  . . . . . *S. quisquiliarum*
- 4.\* Ascospores  $30\text{--}40 \times 12\text{--}13 \mu\text{m}$  . . . . . *S. macrosporum*

After examining of the holotypes of *Anthostoma chionostoma* Speg. and *H. chionostomum* Speg., we agree with Ju & Rogers (1996) that the latter is a good species of *Hypoxyton* Bull. The former is *Leptomassaria simplex* (Nitschke ex Otth) Petrak.

***Leptomassaria simplex*** (Nitschke ex Otth) Petrak, Ann. Mycol. 12: 474, 1941. – Fig. 4A–E.

= *Anthostoma chionostoma* Speg., Anal. Soc. Cient. Argent. 18(6): 267, 1884.

Stromata subperidermal, 0.3–0.6 mm diam, externally barely visible or merely showing ostiolar discs. – Perithecia globose, gregarious, 1–1.5 mm diam. – Asci cylindrical, stipitate,  $190\text{--}210 \times 15\text{--}19 \mu\text{m}$ , with an apical ring IK+,  $4 \times 6 \mu\text{m}$ . – Ascospores unicellular, symmetrical, ellipsoid with rounded ends, brown,  $30\text{--}36 \times 15\text{--}17 \mu\text{m}$ , with a spiral germ slit.

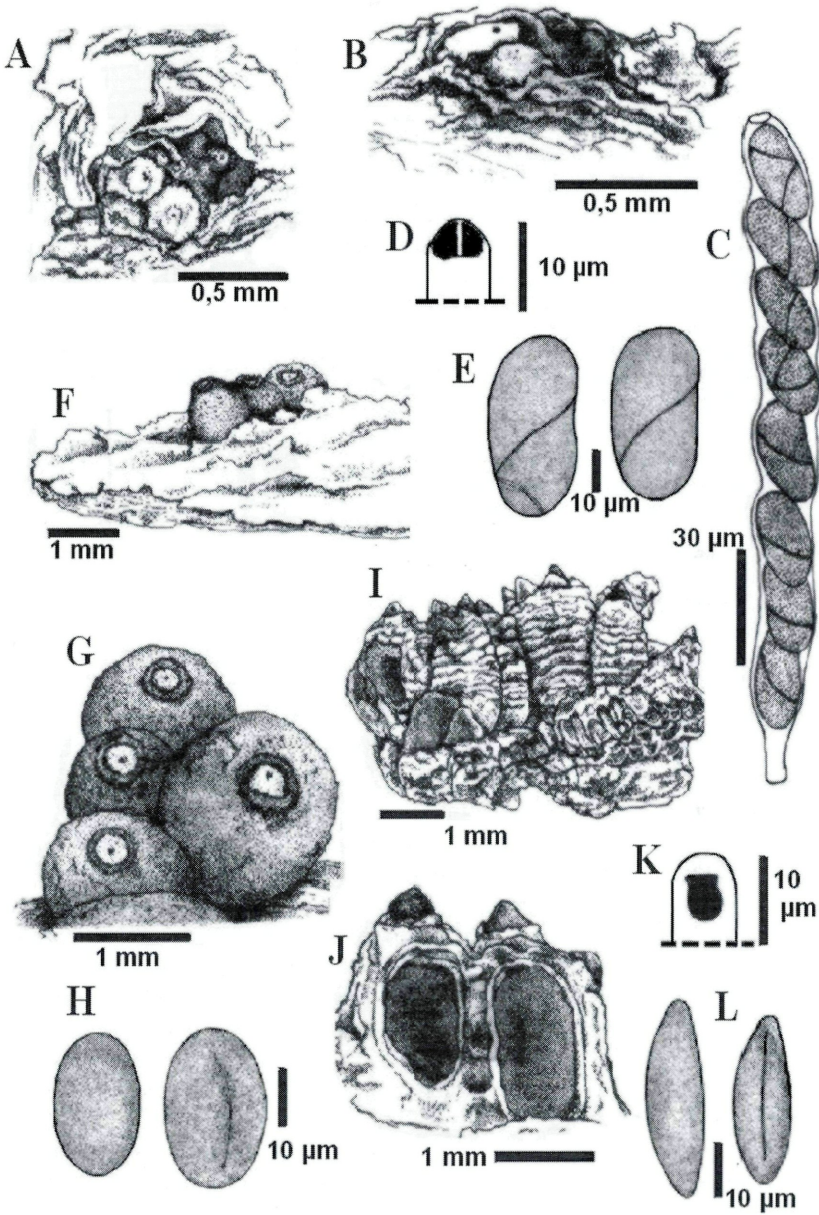


Fig. 4A-E. - *Leptomassaria simplex* (Holotype of *Anthostoma chionostoma*). - A. General aspect. - B. Detail. - C. Ascus. - D. Apical ring, I<sup>+</sup>. - E. Ascospores. - F-H. *Hypoxylon chionostomum* (Holotype). - F. General aspect. - G. Detail. - H. Ascospores. - I-L. *Kretzschmaria micropus* (Holotype of *H. cyclopicum*). - I. General aspect. - J. Stromata in longitudinal section. - K. Apical ring, I<sup>+</sup>. - L. Ascospores.

