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ORIGINAL ARTICLE

New species and reports of *Hypoxylon* from Argentina recognized by a polyphasic approach

Esteban Benjamin Sir[1,2] · **Eric Kuhnert**[3,4] · **Christopher Lambert**[3,4] · **Adriana I. Hladki**[2] · **Andrea I. Romero**[1,5] · **Marc Stadler**[3,4]

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Abstract A preliminary account of *Hypoxylon* species (Xylariaceae) from the hitherto widely unexplored "Yungas" mountain forests of Northwest Argentina is presented. Two new species are described based on extensive morphological, molecular (ITS region of rDNA, partial β-tubulin gene) and chemotaxonomic data. *Hypoxylon spegazzinianum* is close to *H. erythrostroma*, but differs by larger ascospores and a virgariella-like asexual morph. *Hypoxylon calileguense* resembles *H. subgilvum* when growing on wood, but can be distinguished by larger ascospores and a fawn to brick stromatal surface colour. Stromata found on bark have affinities to *H. pelliculosum*, but differ in their stromatal surface colour and conspicuous amyloid apical apparatus. In addition, nine taxa of *Hypoxylon* are reported for Argentina for the first time, and some details on their asexual state and stromatal secondary metabolites are reported. An updated dichotomous key for *Hypoxylon* species from Argentina is provided.

Keywords Argentina · Biodiversity · Secondary metabolites · *Hypoxylon* · Xylariales

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✉ Marc Stadler
Marc.Stadler@t-online.de

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

[2] Mycology Laboratory, Fundación Miguel Lillo, Miguel Lillo 251, San Miguel de Tucumán CP 4000, Tucumán, Argentina

[3] Department Microbial Drugs, Helmholtz-Zentrum für Infektionsforschung GmbH, Inhoffenstrasse 7, 38124 Braunschweig, Germany

[4] German Centre for Infection Research (DZIF), partner site Hannover-Braunschweig, 38124 Braunschweig, Germany

[5] Departmento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales (UBA), PROPLAME-PRHIDEB, UBA-CONICET, Ciudad Universitaria, Pabellón II, 4to. Piso, CP1428EHA Buenos Aires, Argentina

Introduction

The xylariaceous genus *Hypoxylon* (Ascomycota, Xylariales) comprises the largest number of species within the informal "subfamily" Hypoxyloideae. According to Ju and Rogers (1996), the genus is defined by a "nodulisporium-like"[1] asexual state, unipartite stromata, the presence of stromatal tissue below a solid and homogeneous perithecial layer and stromata that are not upright. Other morphological characters, for example, dehiscence of the perispore, the ascal apical apparatus, ascospore size, the germ slit and the KOH-extractable stromatal pigments, are useful to delimit *Hypoxylon* at the species level. In many cases, the complex morphology generates the need of chemotaxonomic techniques and molecular (PCR-based) methods to distinguish species or assign relationships within the genus (Kuhnert et al. 2014a, 2015; Sir et al. 2015).

As reported by Ju and Rogers (1996), the genus is distributed worldwide, showing its highest diversity in the tropics, particularly in the neotropics. For the southernmost part of South America, the first reports of *Hypoxylon* have arisen from collections and descriptions made by the pioneer mycologist Carlos Spegazzini between 1880 and 1922 (Spegazzini

[1] Actually, these authors have distinguished various subtypes of branching patterns according to the complexity of the conidiophores, which are here accepted in the species descriptions; see Stadler et al. (2014) for further explanation.

1880, 1891, 1922). He proposed 33 *Hypoxylon* species and two varieties for this region. Based on the modern concept of the genus, Hladki and Romero (2006, 2009a) studied the taxonomic positions and revised the nomenclature of the proposed species. Moreover, the aforementioned authors collected additional specimens and recognized, in total, 12 species for the Argentine mycobiota (Hladki and Romero 2009b).

An important spot representing the diversity of Xylariaceae is located in "Las Yungas", a subtropical montane forest in the northwest of Argentina (Sir et al. 2012). It covers a narrow strip of 2,700,000 hectares (23–29° S, 64–68° O) from north to south, along the provinces of Salta, Jujuy, Tucumán and Catamarca, located between 300 and 3000 m above sea level (s.l.m.) adjacent to the Bolivian and Chilean borders. Although "Las Yungas" represents a limited ecosystem for the country (less of 0.1 % of the territory), it comprises nearly 50 % of the Argentine biodiversity, according to some estimates (Brown 1995; Brown et al. 2002). However, only eight *Hypoxylon* species were hitherto reported for this area: *H. anthochroum*, *H. lenormandii*, *H. megalosporum*, *H. notatum*, *H rubiginosum* var. *microsporum*, *H. subgilvum* and *H. subrutilum* (Hladki and Romero 2009b).

Here we report the morphological, molecular and chemotaxonomic details of *Hypoxylon* specimens collected during 2012–2014 in the aforementioned Las Yungas region. Based on a polyphasic taxonomic approach, we propose two new species. Moreover, the largest phylogenetic reconstruction of Argentine Xylariaceae using the ITS region of the ribosomal DNA, as well as the partial region of the β-tubulin gene, is presented. In addition, an emended taxonomic key to Argentine *Hypoxylon* is given.

Materials and methods

General

If not indicated otherwise, solvents were obtained in analytical grade from J.T. Baker (Deventer, Netherlands) or Merck (Darmstadt, Germany). All scientific names of fungi follow the entries in Mycobank (www.mycobank.org); hence, no authorities and years of publications are given. Reference specimens and cultures were preserved in LIL (Fundación Miguel Lillo, San Miguel de Tucumán, Argentina) and STMA (HZI culture collection, Helmholtz Centre for Infection Research, Braunschweig, Germany). Additional specimens for comparison were obtained from BPI (US National Fungus Collections, Beltsville, Maryland, USA), LPS (Universidad Nacional de La Plata, Buenos Aires, Argentina), S (Swedish Museum of National History, Stockholm, Sweden), and WSP (Washington State University, Pullman, Washington, USA). Herbaria acronyms are from Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Collection sites

In general, this study is based on specimens collected from different Natural Reserves of "Las Yungas": Calilegua National Park, Las Lancitas Provincial Reserve (Jujuy province), Acambuco Provincial Reserve, Baritú National Park, El Nogalar de los Toldos National Reserve, El Rey National Park (Salta province) and Sierras de San Javier Park (Tucuman province).

Morphological characterization

Structures of the sexual morph were measured from fresh material mounted in distilled water, 5 % KOH and 10 % KOH, respectively (Stadler et al. 2014). Melzer's reagent was used to test for the amyloid reaction. Asexual structures were observed microscopically in water or 5 % KOH. The descriptions of the sexual state and branching pattern of conidiogenous structures were classified according to Ju and Rogers (1996). The colour codes of the stromata and the extractable pigments are reported as proposed by Rayner (1970). Photos of micro-morphological structures were taken through a brightfield microscope at 400–1000× magnification. Pictures of the ultrastructure of the perispore were obtained with a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany) up to a magnification of 25.000×. The cultures of the specimen were obtained from multispore isolates, using yeast-malt-glucose agar (YMG: yeast extracts 4 g/l; malt extract 10 g/l; dextrose 4 g/l; cf. Bitzer et al. 2008) and Difco Oatmeal Agar (OA).

HPLC profiling

For HPLC analyses, stromata were extracted with methanol (Stadler et al. 2001) or acetone, respectively. Samples were analyzed by analytical HPLC (Agilent 1260 Infinity Series) equipped with a diode array detector and an ESI iontrap MS detector (Amazon, Bruker). The instrumental settings were the same as described by Kuhnert et al. (2014b). 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).

Molecular phylogenetic analyses

DNA isolation and sequence generation of the ITS region of the ribosomal RNA (spanning ITS1, 5.8S rDNA, ITS2 as well as partial 18S and 28S rDNA), as well as the ß-tubulin coding gene (TUB) was carried out as described by Kuhnert et al. (2014b). In total, 20 ITS and four β-tubulin sequences of mainly Argentine Xylariaceae specimens (including the newly described species) were obtained. Origin and culture collection numbers of the

Table 1 List of used taxa for phylogenetic reconstruction. GenBank accession numbers, strain ID of public culture collections (if available), origin and reference studies are given. Type specimens are labelled with T (holotype), IT (isotype) or ET (epitype)

Species	GenBank Acc No ß-tubulin	GenBank Acc No ITS	Specimen or strain ID	Origin	Reference
Annulohypoxlyon annulatum	KU159523	KU604559	CBS 140775	Texas (USA)	This study
A. leptascum	KU604580	KU604576	MFLUCC 13-0587	Thailand	This study
A. moriforme	KU159525	KU604561	STMA 14065	Argentina	This study
A. stygium	KP401586	KP401579	MFLUCC 13–0599, BCC 71968	Thailand	Sir et al. (2015)
A. stygium var. *annulatum*	KU159526	KU604575	STMA 14066	Argentina	This study
A. truncatum	KU159524	KU604560	CBS 140777	Texas (USA)	This study
A. urceolatum	KP401585	KP401578	MFLUCC 14-1228	Thailand	Sir et al. (2015)
Creosphaeria sassafras	KU159533	KU60457	STMA 14088	Argentina	This study
Daldinia concentrica	KC977274	AY616683	CBS 113277	Germany	Triebel et al. (2005), Kuhnert et al. (2014a)
D. eschscholtzii	KC977266	JX658484	MUCL 45435	Benin	Stadler et al. (2014), Kuhnert et al. (2014a)
D. placentiformis	KC977278	AM749921	MUCL 47603	Mexico	Bitzer et al. (2008), Kuhnert et al. (2014a)
Hypoxylon calileguense	KU604579	KU604566	STMA 14059	Argentina	This study
	KU604578	KU604565	STMA 14070	Argentina	This study
H. chionostomum	-	KU604563	STMA 14060	Argentina	This study
H. cinnabarinum	AY951709	JN979409	BCRC33810	Mexico	Hsieh et al. (2005)
H. erythrostroma	KC977296	KC968910	MUCL 53759	Martinique	Kuhnert et al. (2014a)
H. fendleri	AY951718	JN979418	BCRC 34064	Taiwan	Hsieh et al. (2005)
	KF300547	KF234421	MUCL 54792	French Guiana	Kuhnert et al. (2014a)
H. flavoargillaceum	KU159532	KU604577	STMA 14062	Argentina	This study
H. fragiforme	AY951719	-	BCRC 34065	France	Hsieh et al. (2005)
	-	AY616690	CBS 114745	Germany	Triebel 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. (2014a)
	KU159535	KU604562	STMA 14052	Argentina	This study
H. haematostroma	KC977291	KC968911	MUCL 53301	Martinique (ET)	Kuhnert et al. (2014a)
	KU159527	KU604569	STMA 14043	Argentina	This study
H. hypomiltum	KC977298	KC968914	MUCL 53312, CBS 129036	Guadeloupe	Kuhnert et al. (2014a)
H. investiens	KC977270	KC968925	CBS 118183	Malaysia	Bitzer et al. (2008), Kuhnert et al. (2014a)
	KU159528	KU604568	STMA 14058	Argentina	This study
H. jaklitschii	KM610304	KM610290	CBS 138916	Sri Lanka (T)	Kuhnert et al. (2015)
H. jecorinum	AY951731	JN979429	N/A	Mexico	Hsieh et al. (2005)
H. laminosum	KC977292	KC968934	MUCL 53305, CBS 129032	Martinique (T)	Kuhnert et al. (2014a)
H. lilloi	KU159537	KU604574	STMA 14142	Argentina (T)	This study
H. lateripigmentum	KC977290	KC968933	MUCL 53304, CBS 129031	Martinique (T)	Kuhnert et al. (2014a)
H. lenormandii	KM610299	KM610283	STMA 14072	Argentina	Kuhnert et al. (2015)
H. lienhwacheense	KU159522	KU604558	MFLUCC 14-1231	Thailand	This study
H. lividicolour	AY951734	JN979432	BCRC 34076	Taiwan (T)	Hsieh et al. (2005)
H. lividipigmentum	KU159529	KU604567	STMA 14044	Argentina	This study
	AY951735	JN979433	BCRC 34077	Mexico (IT)	Hsieh et al. (2005)

