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A new species of *Daldinia* (*Xylariaceae*) from the Argentine subtropical montane forest

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Abstract

Based on a combination of morphological, molecular phylogenetic and chemotaxonomic evidence, a new species of *Daldinia* from Northwest of Argentina is described. *Daldinia korfii* is morphologically related to *D. placentiformis*, but differs in having brown vinaceous, KOH-extractable pigments and the tissue below the perithecial layer is composed of inconspicuous to conspicuous alternating zones in the new taxon.

Key words – Ascomycetes – Yungas – secondary metabolites – Xylariales

Introduction

Daldinia Ces. & De Not. (Ascomycota, Xylariales) comprise a group of hypoxyloid *Xylariaceae* that had been initially erected based on their conspicuous stromata with internal alternating ring zones. Its species are worldwide distributed and principally associated with angiosperms. The genus was first monographed by Child (1932) and Ju et al. (1997). Recently, in the last, comprehensive monograph of *Daldinia*, Stadler et al. (2014) introduced a modern polythetic concept, involving a combination of morphological, phylogenetic and chemotaxonomic data. In total, including the recently described endophytic, insect associated species *D. hawksworthii* S. Pažoutová, Šrůtka & M.Stadler, which is thus far only known from its asexual state (Pažoutová et al. 2013), 48 *Daldinia* species are currently known from around the world. In Argentina, the genus has hitherto been poorly studied, and the only previous reports go back to Spegazzini (1902, 1909) and Hladki (2004).

During a survey *Xylariaceae* in the "Las Yungas" phytogeographic region (Sir et al. 2016) from northwest Argentina, interesting daldinoid specimens were collected. Our studies based on the modern taxonomic concept revealed that the specimens represent an undescribed species, which we introduce in this paper.

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Materials & Methods

The specimens were obtained from different natural reserves from 'Las Yungas" of Jujuy and Salta provinces. The study of sexual state was made according to Stadler et al. (2014). The cultures of the specimen were obtained from multispore isolates as reported by Sir et al. (2016) and the conidiophores branching pattern of the asexual state were classified according to Ju & Rogers (1996). The color codes of the stromata, extractable pigments and cultures are reported as proposed by Rayner (1970). Reference specimens and cultures are preserved in LIL (Fundación Miguel Lillo, San Miguel de Tucumán, Argentina) and STMA (HZI culture collection, Helmholtz Centre for Infection Research, Braunschweig, Germany).

For chemical analyses, stromata were extracted with methanol (Stadler et al. 2001). Samples were analyzed by analytical HPLC (High performance liquid chromatography) using an Agilent 1260 Infinity Series instrument equipped with a diode array detector and an Electrospray ionisation (ESI)-iontrap mass detector (amaZon, Bruker). The instrumental settings were the same as described by Kuhnert et al. (2014a, 2017). Spectra were compared to an internal database with stored signals from standards of known secondary metabolites produced by *Xylariaceae* from previous work (Bitzer et al. 2007, Stadler et al. 2014). Facesoffungi numbers (Jayasiri et al. 2015) and Index Fungorum (http://www.indexfungorum.org/names/names.asp) numbers are provided.

Molecular phylogenetics

DNA Isolation, phylogenetic inference and gene amplification of the ITS fragment were performed as described in detail by Otto et al. (2016), while the β-tubulin fragment was amplified using the protocol of Kuhnert et al. (2014b). Maximum Likelihood trees for both marker genes were calculated based on cured MAFFT (Katoh et al. 2002) alignments and the best fitting substitution models were determined from cured alignments (Gblocks Server by Talavera & Castresana 2007; with low stringency settings for ITS/ ß-tubulin: Minimum number of sequences for a conserved position: 17/18, minimum number of sequences for a flanking position: 17/18, maximum number of contiguous nonconserved positions: 8, minimum length of a block: 5, allowed gap positions: with half) by MEGA modeltest (Tamura et al. 2013). The phylogenetic tree of the ITS region was calculated with PHYML (Guindon et al. 2010); and RAxML (Stamatakis 2014) was used to calculate the phylogenetic tree inferred from ß-tubulin sequences. Both trees were calculated with 1000 bootstrap replicates (BS). The phylogenetic trees were submitted to TreeBase (ß-Tubulin tree; TB2:S20192 ITS tree: TB2:S20199) Sources of reference sequences are listed in Table 1.

