Phylacia korfii sp. nov., a new species of *Phylacia* (*Xylariaceae*) from French Guiana, with notes on three other *Phylacia* spp.

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Summary: During a field trip to French Guiana in June 2012, a distinctive species of *Phylacia* was collected. It appeared to differ from all known species by significantly larger and pear-shaped ascospores and thus it is described as the new taxon *P. korfii* J. Fourn. & Lechat. We also noticed the presence of a germ slit on ascospores and we observed that fragments of the stromatal crust yielded pigments in 10% KOH, two characters so far rarely or not documented in this genus. Similar investigations carried out on three other *Phylacia* spp. showed that recording these characters is taxonomically relevant and should facilitate a better appraisal of the known species and the segregation of new taxa.
Keywords: Ascomycota, *Hypoxyloideae*, Paracou, pyrenomycetes, saproxylic, taxonomy, tropical mycology, *Xylariales*.

Résumé : Lors d'une expédition en Guyane Française en juin 2012, une espèce remarquable du genre *Phylacia* a été récoltée. Elle s'est révélée différente de toutes les espèces connues par des ascospores significativement plus grandes et piriformes et par conséquent elle est décrite comme nouvelle sous le nom de *P. korfii* J. Fourn. & Lechat. Nous avons aussi remarqué que les ascospores présentaient un sillon germinatif et que les stromas libéraient des pigments dans la potasse à 10 %, deux caractères rarement ou non encore signalés dans ce genre. La recherche de ces caractères chez trois autres espèces du genre *Phylacia* a montré que leur mise en évidence présente un intérêt taxinomique et devrait aider à une meilleure évaluation des espèces connues et faciliter la reconnaissance d'espèces nouvelles.
Mots-clés : Ascomycota, *Hypoxyloideae*, mycologie tropicale, Paracou, pyrénomycètes, saproxyliques, taxinomie, *Xylariales*.

Introduction

The genus *Phylacia* Lév. was introduced by Léveillé (1845) for *P. globosa* Lév., a lignicolous fungus collected in Colombia, characterized by carbonaceous stromata encasing closely packed narrowly cylindrical perithecia containing a powdery mass of ascospores and lacking ostioles. The status of *Phylacia* in Ascomycota was controversial since asci were very rarely observed in the perithecia. Patouillard (1903) even considered that *P. bomba* (Mont.) Pat. existed under two forms, with ascospores or conidiospores depending on the presence or absence of asci. Dennis (1957) made a historical review of the genus and resumed Patouillard's views but accepted in *Phylacia* those species in which asci had been observed and those apparently lacking asci. Speer (1980) described two new species from Brazil and Ecuador (Galapagos Islands) and provided illustrations of clavate or subglobose sessile asci arising remotely from long ascogenus hyphae and early deliquescent. Based on the unusual ascogenesis, the absence of ascal apical apparatus, the absence of paraphyses and the lack of ostioles, Speer excluded the genus *Phylacia* from the *Xylariaceae* and introduced the new family *Phylaciaceae* Speer to accommodate it. Rodrigues & Samuels (1989) reinstated *Phylacia* in the *Xylariaceae* after having obtained a geniculosporium-like asexual morph in culture from *P. globosa*, the type species, and from *P. bomba* var. *macrospora* K.F. Rodrigues & G.J. Samuels, and observed a similar asexual morph on immature stromata of *P. poculiformis* (Mont.) Mont. The familial placement of *Phylacia* was more recently confirmed by chemotaxonomic data on metabolites in culture and molecular results showing its affinities with *Daldinia* Ces. & De Not., with daldinioid species of *Hypoxylon* Bull. (Bitzer *et al.*, 2008), with *Rhopalostroma* D. Hawksw. and with *Thamnomyces* Ehrenberg (Stadler *et al.*, 2010). *Rhopalostroma* appears to be the most morphologically similar genus, differing by ostiolate perithecia lying just beneath the outer stromatal crust and a distribution restricted to Africa and Asia (Hawksworth, 1977), while *Phylacia* appears restricted to the Neotropics.

A *Phylacia* resembling *P. bomba* by its dome-shaped sessile stromata but featuring distinctive elongate piriform ascospores was collected during a field trip to French Guiana in June 2012 (Fournier & Lechat, 2015). It is described and illustrated here as a new species, after comparison with the species dealt with in the comprehensive survey by Medel *et al.* (2006).

It is given the name *P. korfii* to pay a grateful tribute to Professor Richard P. Korf, both for the mycologist as for the man himself.