Table 1 (continued)

Species	GenBank Acc No ß-tubulin	GenBank Acc No ITS	Specimen or strain ID	Origin	Reference
H. monticulosum	KP401578	KP401585	MFLUCC 13–0593, BCC 71965	Thailand	Sir et al. (2015), Surup et al. (2014)
	KU604581	KU604572	STMA 16000	Argentina	This study
H. nicaraguense	KC977272	AM749922	CBS 117739	Burkina Faso	Bitzer et al. (2008)
H. ochraceum	KC977300	KC968937	MUCL 54625	Martinique (ET)	Kuhnert et al. (2014a)
H. perforatum	KC977299	KC968936	MUCL 54174	Japan	Kuhnert et al. (2014a)
	KU159531	KU604564	STMA 14051	Argentina	This study
H. pilgerianum	AY951744	JQ009310	BCRC 34985	Taiwan	Hsieh et al. (2005)
H. polyporus	KU159530	KU604570	STMA 14090	Argentina	This study
	KC977283	AM749941	MUCL 49339	Ivory Coast	Kuhnert et al. (2014a)
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. (2014a)
H. samuelsii	KC977286	KC968916	MUCL 51843	Guadeloupe (ET)	Kuhnert et al. (2014a)
H. spegazzinianum	KU604582	KU604573	STMA 14082	Argentina (T)	This study
H. subgilvum	AY951754	JQ009314	BCRC 34094	Hawaii (USA)	Hsieh et al. (2005)
H. trugodes	KF300548	KF234422	MUCL 54794	Sri Lanka (ET)	Kuhnert et al. (2014a)
H. umbilicatum	KU159536	-	STMA 15276	Argentina	This study

strains are summarized in Table 1. Each set of sequences was aligned with a selection of Xylariaceae sequences originating from previously published studies (for details see Table 1). The backbone tree was composed of 43 representatives of the genus *Hypoxylon*, six *Annulohypoxylon* spp., three *Daldinia* spp. and one *Creosphaeria* species (Lopadostomataceae) as outgroup (cf. Senanayake et al. 2015). The phylogenetic analyses were performed for each gene by using maximum likelihood (ML) as an optimality criterion (Kuhnert et al. 2014a). Phylogenetic reconstruction of the ML tree was estimated with the software RAxML (Stamatakis 2006), applying the GTRCAT rate distribution model (with 25 rate categories) and the rapid bootstrap analysis algorithm. The program executed 1000 bootstrap (BS) replicates, the support values of which were mapped onto the most likely tree topology found by an independently executed search. Branch lengths were thereby calculated based on base exchange rates. The following deviations from the protocol (Kuhnert et al. 2014a) were applied in order to improve alignability: 325 reliably alignable base pair positions, 25–62, 279–292, 336–543, 552–616 according to the sequence JX183075 (*H. pulicicidum*), were included in the ITS analysis and 1024 base pair positions, 137–149, 159–195, 239–256, 260–345, 385–456, 523–509 and 523–1320 of JX183072 (*H. pulicicidum*), were considered in the β-tubulin analysis. Both trees were rooted with sequences of *Creosphaeria sassafras*.

Results & discussion

Taxonomic part

The unique combination of morphological, molecular and chemotaxonomic data led to the conclusion that *Hypoxylon spegazzinianum* and *Hypoxylon calileguense* represent undescribed species of the Xylariaceae, and are therefore described as new. In addition, nine new records of *Hypoxylon* species for Argentina are listed and depicted, including updated information on the asexual morph and the chemical composition of the stromata (for previous records on Argentine *Hypoxylon* see Ju and Rogers 1996; Hladki and Romero 2006, 2009b; Kuhnert et al. 2015). Furthermore, a dichotomous key to Argentine *Hypoxylon* species is provided.

Hypoxylon spegazzinianum Sir, Kuhnert, Hladki & A. I. Romero, sp. nov. Fig. 1
MB815821

Etymology In honor of the Italian mycologist Carlos Spegazzini, a pioneer in the exploration of South American fungi.

Known distribution Argentina.

Holotype *Argentina*, Salta, Santa Victoria department, "El Nogalar de los Toldos" National Reserve, on wood or bark of a dead branch, 26 June 2013, Sir & Hladki 466 (LIL, ex-

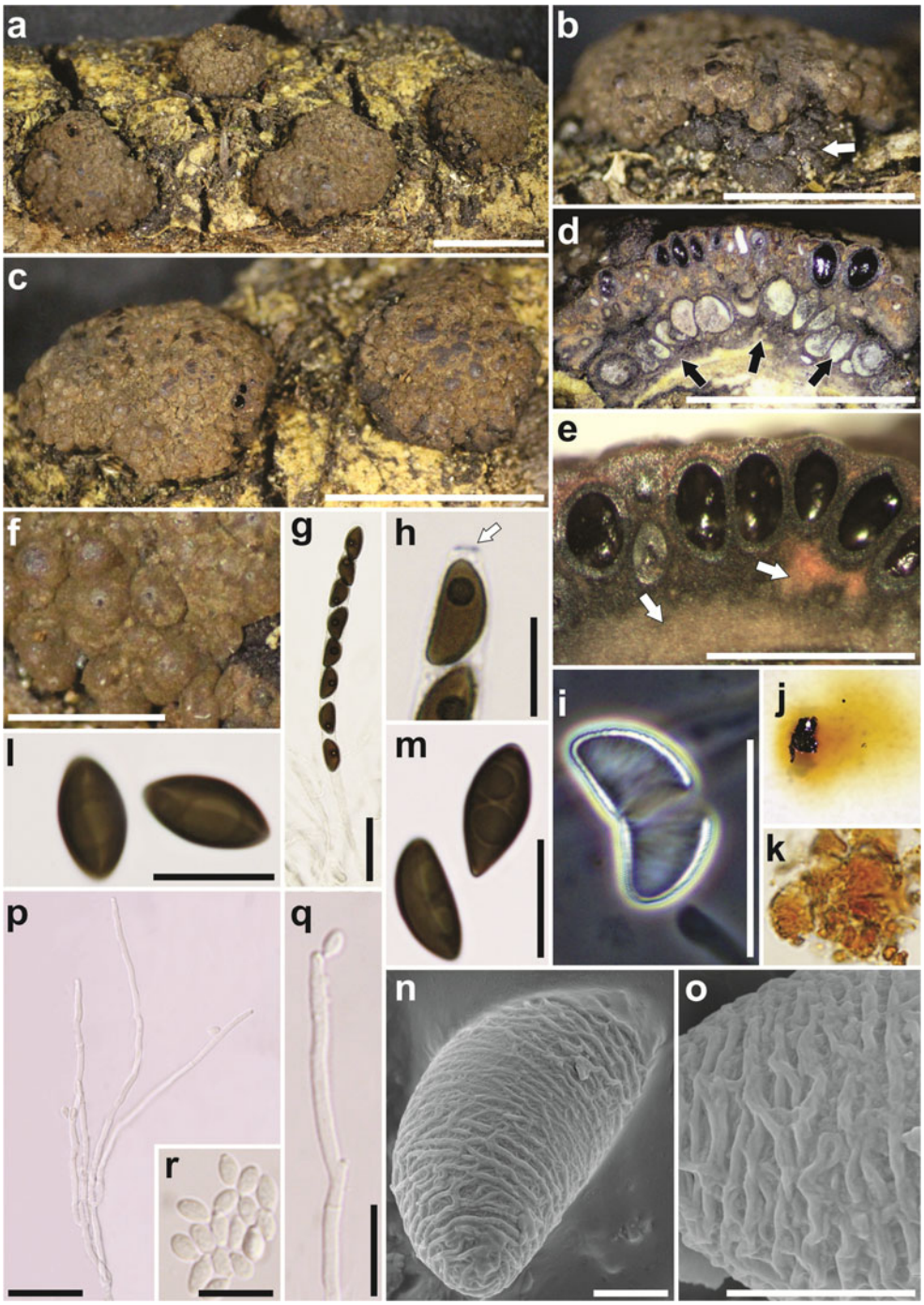


Fig. 1 *Hypoxylon spegazzinianum* (holotype): **a**, **b**, **c**: stromatal habit on the natural substrate. **b**: young stromata growing on older ones (*arrow*). **d**: section through stromata, showing two stromatal layers (*arrow*). **e**: section through stromata showing perithecia and pale brown to orange-brown tissue below the perithecial layer (*arrow*). **f**: stromatal surface in close up with perithecial mounds and ostioles. **g**: asci. **h**: amyloid apical apparatus. **i**: perispore showing conspicuous striated ornamentation under polarized light. **j**: KOH-extractable pigments. **k**: granules in water. **l**: ascospores showing sigmoid germ slit. **m**: ascospores showing acute end. **n**: ascospore under SEM. **o**: detail of striated perispore (SEM). **p**: virgariella-like conidiogenous structure derived from culture. **q**: conidiogenous cell. **r**: conidia. Scale is indicated by bars (**a**, **c**: 5 mm. **b**, **d**: 3 mm; **e**, **f**: 1 mm. **g**, **i**: 20 μm., **h**, **l**, **m**, **p**, **q**, **o**, **r**:10 μm. **n**, **o**: 2 μm

type culture STMA14082, GenBank Acc. No: ITS – KU604573, ß-tubulin – KU604582).

Differs from Hypoxylon erythrostroma by larger ascospores and virgariella-like conidiogenous structures

Sexual state *Stromata* effused-pulvinate, usually growing above old stromata, 3–7 mm long × 5–6 mm broad × 0.7–1 mm thick; with inconspicuous to conspicuous perithecial mounds; surface Sepia (63) with umber tones; black when old, pruinose; umber to orange-brown granules immediately beneath surface and between perithecia; with KOH extractable pigments Orange (7) with Citrine (13) tones; tissue below the perithecial layer inconspicuous, light brown or orange, 0.2–0.5 mm thick. *Perithecia* ovoid, obovoid or spherical, 0.4–0.5 high × 0.2–0.4 mm diam; ostioles lower than the stromatal surface, umbilicate. *Asci* eight-spored, cylindrical, 132–168 μm total length, the spore-bearing parts 84.5–98 μm × 6–8.5 μm broad, stipes 76–41 μm long; with amyloid, discoid apical

apparatus 0.4–0.5 high × 2–2.6 μm broad. *Ascospores* brown to dark brown, usually ellipsoid-inequilateral with narrowly rounded ends, rarely fusoid with nearly acute ends, occasionally slightly curved, (10.7–)11.6–14.0(−15.0) × (4.9–)5.6–7.0(−7.7) μm (N = 60; Me = 13.0 × 6.3 μm), with spore-length sigmoid germ slit on convex side; perispore dehiscent in 10 % KOH, revealing a strongly reticulate ornamentation by light microscopy and SEM (5000×); epispore smooth.