The phylogenetic tree based on the ITS region of the rDNA (Fig. 1) is composed of 34 sequences, consisting of 23 *Hypoxylon*, 9 *Daldinia*, and one *Xylaria* sequences (Table 1). The backbone of the tree is not statistically supported. The *Daldinia* sequences form a monophyletic clade, which is supported by a bootstrap value (BS) of 76%. The sequences of the newly described species *D. korfii* cluster together with *D. placentiformis*, while being deliminated from the *D. eschscholtzii* clade (BS value 53%).

The β-tubulin tree (Fig. 2) consists of the same genus composition as described above. The backbone is supported partially (BS values of 50% and above). The *Daldinia* sequences form a monophyletic clade. and its topology is well supported. EBS476 and EBS473 (*D. korfii)* cluster together (100% BS support), while being separated from EBS030 (*D. placentiformis).* This cluster is differentiated (91%) from EBS067 (*D. korfii).* Even though this slight deviation was observed, we find it fully justified to erect a new species, since the morphological and chemotaxonomic data were in accordance in all examined specimens of *D. korfii.*

Taxonomy

Daldinia korfii Sir & **Lambert, sp. nov.** Figs 3–5

Index Fungorum number: IF552565; Facesoffungi number: FoF 02715

Table 1 List of used strains for molecular phylogeny. Holotypes (T) and epitypes (ET) are indicated accordingly. EBS codes refer to Sir & Hladki numbers.

Species	GenBank Acc No β-tubulin	GenBank Acc No ITS	Specimen or strain ID	Origin	Reference
Daldinia bambusicola	AY951688	KY204022	CBS 122872	Thailand	This study, Hsieh et al. (2005)
D. concentrica	KC977274	AY616683	CBS 113277	Germany	Triebel et al. (2005), Kuhnert et al. (2014b)
D. eschscholtzii	KC977266	JX658484	MUCL 45435	Benin	Stadler et al. (2014), Kuhnert et al. (2014b)
D. placentiformis	KY204015	KY204019	EBS 030	Argentina	This study
D. korfii sp. nov.	KY204014	KY204018	EBS 067	Argentina	This study
D. korfii sp. nov.	KY204016	KY204020	EBS 473	Argentina	This study
D. korfii sp. nov.	KY204017	KY204021	EBS 476	Argentina	This study
D. placentiformis	KC977278	AM749921	MUCL 47603	Mexico	Bitzer et al. (2008), Kuhnert et al. (2014b)
D. theissenii	KX271247	KY204023	CBS 113044	Argentina	Stadler et al. (2014), This study
Hypoxylon calileguense	KU604579	KU604566	STMA 14059	Argentina (T)	Sir et al. 2016, Kuhnert et al. (2017)
H. cinnabarinum	AY951709	JN979409	BCRB 33810	Mexico	Hsieh et al. (2005)
H. erythrostroma	KC977296	KC968910	MUCL 53759	Martinique	Kuhnert et al. (2014b)
H. fendleri	AY951718	JN979418	BCRB 34064	Taiwan	Hsieh et al. (2005)
H. fragiforme	-	AY616690	CBS 114745	Germany	Triebel et al. (2005)
H. fragiforme	AY951719	-	BCRC 34065	France	Hsieh et al. (2005)
H. fulvo-sulphureum	KP401584	KP401576	MFLUCC 13-0589	Thailand (T)	Sir et al. (2015)
H. fuscum	AY951723	JN979423	BCRC 34069	Taiwan	Hsieh et al. (2005)
H. griseobrunneum	KC977281	KC968928	MUCL 53310, CBS 129346	Guadeloupe	Kuhnert et al. (2014)
H. haematostroma	KU159527	KU604596	STMA 14043	Argentina	Sir et al. (2016)
H. hypomiltum	KC977298	KC968914	MUCL 53312	Guadeloupe	Kuhnert et al. (2014b)
H. investiens	KC977270	KC968925	CBS118183	Malaysia	Bitzer et al. (2008), Kuhnert et al. (2014b)
H. laminosum	KC977292	KC968934	MUCL 53305, CBS129032	Martinique (T)	Kuhnert et al. (2014b)
H. lateripigmentum	KC977290	KC968933	MUCL 53304, CBS 129031	Martinique (T)	Kuhnert et al. (2014)
H. lenormandii	KM610305	KM610289	BCC 71961	Thailand	Kuhnert et al. (2015)
H. lienhwacheense	KU159522	KU604558	MFLUCC 14-1231	Thailand	Sir et al. (2016)
H. lilloi	KU159537	KU604574	STMA 14142	Argentina (T)	Kuhnert et al. (2017)
H. lividipigmentum	KU159529	KU604567	STMA 14044	Argentina	Sir et al. (2016)
H. monticulosum	KP401578	KP401585	MFLUCC 13-0593, BCC 71965	Thailand	Sir et al. (2015)
H. ochraceum	KC977300	KC968937	MUCL 54625	Martinique (ET)	Kuhnert et al. (2014b)
H. perforatum	KC977299	KC968936	MUCL 54174	Japan	Kuhnert et al. (2014b)
H. pulicicidum	JX183072	JX183075	MUCL 49879, CBS 122622	Martinique (T)	Bills et al. (2012)