Material examined. – BRAZIL: Guarapí, on trunks, Balansa B. no. 2798, 1881, holotype of *Anthostoma chionostoma* Speg., LPS 6804.

Spegazzini (1884) placed this species in *Anthostoma* Nitschke, a genus belonging to *Diatrypaceae* Nitschke (Eriksson, 1966). Because of its features our attention was turned to other genera of *Xylariaceae* Tul. & C. Tul. and through the key to the species by Rappaz (1995) we could identify it as a *Leptomassaria* Petrak species. This genus is close to *Anthostomella* Sacc. and *Helicogermislita* Lodha & Hawksworth. It is distinguished from *Anthostomella* by having larger ascospores with a spiral germ slit. *Helicogermislita* has ascospores with appendages on both ends (Rappaz, 1995). The Spegazzini's material was collected in Brazil and it is probably the only record of *L. simplex* for South America.

***Hypoxylon chionostomum*** Speg., Bol. Acad. Nac. Cienc. Córdoba 11(4): 506, 1889. – Fig. 4F–H.

Material examined. – BRAZIL: Apiahy, on decayed bark, leg. Puiggari J. no. 2858, 6-1888, holotype, LPS 1679.

The material is in poor conditions. According to Ju & Rogers (1996) the ascospore morphology of *H. chionostomum* is similar to that of *H. megalosporum* Speg. and *H. umbilicatum* Speg. *H. chionostomum* was not, however, transferred from another genus, as cited in Ju & Rogers (1996). These authors cited *Anthostoma chionostoma* Speg. as a nomenclatorial synonym of *H. chionostomum*. In fact, *H. chionostomum* and *A. chionostoma* are based on different types. The initial confusion apparently arose when Miller (1961) listed both species as synonyms of *H. quisquiliarum* (Mont.) Mont. He apparently did not examine the type of either species. Ju & Rogers (1996) further erred by citing a slide from the C. L. Shear collection as the type of *A. chionostoma* rather than the type of *H. chionostomum* as it was clearly labeled.

The study of the holotype of *Hypoxylon cyclopicum* revealed that this species is in fact *Kretzschmaria micropus*. After examining different specimens of *K. micropus* including the lectotype and other types of some of the synonyms studied by Rogers & Ju (1998), we provide the following description as a supplement.

***Kretzschmaria micropus*** (Fr. : Fr.) Sacc., Syll. Fung. II, Addenda ad vol.I.: xxix, 1883. – Fig. 4I–L.

= *Hypoxylon cyclopicum* Speg., Bol. Acad. Nac. Cs. de Córdoba, 11(4): 504, 1889.



Stromata convex or obconic, sessile or attached to substrate with a short strap-like or cylindrical stipe, densely aggregated, 1–3.5 mm diam, surface dull brown and dull blackish brown, with weakly or strongly reticulate cracks, bearing one to several conic spines or sometimes with a solitary small eccentric sterile umbo present in some clubs and occasionally with fine horizontal striations on sides; tissue between and beneath perithecia coriaceous to woody, white to gray, becoming dark brown and disintegrating. – Perithecia spherical to obovate, 0.7–1.6 × 0.9–2 mm, 1–3 perithecia per stroma. – Ostioles sharply or coarsely conical, black, many per stroma, minute or larger than spine. – Asci fragmentary, with apical ring IK+, urn-shaped, 4–8 × 4–5 µm. – Ascospores brown or dark brown, unicellular, ellipsoid-inequilateral, with narrowly or widely rounded ends, smooth, 30–40 × 8–12(–13.5) µm, with germ slit slightly less than spore-length.

Material examined. – ANGOLA: Welwitsch, 1855, type of *Kretzschmaria aggregatum* (Welw. & Curr) Cooke, K(M) 62683. BRAZIL: Apiahy, on decorticated trunk, 1888, J. Puiggari 237, holotype of *Hypoxyton cyclopicum*, LPS 1680. Sao Paulo, 5. 4. 1947, J. Rick, as *Kretzschmaria lichenoides* Rick, GZU-Petrak 2084. Río Grande do Sul, H. Zogg, as *Kretzschmaria spinifera* Ellis & Macbride, GZU-Petrak. No data, J. Rick, holotype of *Kretzschmaria apoda* C.G. Lloyd, BPI 716393, Lloyd herb. 12621 (this specimen was examined because it has also the same “vertical striations on sides” as the type material of *Hypoxyton cyclopicum*). MEXICO: 1895, C. L. Smith, lectotype of *Kretzschmaria lucidula* (Mont.) Dennis (selected by Ju & Rogers 1998), NY. SAN SALVADOR: Sehnem, as *Kretzschmaria spinifera*, LIL 8245.

It should be noted that *K. micropus* has not been found in Argentina so far (Hladki & Romero, 2001).

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