Asexual state *Conidiogenous structures* virgariella-like. *Conidiophores* hyaline, smooth to finely roughened. *Conidiogenous cells* hyaline, smooth, 14–36 × 1.5–2 μm. *Conidia* ellipsoid, hyaline, smooth, 4.5–5 × 2–2.5 μm.

Culture Colonies on OA covering Petri dish in 3 weeks, at first whitish, becoming Sepia (63), velvety to felty, zonate, with entire margins, pale Umber (9). Sporulating regions located at the center of colonies, pale Isabelline (65).

Secondary metabolites (Fig. 2) Stromata of *H. spegazzinianum* contain an unknown major compound, which is related to the mitorubrin family (M = 456). In addition, mitorubrinol acetate und two further secondary metabolites of unknown origin (M = 434 and M = 462) can be detected.

Notes *Hypoxylon spegazzinianum* is closely related to *H. erythostroma*; however, it can be easily distinguished by its stromatal granules, larger ascospores (10.7–15.0 × 4.9–7.7 vs. 7–9.5 × 3–4.5 μm) and virgariella-like condiogeneous structures. In addition, *H. erythrostroma* contains orsellinic acid, mitorubrinol and mitorubrinol acetate in larger amounts. The holotype of the latter was even found to contain rutilins (Quang et al. 2006). In *H. spegazzinianum* only mitorubrinol acetate could be detected in minor quantities and instead an unknown metabolite of the mitorubrin family was detected. The close relationship of both species is confirmed by the phylogenetic analyses based on TUB, where they cluster together with *H. fulvo-sulphureum*, another mitorubrin producing species (Sir et al. 2015).

Notably, the stromata of *Hypoxylon spegazzinianum* were found to grow preferably on older stromata. A similar phenomenon was reported for some specimens of *H. griseobrunneum*, but the species affiliation of the older stromatal layers could not be settled (Kuhnert et al. 2014a). In the case *of H. spegazzinianum*, we found diagnostic characters in the old stromata that unambiguously confirmed their relationship with the new species.

Hypoxylon calileguense Sir, Kuhnert, Hladki & A. I. Romero, sp. nov. Fig. 3

MB815822.

Etymology In reference to Calilegua National Park (Argentina), the locality where the holotype was collected.

Known distribution Argentina.

Holotype *Argentina*, Jujuy, Dept. Ledesma, Calilegua National Park, El Negrito, on bark of a dead small branch, 11 May 2012, Sir & Hladki 009 (LIL, ex-type culture STMA14059, GenBank Acc. No: ITS – KU604566, ß-tubulin – KU604579).

Differs from Hypoxylon subgilvum by having a brick to fawn stromatal surface colour, yellow to yellow-orange granules, larger ascospores and larger conidia. Differs from Hypoxylon pelliculosum by having a brick to fawn stromatal surface colour and conspicuous amyloid apical apparatus

Sexual state *Stromata* effused-pulvinate on wood 10–33 mm long × 4–8 mm broad × 1 mm thick, or glomerate coalescent to effused-pulvinate on bark, 1.8–7 mm long × 1.5–4 mm broad × 1–2 mm thick, plane or with inconspicuous to conspicuous perithecial mounds; surface Brick (59) to Fawn (87), pruinose; yellow to yellow-orange granules immediately beneath surface and between perithecia; with KOH extractable pigments Orange (7); tissue below the perithecial layer inconspicuous, brown to black, 0.2–0.35 mm thick. *Perithecia* spherical to obovoid or compressed-obovoid 0.4–0.6 mm high × 0.2–0.6 mm diam; ostioles slightly higher than the stromatal surface, umbilicate. *Asci* eight-spored, cylindrical, 96.5–155 μm total length, the spore-bearing parts 63–92 μm long × 6–8 μm broad, stipes 32–74 μm long; with amyloid, discoid apical apparatus 0.4–1 μm high × 1.7–2.7 μm broad. *Ascospores* brown to dark brown, ellipsoid-inequilateral, with narrowly rounded ends, slightly curved, (9.5–)10.5–12.4(−14.3) × (4.7–)5.1–6.2(−6.5) μm (N = 60, Me = 11.4 × 5.6 μm); with straight germ slit spore length on convex side; perispore dehiscent in 10 % KOH, with inconspicuous to conspicuous coil-like ornamentation under light microscope, perispore with root ramification-like ornamentation under SEM, epispore smooth.

Asexual state *Conidiogenous structure* nodulisporium-like. *Conidiophores* hyaline, pale yellow to pale brown, smooth to finely roughened. *Conidiogenous cells* hyaline, smooth, 14–30 × 2–3 μm. *Conidia* ellipsoid, pale brown, smooth, 5–8 × 3–4 μm.

Culture Colonies on OA covering Petri dish in 4 weeks, at first whitish, becoming Sepia (63), Yellowish Green (18) and Grey Olivaceous (107), velvety to felty, zonate, with entire margins, reverse Umber (9). Sporulating regions located at the center of colonies, Sepia (63).

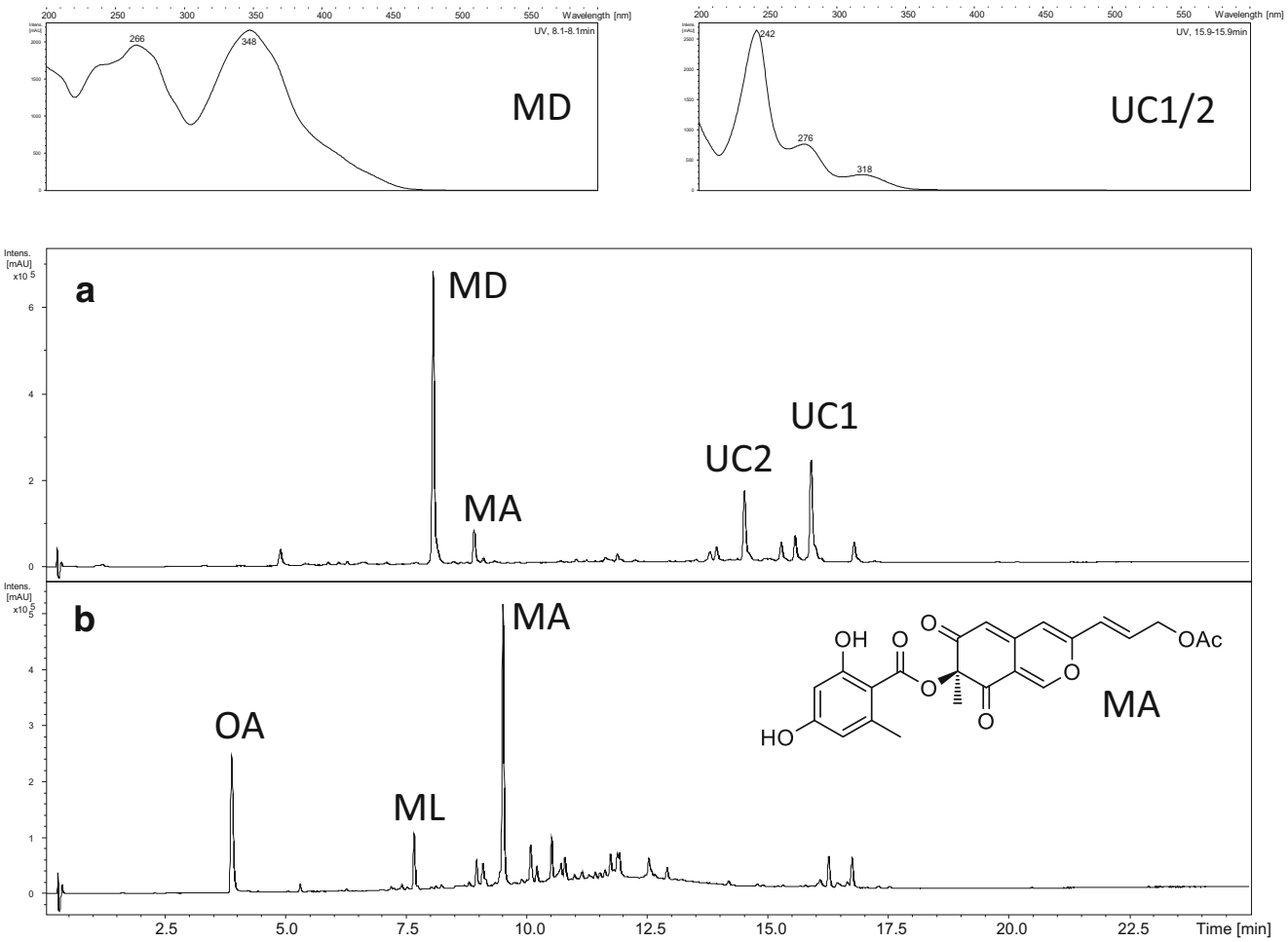


Fig. 2 Stromatal HPLC-UV profiles of *Hypoxylon spegazzinianum* (holotype, **a**) and *H. erythrostroma* (**b**) and corresponding DAD spectra of the unknown main metabolites. The structure of mitorubrinol acetate (MA) is depicted in **b**. OA – orsellinic acid, ML – mitorubrinol, MD – unknown mitorubrin derivative, UC – unknown compound

Secondary metabolites (Fig. 4) Stromata contain orsellinic acid and various other unknown compounds as major constituents which are most likely related to the mitorubrin family of azaphilones (e.g. M = 458, M = 896).

Additional specimens examined *Argentina*, Jujuy, Dept. Ledesma, Calilegua National Park, on wood of a dead branch, 11 May 2012, Sir & Hladki 025b (LIL); Salta, Orán, Road to "Isla de Cañas", on bark of a dead small branch, 24 May 2014, Sir & Hladki 601 (LIL).

Notes The effused stromata of *H. calileguense* could be confused with *H. subgilvum*, but can be distinguished from the latter by its stromatal surface colour, yellow granules, larger ascospores (9.5–14.3 × 4.7–6.5 μm vs 7–11 × 3.5–5 μm) and larger conidia (5–8 × 3–4 μm vs 3–4.5 × 1.5–2 μm). In addition, *H. calileguense* lacks mitorubrinol as stromatal constituent, as well as various other unknown compounds that are present in *H. subgilvum* (Fig. 4). The glomerate stromata resemble *H. pelliculosum*, but the latter differs in having a dark brick to sepia stromatal surface colour, an inconspicuous apical apparatus in Melzer's reagent and smaller ascospores (9.1–11.9 × 5–6.2 vs. 9.5–14.3 × 4.7–6.5 μm).

New reports of *Hypoxylon* species from Argentina

Hypoxylon carneum Petch, Ann. R. bot. Gdns Peradeniya 8: 157 (1924) Fig. 5a–d

For sexual morph descriptions, see Ju and Rogers (1996).

Asexual state Conidiogenous structure nodulisporium-like. Conidiophores reddish-brown to hyaline, smooth to finely roughened. Conidiogenous cells hyaline, smooth, 12–18.5 × 2–3 μm. Conidia ellipsoid, hyaline, generally smooth, 3.5–5 × 2–3 μm.