H. rickii	KC977288	KC968932	MUCL 53309, CBS 129345	Martinique (ET)	Kuhnert et al. (2014b)
H. spegazzinianum	KC604582	KU604573	STMA 14082	Argentina (T)	Kuhnert et al. (2017)
Xylaria hypoxylon	KX271279	KY204024	CBS 122620	Sweden	This study, Stadler et al. 2014a)

Holotype – Argentina, Jujuy Province, Dept. Ledesma, Calilegua National Park, "La Lagunita" trail, 11 May 2012; Sir & Hladki 067 (LIL, ex-type culture STMA 16158, GenBank Acc. No: ITS – KY204018, ß-tubulin – KY204014).

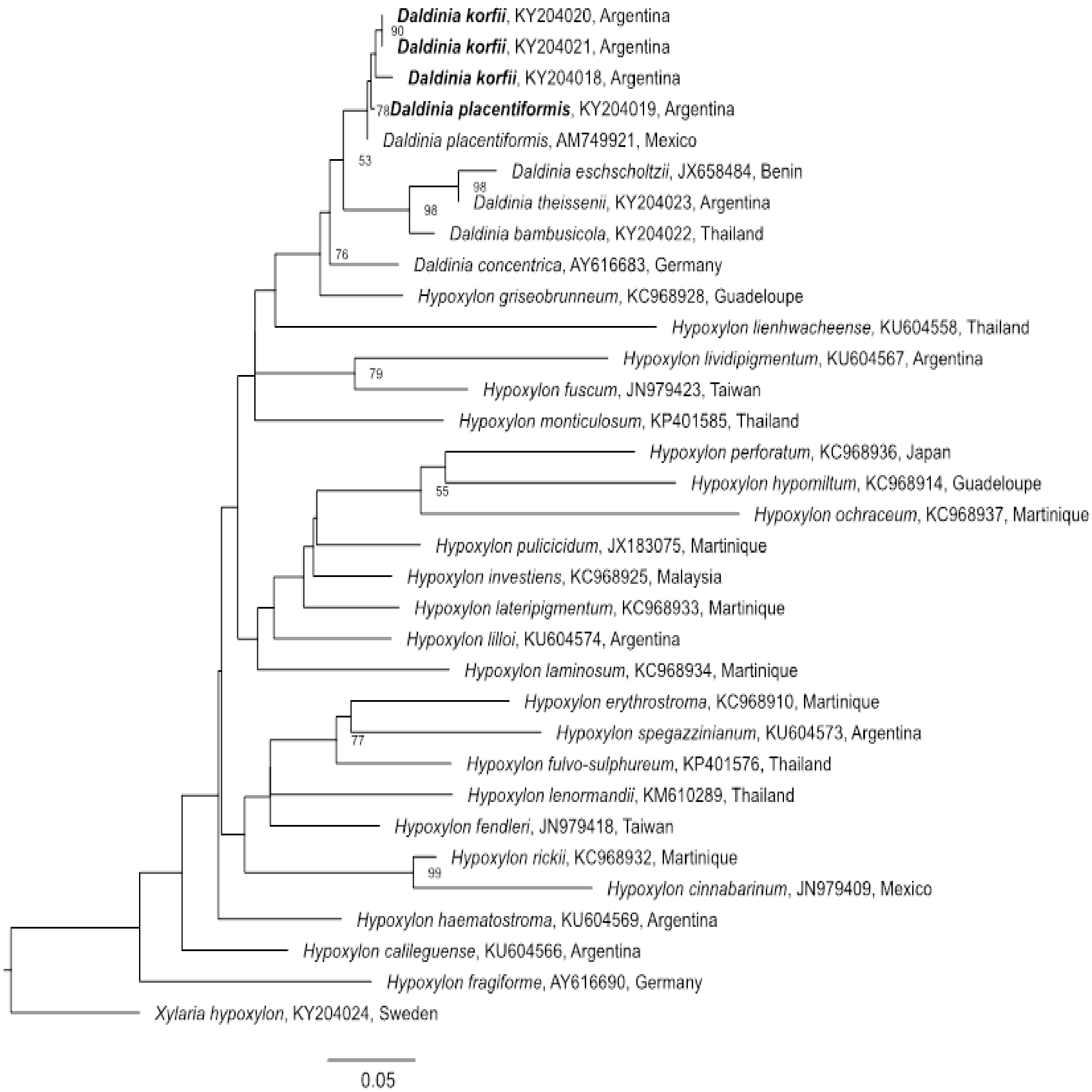

Fig. 1 – Phylogenetic tree (PHYML, substitution model K80+G, BS10000; log L = -5372.23) of selected *Daldinia* and *Hypoxylon* species, inferred from the ITS region. Bootstrap support values of 50% and above are indicated at the respective nodes. *Daldinia* species discussed in this paper are highlighted in bold letters.