We observed germ slits on ascospores and KOH-extractable pigments in the new species. Rogers previously observed what appeared to be a germ slit in a collection of *P. sagrana* from Costa Rica (Medel *et al.*, 2006) and he later published a photograph of an ascospore of *P. sagrana*[1] (Mont.) Mont. featuring a germ slit (Chaverri *et al.*, 2010). We extended our observations to three available specimens of *Phylacia* and our results suggest that germ slits on ascospores and KOH-extractable pigments should be used as differential characters to improve the taxonomy of this poorly known genus.

Material and methods

Material and methods including colour charts follow Fournier & Lechat (2015).

Taxonomy

Phylacia korfii J. Fourn. & Lechat, *sp. nov.* — MycoBank MB814612. Plates 1–2

Diagnosis: Differs from all known *Phylacia* spp. by larger piriform ascospores averaging 18.7 × 8.7 µm and a fairly conspicuous short germ slit.

Holotype: FRENCH GUIANA: Sinnamary, Paracou, CIRAD field station, edge of the parking area, on a corticated trunk, 26 Jun. 2012, *leg*. J. Fournier & C. Lechat, GYJF 12235 (LIP).

Etymology: Named in honour of Professor Richard P. Korf, for his outstanding contribution to mycology and his friendly support to non-professional mycologists.

[1] Following Mycobank and Index Fungorum, we retain here the above orthograph against *sagraeana* that can be encountered in older literature.