Culture Colonies on OA covering Petri dish in 2 weeks, at first whitish, becoming Grey Olivaceous (107), velvety to

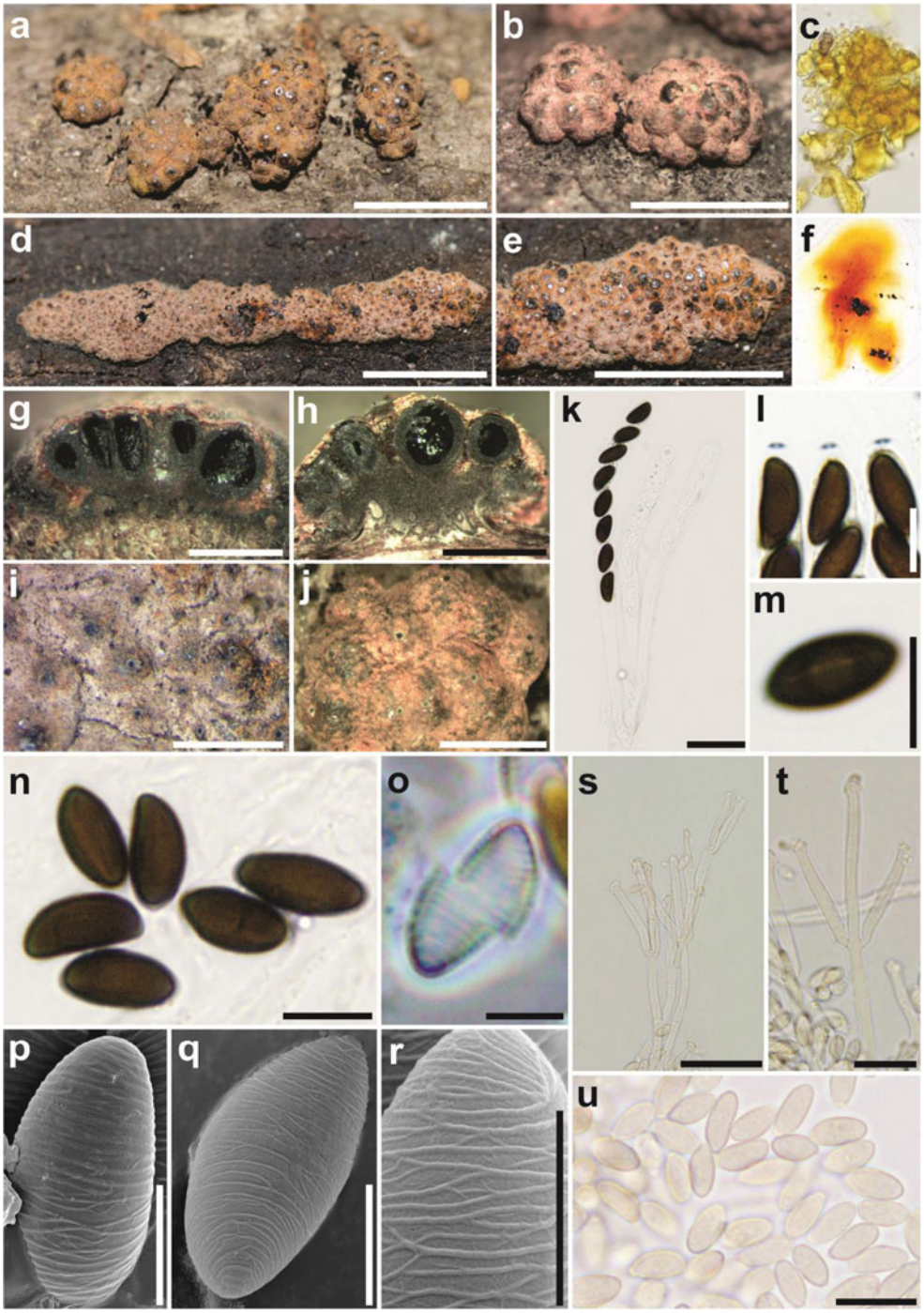


Fig. 3 *Hypoxylon calileguense*. (a, b, c, f, h, j, k, l, m, n, o, p, r, s, t, u: holotype Sir & Hladki 009; d, e, g, i, q, r: Sir & Hladki 025b). **a**, **b**: stromatal habit on bark. **c**: granules in water. **d**, **e**: stromata habit on wood. **f**: KOH-extractable pigments. **g**, **h**: stromata in vertical section showing the perithecia. **i**, **j**: stromatal surface in close up showing inconspicuous to conspicuous perithecial mounds and ostioles. **k**: asci. **l**: amyloid apical apparatus. **m**: ascospore showing straight germ slit. **n**: ascospores in water. **o**: perispore showing ornamentation under polarized light. **p**, **q**: SEM microphotographs of the conspicuous ornamented perispore. **r**: detail of root-like ornamentation of the perispore under SEM. **s**, **t**: nodulisporium-like asexual morph. **u**: conidia. Scale is indicated by bars (**a**, **b**, **d**, **e**: 5 mm. **g**, **h**, **i**, **j**: 1 mm. **k**, **s**: 20 μm. **l**, **m**, **n**, **t**, **u**: 10 μm. **o**, **p**, **q**, **r**: 4 μm)

felty, slightly zonate, with entire margin, reverse Fuscous Black (104), Dull Green (70). Sporulating regions scattered over entire surface of colony.

Specimens examined *Argentina*: Salta, San Victoria, Baritú National Park, on bark of a dead branch, 28 July 2011, Sir & Hladki 152 (LIL). Tucumán, Yerba Buena, Sierras de San Javier Park, on bark of a dead branch, 15 May 2014, Sir & Hladki 758 (LIL).

Known distribution Argentina, France, New Zealand, Sri Lanka, USA, Venezuela.

Notes The collections from Argentina have a pinkish stromatal surface colour as initially described by Petch (1924) and exhibit livid purple KOH-extractable pigments in young stromata (Fig. 2a, c) as reported by Fournier et al. (2010).

Our study revealed a nodulisporium-like conidiogenous structure in culture in this species for the first time (Fig. 5b).

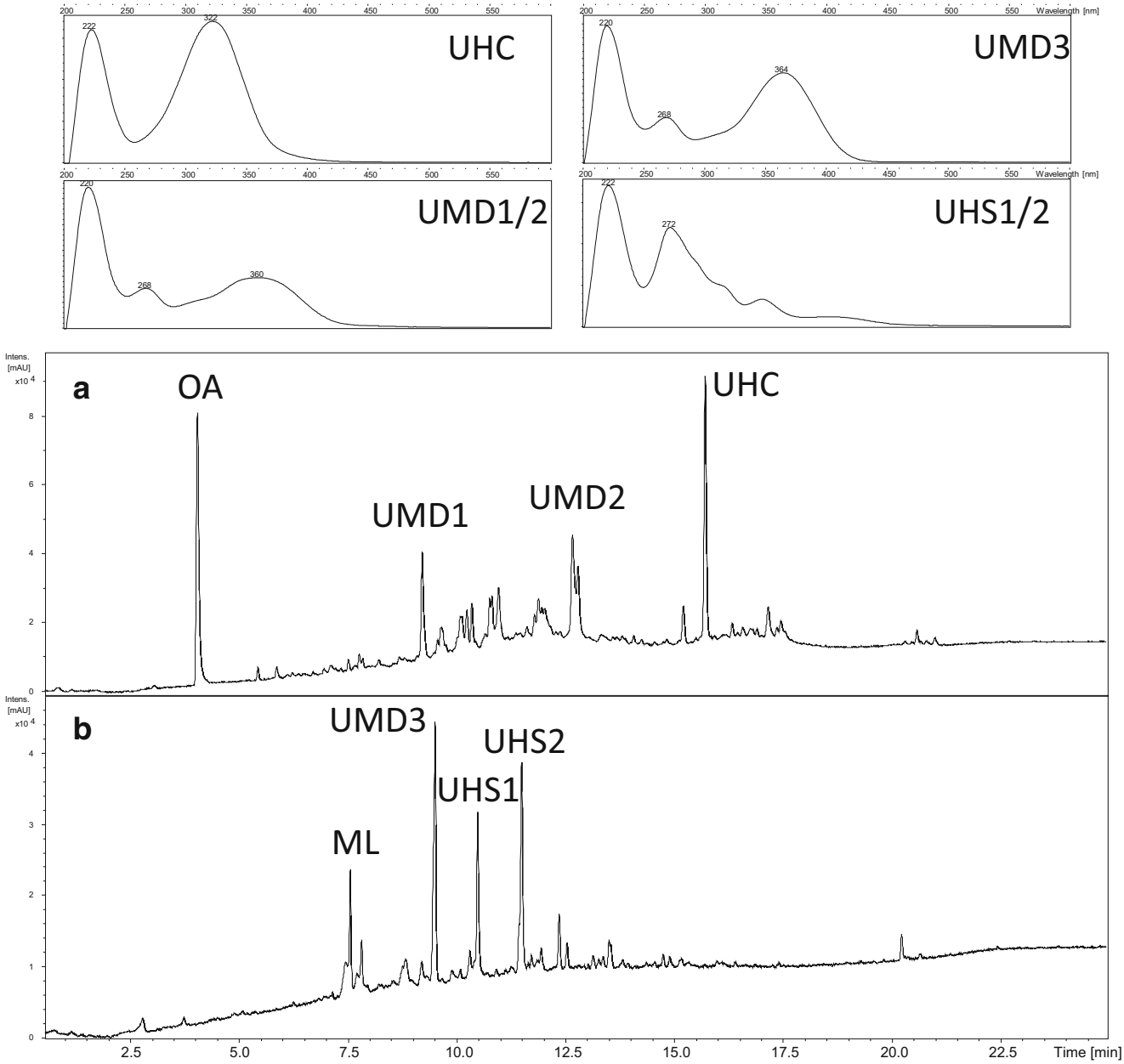


Fig. 4 Stromatal HPLC-UV profiles of *Hypoxylon calileguense* (holotype, **a**) and *H. subgilvum* (holotype, **b**) and corresponding DAD spectra of the unknown main metabolites. OA – orsellinic acid, ML – mitorubrinol, UHC – unknown compound of *H. calileguense*, UHS – unknown compound of *H. subgilvum*, UMD – unknown mitorubrin derivative

The stromata of the examined collections contained the characteristic chemotaxonomic markers carneic acid A and B (Quang et al. 2006, Fig. 6).

Hypoxylon chionostomum Speg., Boln Acad. nac. Cienc. Córdoba 11(4): 506 (1889) Fig. 5w–z

For sexual morph description, see Ju and Rogers (1996).

Asexual state Conidiogenous structure periconiella-like, around or above of the young stromata. Conidiophores brown to pale brown, roughened. Conidiogenous cell pale brown to hyaline, roughened, 9–19 × 3–4 μm. Conidia ellipsoid, pale brown to hyaline, smooth to finely roughened, 4–6 × 3–4 μm.

Culture Colonies on OA covering Petri dish in 4 weeks, at first whitish, becoming sepia (63), velvety to felty, irregularly zoned, with entire margin, reverse Umber (9). No sporulation observed.