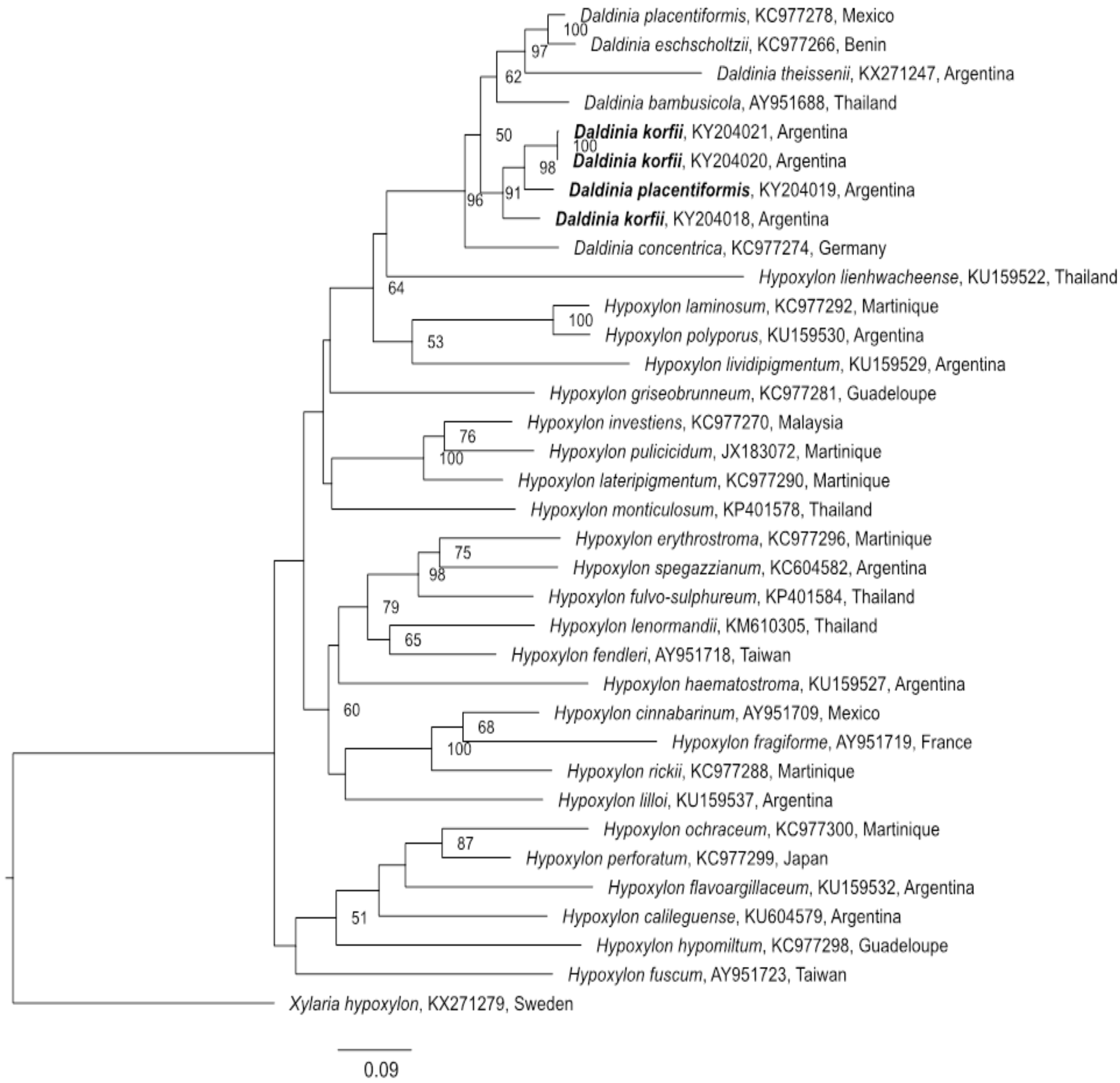

Fig. 2 – Phylogenetic tree (RAxML, GTR+G+I, BS1000; log L = -13927.95) of selected *Daldinia* and *Hypoxylon* species, inferred from the β-tubulin gene region Bootstrap support values of 50% and above are indicated at the respective nodes. *Daldinia* species discussed in this paper are highlighted in bold letters.

Etymology – In honour of Dr. Richard P. Korf, one of the leading mycological taxonomists of the 20th century.

On dead angiosperm wood. Sexual morph – Stromata hemispherical, pulvinate or peltate, the base broadly attached to the substrate or constricted generally, coalescent (Fig. 3a-d), with inconspicuous to conspicuous perithecial mounds (Fig. 3k), 8−55 mm long × 8−30 mm broad × 5−13 mm thick. Surface Brown Vinaceous (84), pruinose, dark red-orange granules forming a thin crust above perithecia, with Brown Vinaceous (84) to Dark Vinaceous (82) KOH-extractable pigments (Fig. 1e); the tissue between perithecia pale brown to brown; the tissue below the perithecia with layer composed of alternating zones (Fig. 3g), darker zones gray-brown, pithy, 0.4−1.2 mm thick, formed by cylindrical cells oriented