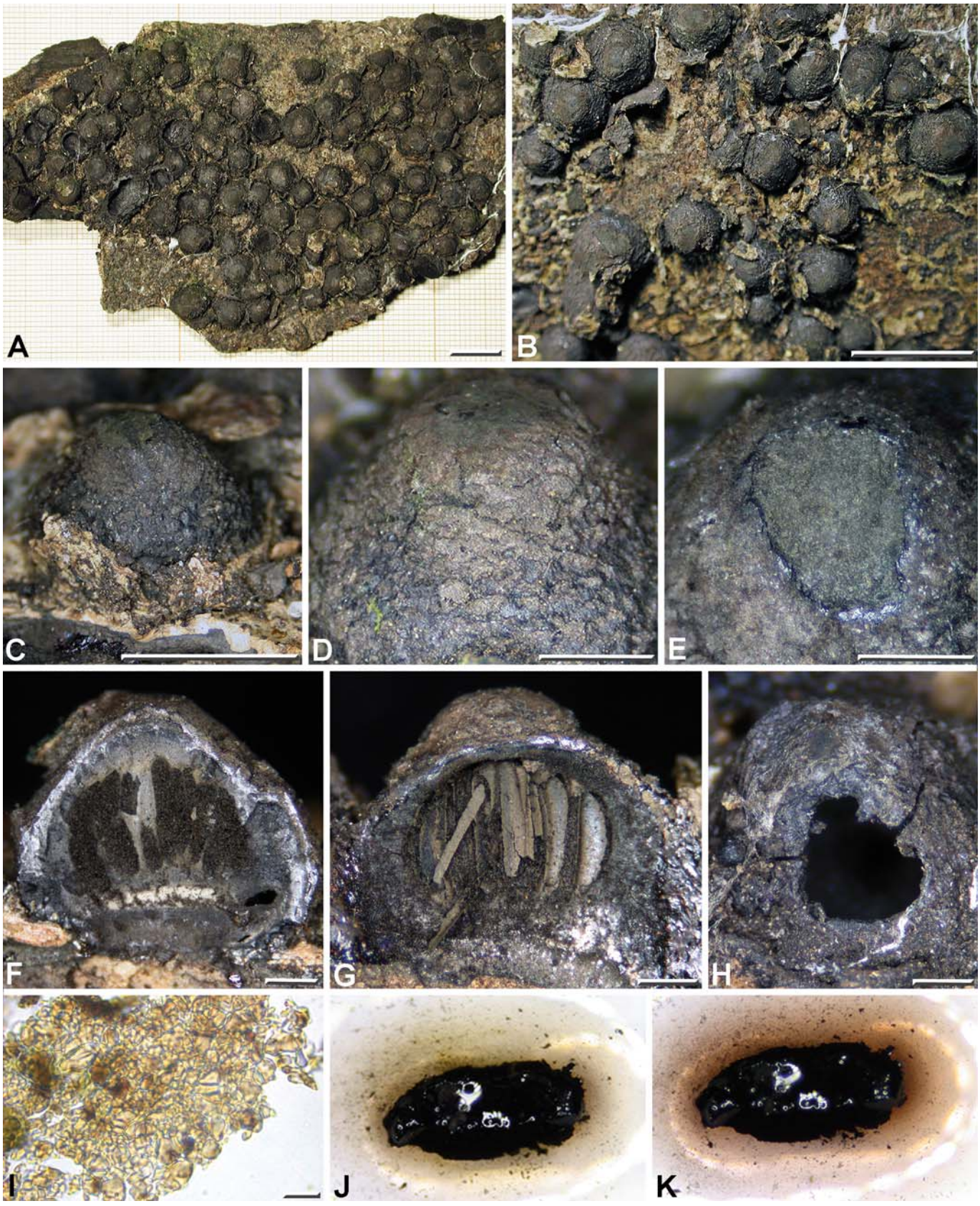

Plate 1 — *Phylacia korfii*

Holotype GYJF 12235. A: Stromata on bark in surface view; B, C: Stromata in close-up showing the erumpent habit with the ruptured periderm around their base; D: Apex of a stroma in close-up showing the scaly-cracked surface and the absence of ostioles; E: Apex of a stroma in close-up showing the outer crust flaking off; F, G: Stromata in vertical section showing the perithecia and their powdery contents (note the absence of ostiolar canals); H: Weathered empty stroma with a hole on the side; I: Waxy stromatal granules in water; J, K: KOH-extractable pigments after 10 and 30 min incubation respectively. Scale bars: A, B = 10 mm; C = 5 mm; D-H = 1 mm; I = 10 µm.