Specimens examined *Argentina*: Salta, Santa Victoria, El Nogalar de los Toldos National Reserve, La Usina, on

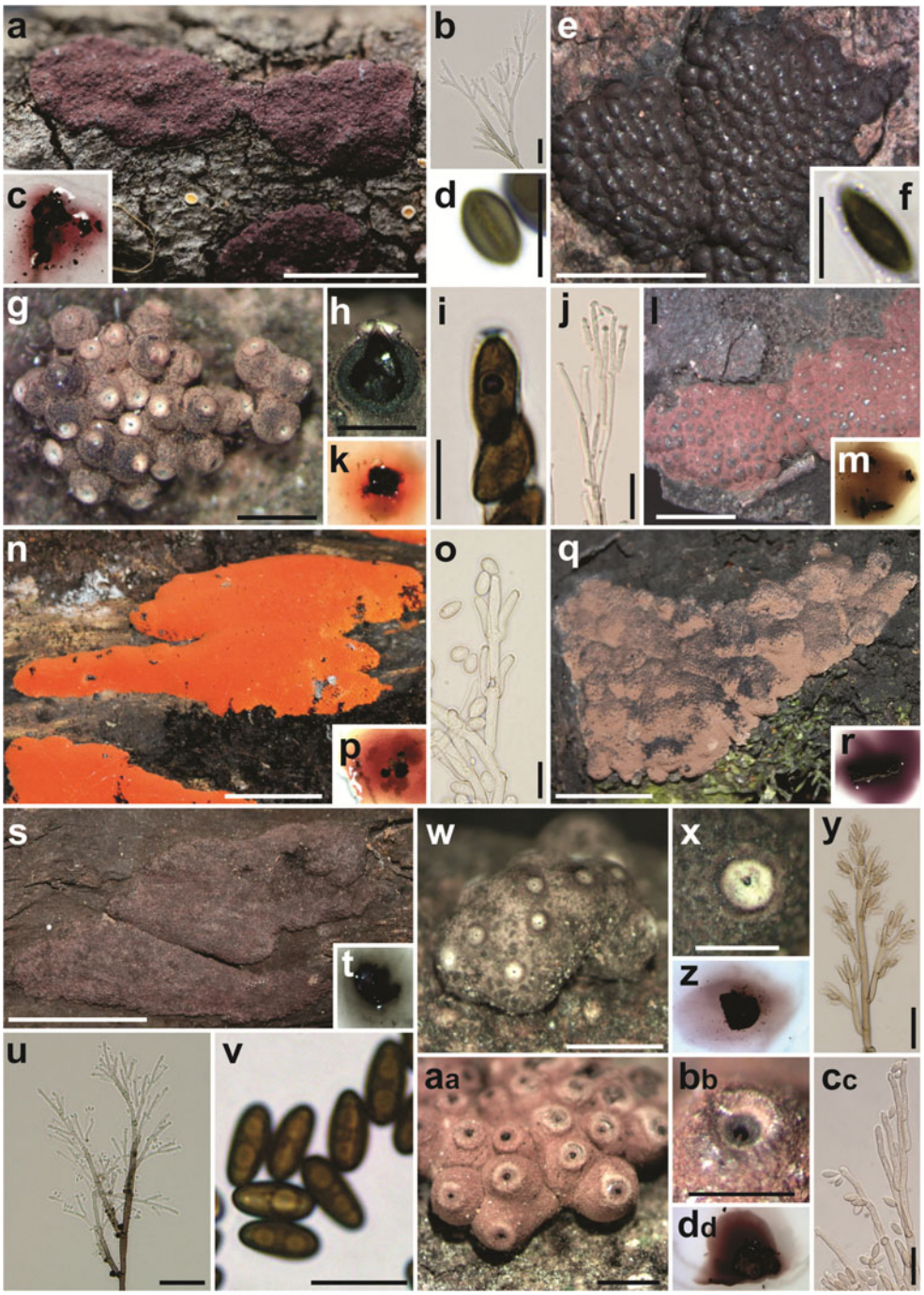


Fig. 5 New reports of *Hypoxylon* species from Argentina. **a-d**: *H. carneum*. **a**: stromata habit. **b**: nodulisporium-like asexual morph from culture. **c**: KOH-extractable pigments from young stromata. **d**: ascospore showing germ slit with a dotted band. **e-f**: *H. monticulosum*. **e**: stromatal habit. **f**: ascosprore showing sigmoid germ slit. **g-k**: *H. flavoargillaceum*. **g**: glomerate stromata on substrate. **h**: stromata in vertical section showing perithecia and subperithecial tissue disc. **i**: amyloid apical apparatus. **j**. virgariella-like asexual morph from culture. **k**: KOH-extractable pigments. **l-m**: *H. griseobruneum*. **l**: stromata. **m**: KOH-extractable pigments. **n-p**: *H. haematostroma*. **n**: stromata on wood. **o**: periconiella-like asexual morph from culture. **p**: KOH-extractable pigments. **q-r**: *H. lividipigmentum*. **q**: stromata on bark. **r**: KOH-extractable pigments. **s-v**: *H. investiens*. **s**: stromata on wood. **t**: KOH-extractable pigments. **u**: periconiella-like asexual morph from culture. **v**: ascospores. **w-z**: *H. chionostomum*. **w**: stromata habit, **x**: details of pale area of ostiole. **y**: periconiella-like asexual morph from natural substrate. **z**: KOH-extractable pigments **aa-cc**: *H. umbilicatum*. **aa**: stromata showing conspicuous perithecial mounds. **bb**: detailed view of sunken ostioles. **cc**: virgariella-like asexual morph from culture. **dd**: KOH-extractable pigments. Scale is indicated by bars (**a**, **e**, **l**, **n**, **q**, **s** – 1 cm. **b**, **d**, **f**, **j**, **o**, **v**, **cc** – 10 μm. **h**, **x**, **bb** - 0.5 mm. **u**, **y** – 20 μm)

bark of a dead branch, 29 Dec. 2011, Sir & Hladki 090, 095, 292 (LIL). *Brazil*: Aphiahy, on decayed bark, leg. Puiggari J. n° 2858, 6–1888 (Holotype of *H. chionostomum*, LPS 1679).

Known distribution Argentina and Brazil.

Notes *H. chionostomum* was hitherto only known from a Brazilian specimen, described by Spegazzini in the 19th century. This fungus is part of a group of *Hypoxylon* with large ascospores that seems to be endemic to the Neotropics. It can be differentiated from *H. megalosporum* by its less subperithecial ostiolar disc, dull reddish brown stromatal granules, diluted vinaceous grey KOH-extractable pigments and smaller ascospores. It differs from *H. umbilicatum* by having spherical perithecia with ostioles not surrounded by a white area, smaller ascospores and periconiella-like conidiogenous structures.

The stromata of *H. chionostomum* contain mainly BNT, and some minor constituents that are probably related to the daldinin class (Fig. 6). BNT is widespread within the informal subfamily Hypoxyloideae and is often responsible for the purple colour of the KOH-extractable pigments (Stadler and Fournier 2006).

Hypoxylon flavoargillaceum J.H. Mill., in Chardón & Toro, Monograph Univ. Puerto Rico, Series B 2: 200 (1934) Fig. 5g–k

For sexual morph descriptions, see Ju and Rogers (1996).

Asexual state Conidiogenous structure virgariella-like. Conidiophores pale brown to hyaline, finely roughened to smooth. Conidiogenous cells hyaline to pale brown, smooth to finely roughened, 11–27 × 2–3 μm. Conidia ellipsoid, hyaline, smooth, 5–6.5 (−7) × 2–3 μm.

Culture Colonies on OA covering Petri dish in 3 weeks, at first whitish, becoming Peach (4), velvety to felty, zonate, with entire margin, reverse Brick (59) and Dark Vinaceous (82). Sporulating in Salmon (41) areas.

Specimens examined *Argentina*: Jujuy, Santa Barbara, Las Lancitas Provincial Reserve, on bark of a dead branch of *Celtis tala* (Cannabaceae), associated with *H. umbilicatum*, 27 Apr. 2014, Sir & Hladki 679, 687 (LIL). Salta, Santa Victoria, El Nogalar de los Toldos National Reserve, La Usina, on bark of a dead branch, 27 Dec. 2011, Sir & Hladki 110 (LIL); 27 Dec. 2014 Sir & Hladki 102 (LIL). Anta, El Rey National Park, on bark of a dead branch of *Celtis tala*, 14 May 2012, Sir & Hladki 208 (LIL); Lagunas de los Patitos, on bark of a dead branch of *Celtis tala*, 14 May 2014, Sir & Hladki 215 (LIL); 29 Apr. 2014, Sir & Hladki 694, 700, 705 (LIL). La Candelaria, on bark of a dead branch, 03 May 2015, Sir Hladki & Robledo 401 (LIL); General José de San Martin, Acambuco Provincial Reserve, on bark of a dead branch of *Celtis tala* , 22 Apr. 2014, Sir & Hladki 503 (LIL).

Known distribution Argentina, Colombia and Venezuela.

Notes Originally, *H. flavoargillaceum* was collected in Colombia and Venezuela. This species has stromata similar to *H. notatum* Berk. & M.A. Curtis, but these two species differ in the latter having an ascal apical apparatus highly reduced or even lacking and not bluing in Melzer's reagent. The specimens from Argentina were found on branches of *Celtis tala* (Cannabaceae), coexisting with *H. umbilicatum* and infrequently with *H. megalosporum*. The asexual state is reported for the first time. The Argentine material is characterized by the presence of an unknown main compound (M = 370) besides BNT (Fig. 6).

Hypoxylon griseobrunneum (B.S. Mehrotra) J. Fourn., Kuhnert & M. Stadler, Fungal Diversity 64: 194 (2013) Fig. 5l–m

For sexual and asexual morph descriptions, see Kuhnert et al. (2014a) and Fournier et al. (2015).

Specimens examined *Argentina*: Jujuy, Dept. Ledesma, Calilegua National Park, "La Lagunita" trail, on wood and bark of a dead branch, 11 May 2012, Sir & Hladki 012 (LIL). Santa Barbara, on bark of a dead branch, "Las Lancitas" Provincial Reserve, 13 May 2012, Sir & Hladki 268 (LIL).

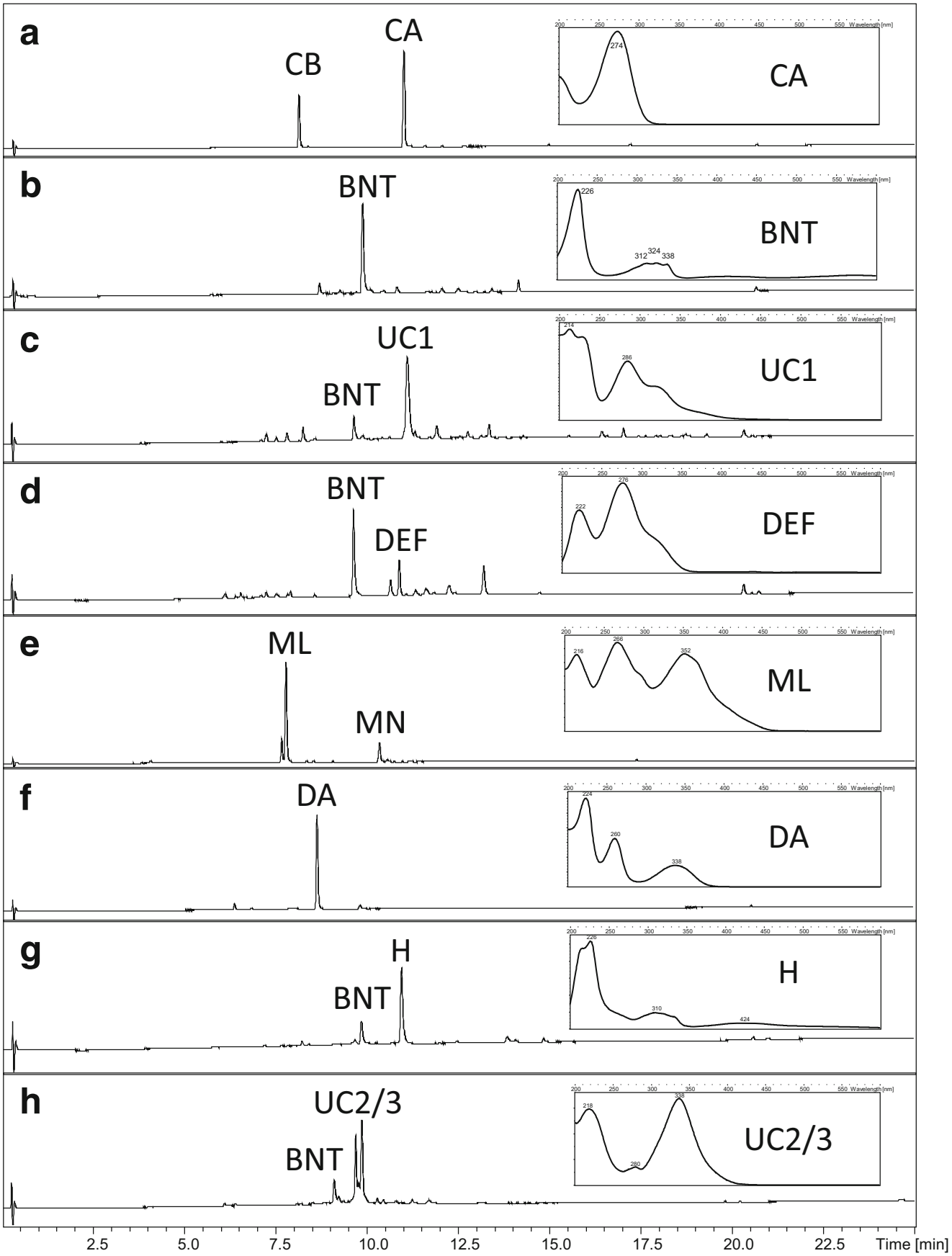
Known distribution Central and South America including the Caribbean, and India (holotype location).