vertically (Fig. 3h and i); the dark-brown zone, pithy, 0.7–1.6 mm thick, forming by cylindrical cells oriented horizontally (Fig. 3h and j). Perithecia lanceolate (Fig. 1l), 1.1−1.8 × 0.3−0.7 mm, ostioles inconspicuous, punctiform to umbilicate (Fig. 3k). Asci 8-spored, cylindrical, 169−311 µm total length, the spore-bearing parts 62−100 × 7−11 µm, the stipes 72.5−228 µm long. (Fig. 4a), with amyloid, discoid apical apparatus

(Fig. 2, c), 0.5−1 × 1.7−3 µm. Ascospores brown to dark brown, ellipsoid-inequilateral with narrow rounded ends (Fig. 4b), (10.3) 11−14 (16) × (4.8) 5.2−6.2 (7) µm, (N = 181, Me = 12.5 × 5.8 µm), with straight germ slit spore-length on convex side (Fig. 4f); perispore dehiscent in 10% KOH, smooth (Fig. 4d-e); epispore smooth. Asexual morph – Conidiophores with virgariella-like to nodulisporium-like branching pattern as defined in Ju and Rogers (1996) (Fig. 4j-l), erect, 200-300µm high, melanized main axis in some cases, hyaline to pale green, smooth to roughened. Conidiogenous cells cylindrical, hyaline to pale green, smooth to finely roughened (Fig. 4m), 11−26 × 2−3.5 µm. Conidia hyaline, smooth, ellipsoid, 5−8 × 2−4 µm (Fig. 4n).