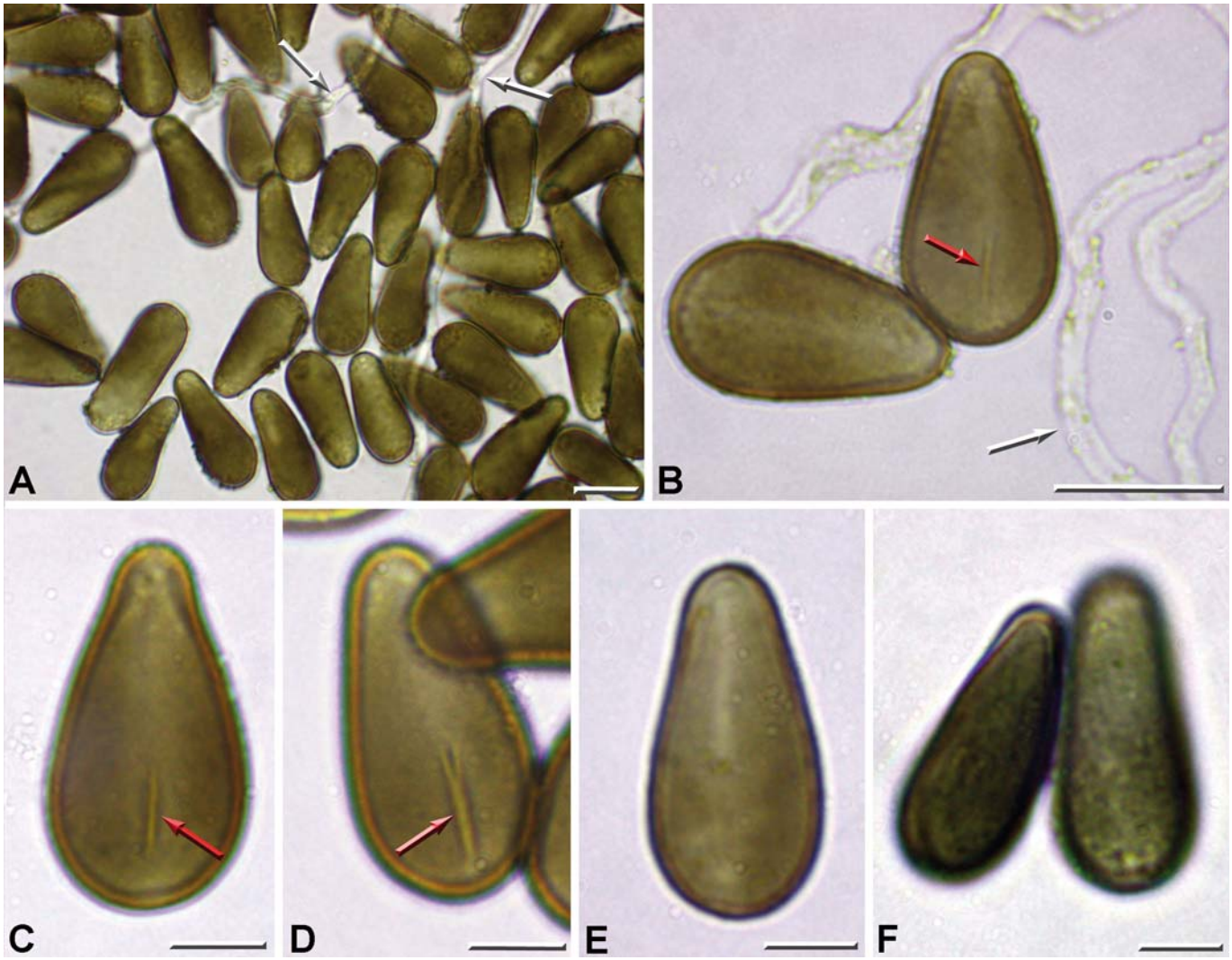

Plate 2 — *Phylacia korfii*

Holotype GYJF 12235. A: Ascospores in water, with ascogenous hyphae (white arrows); B-D: Ascospores in PVA-lactophenol with ascogenous hyphae (white arrows) and germ slits (red arrows); E: Ascospore in PVA-lactophenol with a paler band on narrower half; F: Two ascospores in 10% KOH showing the absence of dehiscent perispore. Scale bars: A, B = 10 µm; C-F = 5 µm.

Stromata gregarious, corticolous, individually erumpent through the host periderm which is ruptured in a stellate pattern around the base, rarely fused by two, broadly conical to dome-shaped with a bluntly rounded apex, the base broadly attached or slightly constricted, 5–8 mm diam × 4–5 mm high; surface dull black, roughened, with thick greyhish brown scales in upper half; stromatal wall strongly carbonaceous, ca. 0.4 mm thick, up to 0.85 mm thick at base, externally coated by a continuous reddish brown crust 80–120 µm thick composed of ochreous granules, flaking off at apex, yielding dilute honey (64, oac847) KOH-extractable pigments after 5-10 min incubation, gradually turning dilute vinaceous (57,oac539) after 20-30 min; the tissue above the perithecia brownish grey, powdery; the tissue beneath perithecia carbonaceous, blackish, ca. 1.5 mm thick, occasionally encasing a thin layer of whitish soft-textured tissue located just beneath the perithecia. **Perithecia** cylindrical, 1.4–2 × 0.4 mm, with brittle pale brown walls, not separated by carbonaceous tissue, deeply immersed under the crust, lacking ostiolar canals. **Ostioles** absent.

Asci deliquescent, not observed; long ascogenous hyphae ca. 2 µm wide, hyaline, apparently non-septate, interspersed among masses of ascospores. **Paraphyses** not observed. **Ascospores** (16.6–) 17.7–19.9 (–20.4) × (7.4–) 7.9–9.3 (–10.3) µm, Q = (1.9–) 2–2.3 (–2.4); N = 60 (Me = 18.7 × 8.7 µm; Qe = 2.2), forming a blackish powdery mass in a filamentous matrix accumulating in the perithecia, elongate-piriform with broadly rounded ends, olivaceous brown when fresh, yellowish brown when dry, thick-walled, with a straight to slightly oblique germ slit much less than spore-length, 4–6 µm long, located towards the broadest end; no perispore dehiscent in 10% KOH; epispore turning dark olivaceous green in 10% KOH, possibly slightly roughened.

Asexual morph on the natural substrate not observed.

Other specimens examined: *Phylacia bomba*: FRENCH WEST INDIES: MARTINIQUE: Trinité, Pointe Bateau, meso-xerophilic coastal rainforest, corticated wood, 3 Dec. 2006, *leg*. C. Lechat, CLL 6163 (LIP). *Phylacia poculiformis*: FRENCH GUIANA: Sinnamary, Saint-Elie track, mesophilic lowland rainforest, on dead wood, 28 Apr. 2008, *leg*. C. Lechat, CLL 8015 (LIP). *Phylacia* cf. *sagrana*: PANAMA: Prov. de Chiriquí, David, Chiriquí F.C.A., 35 m, bark of *Khaya senegalensis* (Desr.) A. Juss. (*Meliaceae*), 29 Oct. 2006, *leg*. A. Carmona, AC 030 (PMA).

Known distribution: French Guiana.