Notes *Hypoxylon griseobruneum* is characterized by a brown vinaceous stromatal surface, dull olivaceous yellow granules and KOH-extractable pigments fawn with vinaceous tinges (Kuhnert et al. 2014a). Various stromata of the specimen Sir & Hladki 268 exhibit multiple layers of stromata, a feature that was already reported by Kuhnert et al. (2014a). The latter also assigned a virgariella-like conidiogenous structure in culture for *H. griseobrunneum*. However, Fournier et al. (2015) found nodulisporium-like conidiogenous structures on the natural substrate, which was confirmed by the asexual state developed in cultures of the Argentine strains. Secondary metabolite profiles of the stromata are in accordance with previous reports (Kuhnert et al. 2014a) with BNT and daldinins E/F as prevailing constituents (Fig. 6).

Hypoxylon haematostroma Mont., in Sagra, Historia física, politica y naturál de la isla de Cuba 9: 344 (1845) Fig. 5n–p

For sexual and asexual morph descriptions, see Ju and Rogers (1996).

Specimens examined *Argentina*: Jujuy, Libertador General San Martin, Calilegua National Park, on wood of a dead branch, 11 May 2012, Sir & Hladki 005, 007 (LIL); on bark, 25 May 2015, Sir & Hladki 857 (LIL); "La Lagunita" trail, on wood of a dead branch, 26 Apr. 2014, Sir & Hladki 640 (LIL); "La Junta" trail, on wood of a dead branch, 27 Apr. 2014, Sir & Hladki 669 (LIL); Santa Barbara, Las Lancitas Provincial Reserve, on bark of a dead branch, 27 Apr. 2014, Sir & Hladki 690 (LIL). Salta, General José de San Martin, Acambuco Provincial Reserve, on dead wood and bark, 22 Apr. 2014, Sir & Hladki 515, 530 (LIL); on wood and bark of a dead


a
CB
CA
CA
274
b
BNT
BNT
226
312
324
338
c
BNT
UC1
UC1
214
286
d
BNT
DEF
DEF
222
276
e
ML
MN
ML
216
266
352
f
DA
DA
224
260
338
g
BNT
H
H
226
310
424
h
BNT
UC2/3
UC2/3
218
280
338
200 250 300 350 400 450 500 550 Wavelength [nm]
2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 Time [min]

◀ **Fig. 6** Stromatal HPLC-UV profiles of some new *Hypoxylon* records from Argentina and the DAD spectra of the characteristic main compounds (CA – carneic acid A, CB – carneic acid B, BNT – binaphthalene tetrol, UC1-3 – unknown compounds, DEF – daldinin E/F, ML – mitorubrinol, MN – mitorubrin, DA – daldinone A, H – hypoxylone). **a** – *H. carneum* (Sir & Hladki 152), **b** – *H. chionostomum* (Sir & Hladki 095), **c** – *H. flavoargillaceum* (Sir & Hladki 208), **d** – *H. griseobrunneum* (Sir & Hladki 268), **e** – *H. haematostroma* (Sir & Hladki 001), **f** – *H. investiens* (Sir & Hladki 004), **g** – *H. lividipigmentum* (Sir & Hladki 014), **h** – *H. umbilicatum* (Sir & Hladki 561)

branch, 23 Apr. 2014, Sir & Hladki 548, 552, 567 (LIL); Orán, road to Islas de Cañas, on bark of a dead branch, 24 Apr. 2014, Sir & Hladki 606 (LIL); Anta, El Rey National Park, Laguna de los Patitos trail, on wood of a dead branch, 14 May 2012, Sir & Hladki 001 (LIL); "El Chorro de los Loros" trail, on wood of a dead branch, 15 May 2012; Sir & Hladki 234 (LIL); on wood and bark of a dead branch, 29 Apr. 2014, Sir & Hladki 732 (LIL); on wood and bark of a dead branch, 28 May 2015, Sir & Hladki 864, 882 (LIL). Tucumán, Yerba Buena, Horco Molle, Sierras de San Javier Park, on dead wood, 21 May 2013, Sir & Hladki 434 (LIL); on dead wood and bark, 15 May 2014, Sir & Hladki 763 (LIL),. *Costa Rica*: Siggers, P.V., on wood (BPI 10733). *Cuba*: Ramón de la Sagra, on wood, (BPI 55132; Isotype of *H. haematostroma*). *Honduras*: Tela, 4 Sep. 1923, Reinking, O. A., on wood (BPI 11327, 55129). *Philippines*: Luzon, Mt. Maquiling, 21 Sep. 1920, Rocafort, A., on wood (BPI 55125); Luzon, Mt. Maquiling, 4 Nov. 1920, Rocafort, A., on wood (BPI 55127). *Tahiti*: June 1922, Parks, H. E., on wood (10747).

Known distribution Pantropical.

Notes *Hypoxylon haemastostroma* is widely distributed in the subtropical and tropical regions but seems to be particularly frequent in the Caribbean (Kuhnert et al. 2014a; Fournier et al. 2015; Cruz and Cortez 2015). The Argentine collections are in accordance with the concept of *H. haematostroma* defined by Ju and Rogers (1996), except for having slightly larger ascospores (15.1–23.5 × 6.0–8.9 μm vs 13.5–19 × 7–8.5 μm). The variation of the ascospore size range was also reported by Fournier et al. (2015). The stromatal HPLC profiles of the Argentine samples are characterized by the presence of mitorubrin and mitorubrinol as main metabolites (Fig. 6) which is in agreement with the data of Hellwig et al. (2005).

Hypoxylon investiens (Schwein.) M.A. Curtis, Goel. Nat. Hist. Surv. N. Carol., Pt 3: 140 (1867) Fig. 5s–v

For sexual and asexual morph, descriptions see Ju and Rogers (1996).

Specimens examined *Argentina*: Jujuy, Dept. Ledesma, Calilegua National Park, La Lagunita trail, on wood, 11 May 2012, Sir & Hladki 026 (LIL). Salta, Anta, El Rey National Park, on wood, 14 May 2012, Sir & Hladki 004 (LIL).

Known distribution Pantropical.

Notes This species can be recognized by its brown vinaceous or dark vinaceous stromatal surface, nearly equilateral ascospores, indehiscent perispore and dark green KOH-extractable pigments. The typical variety differs from *H. investiens* var. *magnisporum* by its smaller ascospores. The studied collections contain daldinone A as the prevailing stromatal constituent, which agrees with previous reports (Hellwig et al. 2005; Stadler and Fournier 2006, Fig. 6).

Hypoxylon lividipigmentum F. San Martín, Y.M. Ju & J.D. Rogers, in Ju & Rogers, Mycol. Mem. 20: 145 (1996) Fig. 5q–r

For sexual and asexual morph descriptions, see Ju and Rogers 1996.

Specimens examined *Argentina*: Jujuy, Dept. Ledesma, Calilegua National Park, La Lagunita trail, on a dead corticated branch, 11 May 2012, Sir & Hladki 006 (LIL); on a dead corticated branch, 26 Apr. 2014, Sir & Hladki 622 (LIL); Sendero del Cielo, on a dead corticated branch, 12 May 2012, Sir & Hladki 014 (LIL); La Junta trail, 27 Apr. 2014, Sir & Hladki 653, 655 (LIL). Salta, Anta, Parque Nacional El Rey, road to Chorro de los Loros, 14 May 2012, Sir & Hladki 203 (LIL); on a dead corticated branch, 27 Apr. 2014, Sir & Hladki 711, 715 (LIL); General José de San Martin, Acambuco Provincial Reserve, on wood and bark, 23 Apr. 2014, Sir & Hladki 543, 544, 556, 560, 563 (LIL). Tucumán, Trancas, La Higuera, Cerro Alto de la Totora, crossing the river La Higuera, 23 Mar. 99, Hladki 2672, 2684, 2685, 2697, 2769 (LIL). *Mexico*: Quintana Roo state, OtMn P. Blanco municipality, Ejido La Unión, 8 Dez. 86, San Martin 96, on wood (WSP 69627, Isotype of *H. lividipigmentum*).

Known distribution Argentina, Mayotte, Mexico, Panama, Venezuela.

Notes So far, this fungus was reported from Mexico, Venezuela (Ju and Rogers 1996), Panama (Carmona et al. 2009) and Mayotte island (Fournier et al. 2014), restricting its distribution to the tropics. However, the Argentine collections are the first records from a subtropical region, showing that the species is widespread in the warmer climates of America. All studied collections of *H. lividipigmentum* contain mainly hypoxylone and BNT in their stromatal extracts (Fig. 6). Hypoxylone was orignally isolated from stromata of *H. sclerophaeum* (Bodo et al. 1983) and was also reported from *H. laminosum, H. polyporus and H. nicaraguense*

(Kuhnert et al. 2014a). The latter species belong to the so called "*H. polyporus* complex" (comprising *H. polyporus*, *H. nicaraguense*, and *H. tortisporum*) and its sequences form a separated clade within both phylogenetic reconstructions. Interestingly, *H. lividipigmentum* forms a basal lineage to this complex in the TUB tree, which might explain the distribution pattern of this compound.

Hypoxylon monticulosum Mont., Syll. gen. sp. crypt. (Paris): 214 (1856) Fig. 5e, f
For sexual and asexual morph descriptions, see Ju and Rogers (1996).