Cultures – Colonies on OA covering Petri dish in 3 weeks, at first whitish becoming Olivaceous Gray (121) velvety to felty, azonate with entire margin (Fig. 4g), reverse, Dull Green (21), Olivaceous (48); no conidiogenous structures were observed. Colonies on YMG covering Petri dish in 4 weeks at first whitish becoming Olivaceous Gray (121) velvety to felty, azonate with entire margin (Fig. 4g-h), reverse Olivaceous Black (106). Sporulation regions Dull Green (70).

Secondary metabolites – The stromata contain BNT (binaphthalene tetrol), concentricol B and different cytochalasins as main metabolites (Fig. 5).

Host – Native angiosperms from "Las Yungas"

Additional material examined – Argentina, Jujuy province, Dept. Ledesma, Calilegua National Park, "La Lagunita" trail, 11 May 2012; Sir & Hladki 066 (LIL); "Sendero del Cielo", 12 May 12, Sir & Hladki 032 (LIL); "La Lagunita" trail, 26 April 2014, Sir & Hladki 631 (LIL). Dept. Santa Bárbara, Las Lancitas Provincial Reserve, 13 May 2012; Sir & Hladki 288 (LIL), 27 April 2014; Sir & Hladki 683, 684 (LIL). Salta province, Depto. Anta, El Rey National Park, "El Chorro de los Loros" trail, 29 April 2014, Sir & Hladki 710, 723 (LIL). Dept. Gral. José de San Martin, near to Acambuco Provincial Reserve, 23 April 2014, Sir & Hladki 547 (LIL). Santa Victoria, El Nogalar de los Toldos National Reserve, 26 June 2013; Sir & Hladki 473 (cultured, code STMA 14089); same locality, Sir & Hladki 476 (cultured, code STMA 14092 LIL).

Notes – *Daldinia korfii* can be identified by its massive subperithecial tissue with inconspicuous to conspicuous concentric zones and KOH-extractable pigments brown vinaceous. Our microscopic study revealed that the concentric zones correspond to different orientations of the hyphae (Fig. 1e−h).

Daldinia placentiformis (Berk. M.A. Curtis) Theiss. appears to be closely related to the new species but differs in having green olivaceous KOH-extractable pigments and by the absence of concentric zones in its subperithecial tissue. In addition, its stromata contain daldinone A as major secondary metabolite (Hellwig et al. 2005). The discovery of the new species provides additional evidence that some members of the "*H. placentiforme* complex" sensu Ju and Rogers (1996) are related to *Daldinia*, while other species with highly similar stromatal macromorphology such as *H. polyporus* (Starb.) Y.M. Ju & J.D. Rogers may represent a different phylogenetic lineage in the hypoxyloid *Xylariaceae*.

Bitzer et al. (2008) reported a somewhat aberrant specimen matching the features of *H. placentiforme* sensu Ju & Rogers (1996) from South Africa (MUCL 47603) with larger amounts of BNT (and purple stromatal pigments as verified in the current study). We noted that the conidiogenous cells and conidia of this material have similar dimensions to those of the new taxon. However, their ascospores are shorter [10–12.5(–13.5) × 6–7 vs (10.3) 11−14 (16) × (4.8) 5.2−6.2 (7) µm] (see Table 2). Moreover, *D. korfii* contains cytochalasins and concentricol B as stromatal metabolites. These are chemotaxonomic marker molecules for *D. concentrica* (Bolton: Fr.) Ces. & De Not., *D. eschscholtzii* (Ehrenb.: Fr.) Rehm and some members of the *D. eschscholtzii* group (Quang et al 2002; Stadler et al. 2014) but they have never been found in typical *D. placentiformis*. The South African fungus resembling *D. placentiformis,* which may warrant erection of another new species as more collections become available, was reported to contain only BNT and further metabolites with similar UV/Vis spectra that presumably constitute other binaphthyl derivatives, but neither cytochalasins nor concentricols were detected in its stromata (cf., Bitzer et al. 2008).