Discussion: The cleistothecial carbonaceous stromata of this xylariaceous fungus featuring a thin outer crust of waxy granules yielding pigments in 10% KOH combined with long cylindrical

perithecia devoid of ostioles, deliquescent asci and pale brown one-celled ascospores with broadly rounded ends released in mass within the perithecia conform well to the genus *Phylacia* as defined by Dennis (1957) and Rodrigues & Samuels (1989). Unlike this new taxon, most species of *Phylacia* have stipitate, substipitate, turbinate or roughly cylindrical stromata that are higher than broad. Stromatal morphology is regarded as highly discriminant in *Phylacia* (Medel *et al.*, 2006) and only *P. bomba* (Mont.) Pat. and its variety *macrospora* feature hemispherical to pulvinate sessile stromata like those of our new taxon. Since its ascospores are significantly larger than those of *P. bomba* and its variety *macrospora* (17.7–19.9 × 7.9–9.3 µm vs. 10–13 × 5–7 µm and 13.5–15.5 × 5.4-7 µm respectively) and elongate piriform vs. oblong ellipsoid, we feel justified to segregate the new species *P. korfii*.

Moreover, the ascospores dimensions of *P. korfii* are significantly larger than those of all other known *Phylacia* spp. (Medel *et al.*, 2006) and their piriform outline strikingly contrasts with the ellipsoid to oblong shape encountered in all other species. Ascospores of *Phylacia* were said to lack a germ slit (Rodrigues & Samuels, 1989; Bitzer *et al.*, 2008) and this character was used to separate this genus from *Rhopalostroma* (Hawksworth, 1977; Stadler *et al.*, 2012). This statement was resumed by Medel *et al.* (2006) but with reservations "*lacking germ slits or perhaps easily discernible slits*"; the presence of a germ slit on ascospores of *P. sagrana* from Costa Rica was later confirmed by Rogers (see earlier herein). Indeed a thorough observation of ascospores enabled us to confirm the presence of a germ slit on ascospores of the limited number of *Phylacia* spp. examined. The observation of fresh ascospores of *P. korfii* in water did not reveal the germ slits distinctly but they became conspicuous after mounting in PVA-lactophenol followed by a 48 h incubation in order to clear out the ascospores contents. Alternatively, observation of dry material in which ascospores lost their vacuolar contents likewise allows to see the germ slit distinctly. The presence of paler bands on ascospore surface (Plate 2, figs. B and E) is difficult to interpret and may be misleading but the location of these paler bands differs from the typical position where the germ slit occurs in this species.

Similar observations were carried out on three available specimens viz: *P. bomba* from Martinique, *P.* cf. *sagrana* from Panama and *P. poculiformis* from French Guiana. The collection from Panama is tentatively referred to *P. sagrana* because its stromata are subpiriform with a broadly conical apex and occur in bunches but instead of being sessile on a broad base like in the typical *P. sagrana* they are substipitate to sometimes clearly stipitate (Carmona-Quintero,