Specimens examined *Argentina*: Jujuy, Libertador General San Martin, Calilegua National Park, on a dead corticated branch, 26 Apr. 2014, Sir & Hladki 620 (LIL); on a dead corticated branch, 24 May 2015, Sir & Hladki 903 (LIL); La Junta trail, on bark, 27 Apr. 2014, Sir & Hladki 667, 674 (LIL). Santa Barbara, Las Lancitas Provincial reserve, 13 May 2012, Sir & Hladki 261, 265, 279 (LIL, BAFC); 27 Apr. 2014, Sir & Hladki 678 (LIL). Salta, Anta, El Rey National Park, 14 May 2012, Sir & Hladki 242 (LIL). La Candelaria, El Jardín, on a dead corticated branch, 03 May 2013, Sir, Hladki & Robledo 400 (LIL); on a dead corticated branch, 28 May 2015, Sir & Hladki 886 (LIL). General Güemes, on a dead corticated branch, 02 May 2013, Sir, Hladki & Robledo 368, 379 (LIL, BAFC). General José de San Martin, Acambuco Provincial Reserve, on wood and bark, 21 May 2015, Sir & Hladki 837, 840, 843 (LIL). Santa Victoria, Baritú National Park, on a dead corticated branch, 27 Apr. 2013, Sir & Hladki 486 (LIL, BAFC); Orán, road to Islas de Cañas, on a dead corticated branch, 24 May 2014, Sir & Hladki 586 (LIL); on a dead corticated branch, 23 May 2015, Sir & Hladki 839 (LIL). *Paraguay*: Guarapi, Balanza, B. 3424, on dead branches, Sep. 1883, Balanza n°3996 (holotype of *H. anthracoderma*, LPS 1677).

Known distribution Pantropical.

Notes As mentioned in Ju and Rogers (1996), *H. monticulosum* is commonly found in the tropics and subtropics. This species together with *H. haematostroma*, *H. lividipigmentum* and *H. lenormandii* were the most common taxa within the genus *Hypoxylon* along "La Yunga". As previously reported *H. monticulosum* is usually absent of extractable stromatal metabolites (Hellwig et al. 2005). In this respect, the Argentine collections are typical. The respective cultures produced sporothriolide, which is a chemotaxonomic marker of the species (Surup et al. 2014).

Hypoxylon umbilicatum Speg., Boln Acad. nac. Cienc. Córdoba 11(4): 507 (1889) Fig. 5a-d

For sexual morph descriptions, see Ju and Rogers (1996).

Asexual state Conidiogenous structure virgariella-like. Conidiophores pale reddish-brown, finely roughened. Conidiogenous cells hyaline, smooth to finely roughened, 12–27 × 2.5–3 μm. Conidia ellipsoid, reddish brown, smooth to finely roughened, 5.5–8 × 3–3.5 μm.

Culture Colonies on YMG covering Petri dish in 4 weeks, at first whitish, becoming Fawn (87), velvety to felty, zonate, with entire margin, reverse Umber (9). Sporulating regions Salmon (41) to Brick (59).

Specimens examined *Argentina*: Salta, General José de San Martin, Acambuco Provincial Reserve, on bark of a dead branch of *Celtis tala* (Cannabaceae), 23 Apr. 2014, Sir & Hladki 554, 561 (LIL). Anta, El Rey National Park, road to Chorro de los Loros, on bark of a dead branch, 29 Apr. 2014, Sir & Hladki 704, 773 (LIL). Tucumán. Chicligasta, Los Alisos National Park, on bark of a dead branch of *Celtis tala* (Cannabaceae), 18 May 2015, Sir & Hladki 919, 943 (LIL). Yerba Buena, Horco Molle, Sierras de San Javier Park, on bark of a dead branch of *Celtis tala*, 21 May 2014, Sir & Hladki 439 (LIL). Trancas, on bark of a dead branch of *Celtis tala*, 15 Jun. 2015, Medina & Hladki s/n (LIL). *Brazil*: São Pablo, Apiahy, on logs, May 1888, Puiggari n° 2858 (Holotype of *H. umbilicatum*, LPS 1952).

Known distribution Argentina and Brazil.

Notes The combinations of glomerate stromata (sometimes also effused pulvinate) with conspicuous perithecial mounds, brick or dark brick surface colour, obovoid perithecia and very conspicuous sunken ostioles are useful morphological characters to separate *H. umbilicatum* from other large-spored species (ascospores > 25 μm, see also notes on *H. chionostomum*). In addition, *Hypoxylon umbilicatum* has a rather unique stromatal HPLC profile with two unknown large molecules (M = 638) and BNT as major metabolites (Fig. 6). The asexual morph is reported for the first time.

Dichotomous key to Hypoxylon species from Argentina

1 a. Ascospores averaging more than 25 μm in length **2**
1 b. Ascospores averaging less than 25 μm in length **4**

2 a. Perithecia obovoid with ostioles sunken, without a pale area, ascospores (29.9–)34.6–42.9(−48.2) × (16.0–)19.9–27.2(−29.0) μm ***Hypoxylon umbilicatum***
2 b. Perithecia spherical or subglobose, ostioles not sunken, but with a pale area **3**

3 a. With conspicuous subperithecial ostiolar disc, stromatal granules dark brown, KOH-extractable pigments isabelline, ascospores 28.0–35.5(−41.5) × (17.0–)18.0–21.0(−22.0) μm ***Hypoxylon megalosporum***

3 b. Without subperithecial ostiole disc, stromatal granules dull reddish brown, KOH extractable pigments dilute vinaceous grey, ascospores (27.5–)28.0–32.0(−33.0) × (15.5–)17.0–20.0(−21.0) μm ***Hypoxylon chionostomum***

4 a. KOH extractable pigments purplish or without apparent pigments **5**

4 b. KOH-extractable pigments with other than above colours **9**

5 a. Stromata peltate, ascospores light brown to brown, germ slit much less than spore length, perispore not dehiscent in 10 % KOH ***Hypoxylon polyporus***

5 b. Stromata effused-pulvinate, ascospores brown to dark brown, germ slit spore length, perispore dehiscent in 10 % KOH **6**

6 a. Mature stromata carbonaceous, without KOH-extractable pigments; ostiole papilate, ascospores (8.7–)9.9–12.0(−12.5) × (3.6–)4.4–5.4(−5.7) μm, with sigmoid germ slit spore-length ***Hypoxylon monticulosum***

6 b. Mature stromata waxy to woody, not carbonaceous, ostile umbilicate, ascospores with straight or slightly sigmoid germ slit **7**

7 a. Stromatal surface chestnut or sepia, KOH extractable pigments dark livid, ascospores (7.9–)11.0–13.5(−14.5) × (5.0–)5.2–6.0(−7.0) μm ***Hypoxylon lividipigmentum***

7 b. Stromatal surface purplish gray or vinaceous gray, KOH-extractable pigments livid purple or absent, ascospores less than 11 μm long **8**

8 a. Ascospores with straight germ slit at the center of a dotted band, perispore dehiscent, smooth; conidiogenous structure nodulisporium-like ***Hypoxylon carneum***

8 b. 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, conidiogenous structure virgariella-like ***Hypoxylon lilloi***[2]

9 a. Ascospores nearly equilateral to slightly inequilateral, with germ slit on flattened side, perispore indehiscent in 10 % KOH **10**

9 b. Ascospores conspicuously inequilateral, with germ slit in convex side perispore dehiscent in 10 % KOH **12**

10 a. Stromata pulvinate with inconspicuous to conspicuous perithecial mounds, surface sulfur yellow, KOH-extractable pigments red livid or vinaceous livid, ascospores 11–12 × 5–6 μm ***Hypoxylon kermesi***

10 b. Stromata effused-pulvinate, plane or with incospicuous perithecial mounds, surface dark vinaceous to brown vinaceous, KOH-extractable pigments dull green or dark green **11**

11 a. Ascospores 9–11 × 4–5 μm ***Hypoxylon investiens* var. *magnisporum***

11 b. Ascospores (7.0–)7.5–10.0 × (3.0–)3.3–4.2(−4.5) μm ***Hypoxylon investiens* var. *investiens***

12 a. Stromata hemispherical, pulvinate, effused, effused-pulvinate; plane or with inconspicuous to conspicuous perithecial mounds **13**

12 b. Stromata glomerate to effused-pulvinate with conspicuous to very conspicuous perithecial mounds, sometimes with the tendency to be perithecioid (approaching rosellinioid) **23**

13 a. Stromatal granules orange, ochraceous, orange-red, amber, yellowish brown or brown; KOH-extractable pigments orange, scarlet or rust **14**

13 b. Stromatal granules dull reddish brown, dark brown, blackish or black granules; KOH extractable pigments olivaceous, gray olivaceous, greenish olivaceous, dull green, dark green, amber, isabelline or fawn **20**

14 a. Stromatal thickness up to 6 mm, ascospores usually greater than 15 μm long **15**

14 b. Stromatal thickness up to 2 mm, ascospores usually less than 15 μm long **16**

15 a. Perithecia long tubular, more than 1.5 mm high, ascospores (15.1–)15.9–18.6(−23.5) × (6.0–)7.1–7.9(−8.9) μm, with smooth perispore, straight germ slit; conidiogenous structure periconiella-like ***Hypoxylon haematostroma***

15 b. Perithecia spherical, ovoid, obovoid or tubular, less than 1.5 mm high, ascospores 14.5–17 × 6.5–7 μm with faintly striated to striated perispore, straight or slightly sigmoid germ slit; conidiogenous structure virgariella-like ***Hypoxylon crocopeplum***

16 a. Ascospores up to 12 μm long and up to 6 μm width **17**

16 b. Ascospores up to 15 μm long and up to 8 μm width **19**

17 a. Stromatal granules orange red, perithecia obovoid to tubular 0.2–0.7 mm high × 0.1–0.3 mm diam, ascospores with straight to slightly sigmoid germ slit; faintly striated to striated perispore ***Hypoxylon subgilvum***

[2] The characteristics of this species are reported concurrently in by Li et al (2016, in press).

17 b. Stromatal granules yellowish brown or brown, perithecia spherical to obovoid, not tubular, up to 0.6 mm high and up to 0.5 mm diam, ascospores with straight germ slit, smooth perispore **18**

18 a. Ascospores (8–)9–12 × 4–5.5 μm
Hypoxylon rubiginosum* var. *rubiginosum

18 b. Ascospores 7.5–9 × 3.5–5 μm
Hypoxylon rubiginosum* var. *microspora

19 a. Ascospores (10.7–)11.6–14.0(−15.0) × (4.9–)5.6–7.0(−7.7) μm, sigmoid germ slit; conidiogenous structure virgariella-like, conidia 4.5–5 × 2–2.5 μm
Hypoxylon spegazzinianum

19 b. Ascospores (9.5–)10.5–12.4(−14.3) × (4.7–)5.1–6.2(−6.5) μm, straight germ slit; conidiogenous structure nodulisporium-like, conidia 5–8 × 3–4 μm
Hypoxylon calileguense

20 a. Ostioles umbilicate with white tissue surrounding the ostioles; conidiogenous structure virgariella-like
Hypoxylon perforatum

20 b. Ostioles umbilicate without white tissue surrounding the ostioles; conidiogenous structure nodulisporium-like **21**

21 a. Stromata pulvinate to effused-pulvinate (rare glomerate) with inconspicuous to conspicuous perithecial mounds, surface sepia, chestnut, perithecia spherical 0.2–0.5 mm diam
Hypoxylon subrutilum