Fig. 3 − *Daldinia korfii* (Holotype) a, b, d. stromatal habit. c. stromata showing ventral insertion point (white arrow). e. KOH-extractable pigments in 10% KOH. f. stromatic granules. g. stroma in longitudinal section showing internal concentric zones (1 and 2). h. cells of internal concentric zones, in 1 cells oriented vertically, in 2 cells oriented horizontally. i. detail of cells oriented vertically. j. detail of cells oriented horizontally. k. stromatal surface l. perithecia in longitudinal section. Scale bars: a, d= 3 cm. b, c, g= 0.5 mm. h= 40 µm. i, j= 10 µm. k, l= 1 mm.

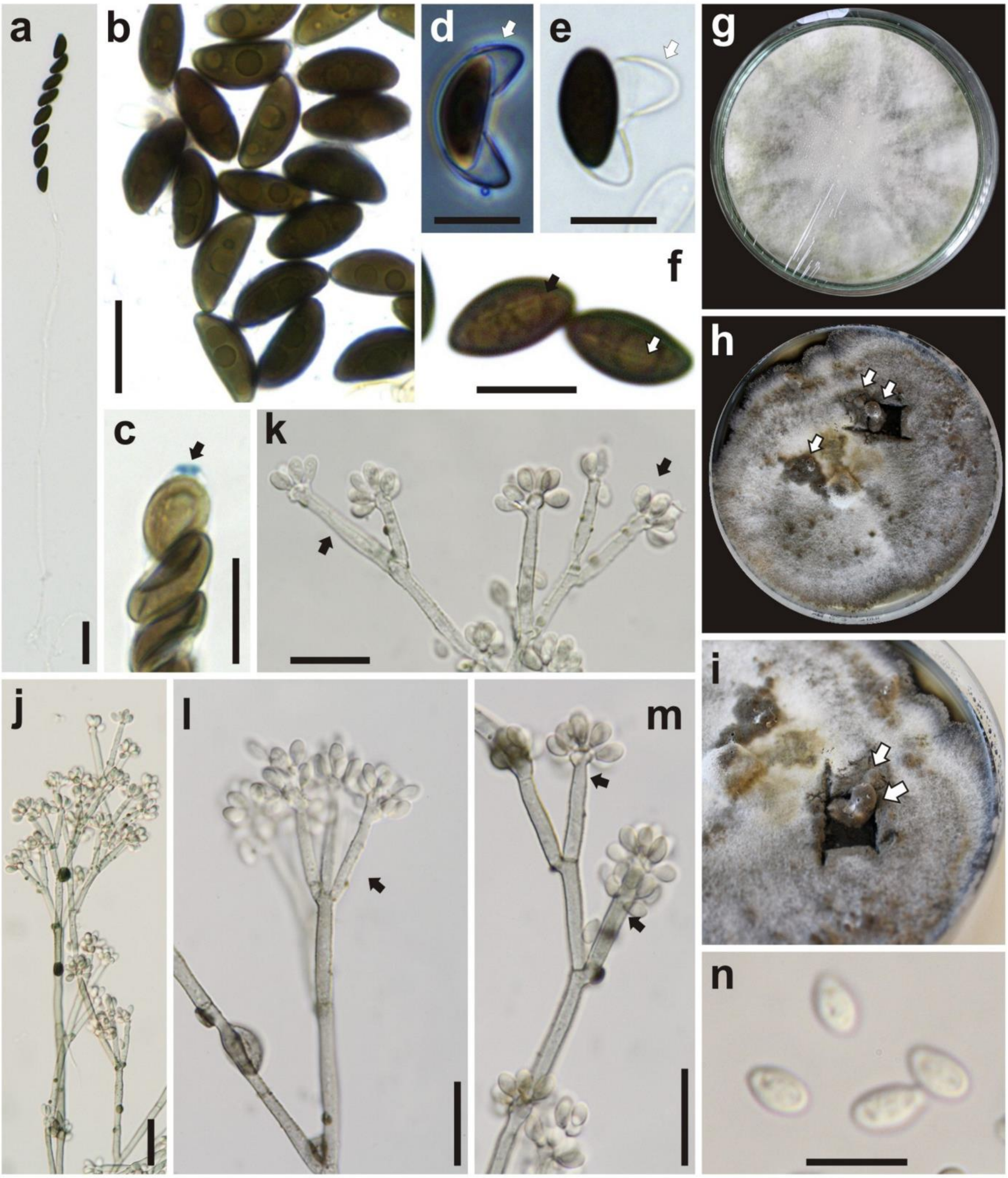

Fig. 4 – *Daldinia korfii*. a. asci. b. ascospores. c. amyloid apical apparatus (black arrow). d. ascospore with dehiscent perispore in polarized light (white arrow). e. ascospore in KOH showing dehiscent perispore (withe arrow). f. ascospores showing germ slit (arrows). g. culture on OA after 3 weeks. h, i. culture on YMG after 4 weeks, showing areas of production of immature stromata (white arrows). j. anamorphs showing conidiophores with virgariella-like to