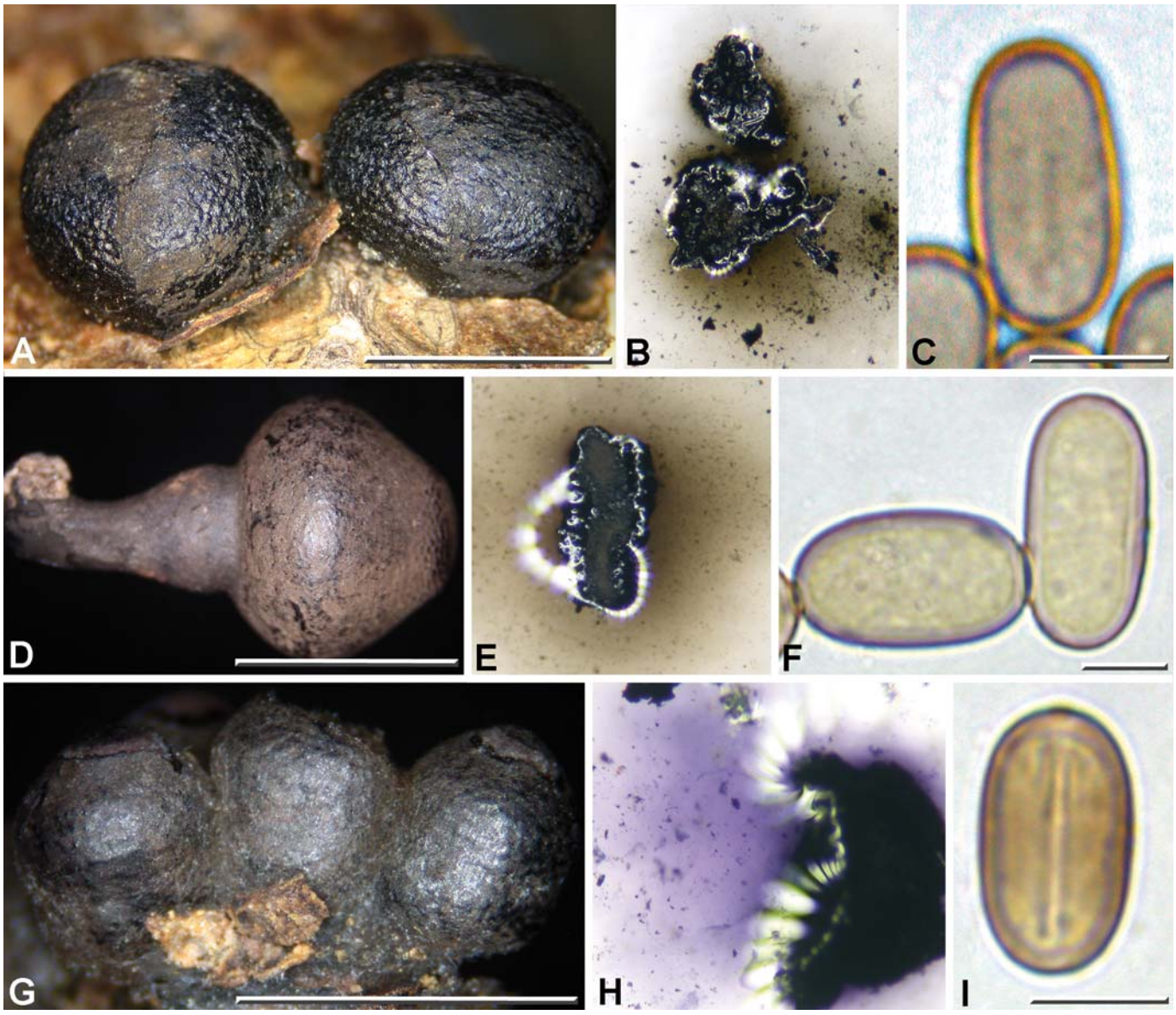

Plate 3 — *Phylacia* spp.

A-C: *P. bomba* CLL 6163; D-F: *P. poculiformis* CLL 8015; G-I: *P.* cf. *sagrana* AC 030.A, D, G: Stromata in side view; B, E, H: KOH-extractable pigments after 1 min incubation; C, F, I: Ascospores in PVA-lactophenol. Scale bars: A, D, G = 5 mm; C, F, I = 5 µm.

2008). It is unknown whether this slight difference is taxonomically significant.

The ascospores of *P. bomba* and *P.* cf. *sagrana* apparently lack a germ slit when observed in water but appear to have a short inconspicuous central germ slit when observed in PVA-lactophenol for the former (Plate 3, fig. C), more conspicuous and almost spore-length for the latter (Plate 3, fig. I). Conversely, the ascospores of *P. poculiformis* that are thin-walled, do not show a visible germ slit under the same conditions of observation (Plate 3, fig. F).

The subsurface of stromata of *Phylacia* consists of a thin crust composed of coloured waxy granules that yield pigments in 10% KOH, just like in other *Hypoxyloideae* like *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh, *Daldinia*, *Hypoxylon*, *Rhopalostroma* and *Thamnomyces*. As the KOH-extractable pigments prove highly useful at species delimitation in these genera, some preliminary comparative observations on *Phylacia* are presented here. *Phylacia bomba* and *P. poculiformis* have similar greyish sepia KOH-extractable pigments but in the latter they are preceded by a fugacious olivaceous pigment. *Phylacia* cf. *sagrana* exhibits greyish violet pigments that would be interesting to compare with those of typical *P. sagrana* and its supposed close relative *P. surinamensis* (Berk.) Dennis. In contrast, the new taxon *P. korfii* yields faint pigments that need a prolonged incubation to develop and evolve from honey to vinaceous with time.

The current taxonomy of *Phylacia* spp. is based only on the combination of external stromatal morphology which is inevitably variable and ascospores dimensions which largely overlap (Medel *et al*., 2006). As noticed by Dennis (1957) the genus is poorly known and species concepts in *Phylacia* should be revised when more information on more specimens is available. When this is carried out, we suggest to take into account the KOH-extractable pigments, the germ slit morphology and the internal configuration of the stromata in addition to the classical aforementioned characters.

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