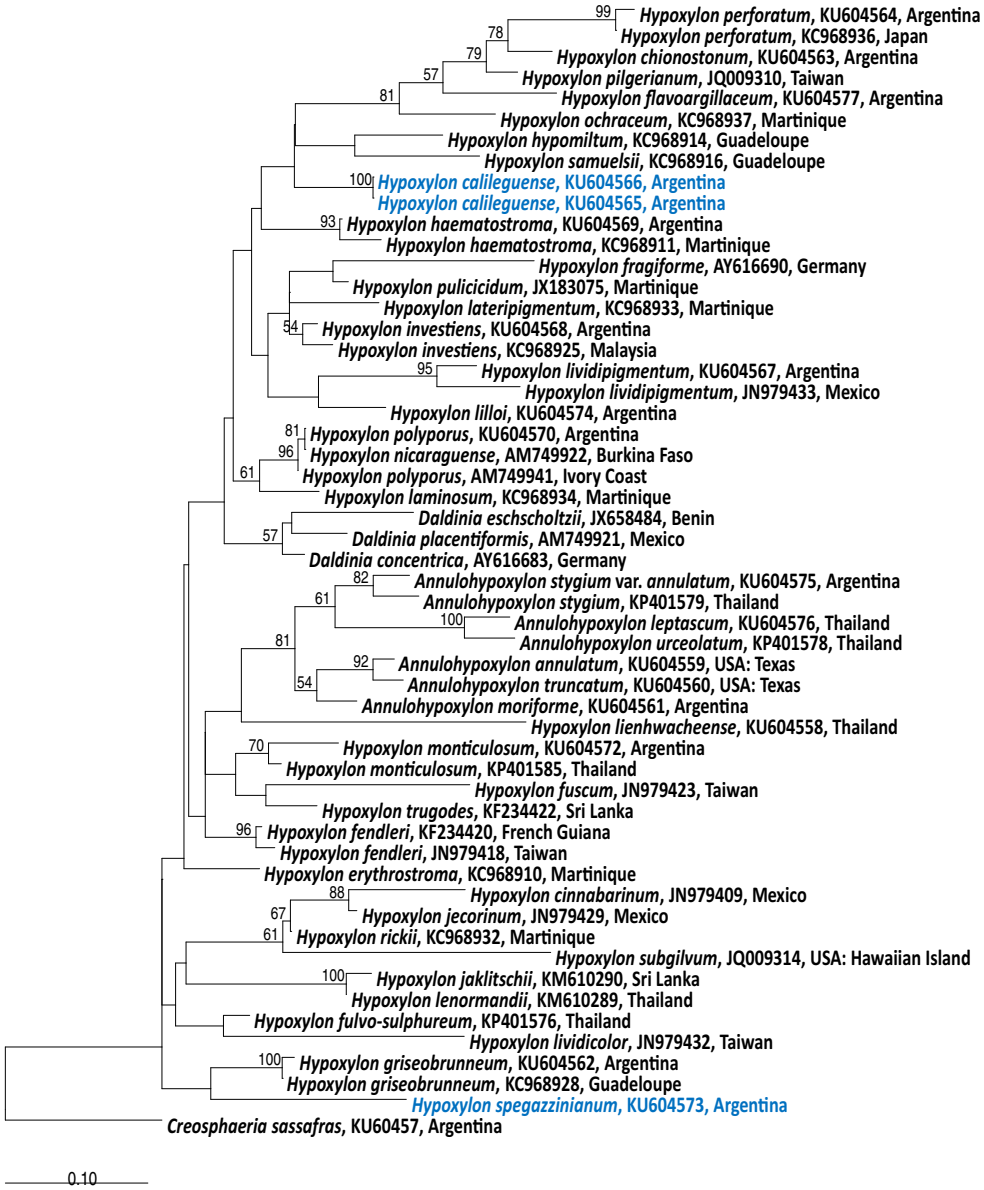


Fig. 7 Phylogenetic relationships among Argentine *Hypoxylon* species and related Xylariaceae as inferred from internal transcribed spacer (ITS) rRNA gene sequences. Maximum Likelihood bootstrap support values above 50 %, from 1000 RAxML replicates are assigned to the tree topology of the most likely tree found by RAxML. Species names are followed by the GenBank accession number and countries of origin. Sequences of the newly described species are highlighted in blue

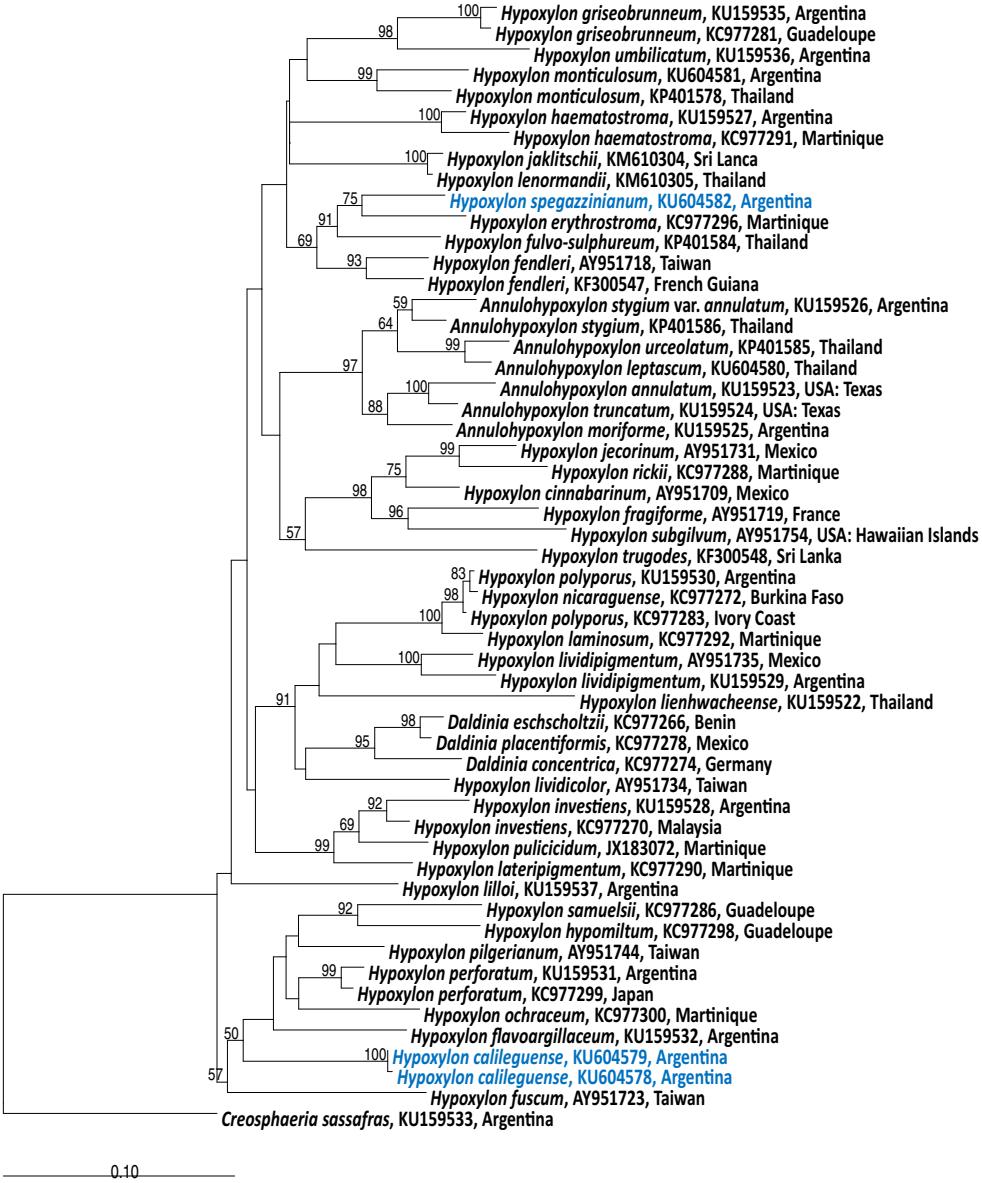


Fig. 8 Phylogenetic relationships among Argentine *Hypoxylon* species and related Xylariaceae as inferred from β-tubulin gene sequences. Maximum Likelihood bootstrap support values above 50 %, from 1000 RAxML replicates are assigned to the tree topology of the most likely tree found by RAxML. Species names are followed by the GenBank accession number and countries of origin. Sequences of the newly described species are highlighted in blue

21 b. Stromata effused-pulvinate, plane or with inconspicuous perithecial mounds, surface brown vinaceous or dark vinaceuos, perithecia obovoid to tubular 0.2–0.3 mm diam **22**

22 a. Stromatal granules dull reddish brown or blackish; ascospores 10–12.5 × 4.2–5.6 µm with straight to slightly sigmoid germ slit spore-length

Hypoxylon anthochroum

22 b. Stromatal granules dull olivaceous yellow; ascospores 9.7–13.0 × 4.0–5.7 µm, with straight germ slit spore-length ***Hypoxylon griseobrunneum***

23 a. Ostioles slightly higher than the stromatal surface or with small papilla, ascospores 11–17 × 4–6 with sigmoid germ slit; conidiogenous structure nodulisporium-like ***Hypoxylon lenormandii***

23 b. Ostioles umbilicate most often opening at the centre of a raised disc, ascospores with straight to slightly sigmoid germ slit, conidiogenous structure virgariella-like **24**

24 a. Apical apparatus bluing in Melzer's iodine reagent, discoid, ascospores 13.9-21 × 6.8-10.2 µm, conidia 5–6.5(−7) × 2–3 µm ***Hypoxylon flavoargillaceum***

24 b. Apical apparatus highly reduced or lacking, not bluing in Melzer's iodine reagent, ascospores 14.5–15.5 × 6.5–8 µm, conidia 4.5–5.5 × 3–3.5 µm ***Hypoxylon notatum***

Molecular phylogeny

The phylogenetic reconstructions were composed of each 54 ITS (Fig. 7) and TUB sequences (Fig. 8), respectively, derived

from various representatives of the Xylariaceae, including the two newly described species. The majority of taxa belongs to the genus *Hypoxylon* (43 sequences of each marker gene), followed by *Annulohypoxylon* (seven sequences of each gene), *Daldinia* (three sequences of each gene) and *Creosphaeria* (one sequence of each gene). In both trees, the backbone was statistically not supported (less than 20 % BS support). The genera *Annulohypoxylon* and *Daldinia* appear monophyletic in both reconstructions with moderate to high BS support (57 % – 97 %). Whereas the TUB results are in accordance with previous reports, the monophyly of *Annulohypoxylon* based on ITS sequences disagrees with those where *Annulohypoxylon* usually shows a paraphyletic behaviour (Kuhnert et al. 2014a, b; Sir et al. 2015). However, herein the selection of representatives is limited and therefore the monophyly can be seen as coincidence. In the ITS tree, *H. spegazzinianum* clusters with *H. griseobrunneum* without support, and in the TUB tree, it forms a clade with *H. erythrostroma* and *H. fulvo-sulphureum* (91 % BS support). There is no obvious morphological and chemotaxonomic relationship between the new species and *H. griseo brunneum*. However, the proximity to *H. erythrostroma* is in accordance with chemical and morphological data (see taxonomic part). Sequences of *H. calileguense* build up a separated clade within the ITS tree nested between *H. haematostroma* and *H. hypomiltum*. Similar to *H. spegazzinianum*, this positioning disagrees with morphological and chemical data. In the TUB calculation, *H. calileguense* also forms a separated branch close to *H. fuscum* and *H. flavoargillaceum* as part of a supported major clade including sequences of *H. perforatum*, *H. hypomiltum* and *H. ochraceum* (87 % BS support). The relationship of the new species with *H. fuscum* and *H. flavoargillaceum* is not reasonable from a morphological and chemotaxonomic point of view. However, the other phylogenetically related species produce mitorubrin type secondary metabolites in their stromata such as hypomiltin, which is the major stromatal pigment of *H. hypomiltum* and *H. perforatum* (Hellwig et al. 2005). In this respect, it can be concluded that phylogenies based on β-tubulin reflect the morphological and chemical evolution much better within the Xylariaceae than the still widely employed ITS region of the ribosomal DNA.

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