nodulisporium-like branching patterns. k. virgariella-like branching pattern (arrows). l. nodulisporium-like branching pattern (arrow). m. conidiogenous cells (arrows). n conidia. Scale bars: a, j-m= 20 µm. b-f, n= 10 µm.

Table 2 Comparison of *Daldinia korfii* and *D. placentiformis*

	Ascospores size (µm)	**KOH-extractable pigments**	**Anamorph (Cc and Co in µm)**	**References**
D. placentiformis	14.5−16 × 6.5−7	Dull Green*	nodulisporium-like Cc: 11−20 × 2−3.5 Co:4.5−6.5 × 2.8−3.5	Stadler et al. (2014)
***D.* cf. *placentiformis* (MUCL 47603)**	10–12.5(–13.5) × 6–7	Purple	nodulisporium-like Cc: 14–25 ×2.5–3.5 Co: 5.5–7.5 × 3.5–4	Bitzer et al. (2008)
D. korfii	(10.3) 11.0−14.0 (16.0) × (4.8) 5.2−6.2 (7.0)	Brown Vinaceous*	nodulisporium-like Cc: 11−26 × 2−3.5 Co: 5−8 × 2−4	This study

Cc − Conidiogenous cells. Co – Conidia. * − following Rayner (1970)

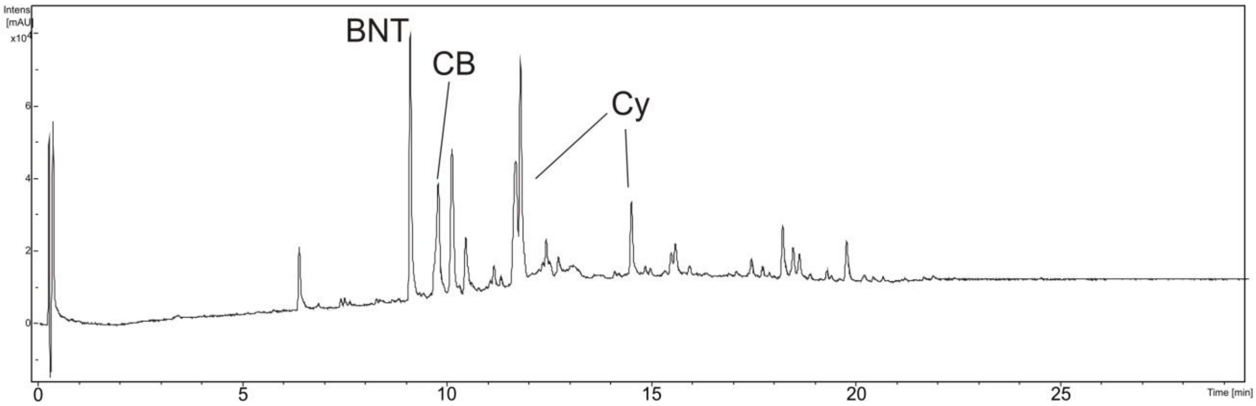

Fig. 5 − Stromatal HPLC-UV profiles of *D. korfii*. (Holotype) (BNT –binaphthalene tetrol; CB – Concentricol B, Cy – cytochalasins).

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