



Three new species of *Hypoxylon* and new records of Xylariales from Panama

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Abstract

Three new species of *Hypoxylon* (Hypoxylaceae, Xylariales) are described based on a polyphasic approach that included morphological examination, molecular phylogeny and chemotaxonomic studies of specimens collected in Panama. Specifically, we compared the sexual morph (stromata, asci, and ascospores) of the specimens collected with known species of *Hypoxylon* and inferred a phylogeny of this genus based on a Randomized Axelerated Maximum Likelihood (RAxML) analysis of partial β -tubulin (TUB2) DNA sequences and the internal transcribed spacer regions (ITS). *Hypoxylon baruense* sp. nov. is phylogenetically related to *H. subgilvum* but differs in the shape of stromata, and by having larger ascospores and a smooth perispore. *Hypoxylon bellicolor* sp. nov. is phylogenetically well differentiated, featuring effused–pulvinate stromata with orange granules and ostioles lower than the stromatal surface. *Hypoxylon sporistriataticum* sp. nov., is phylogenetically related to *H. shearii* var. *minor* but differs in the shape of the stromata, in having ostioles higher than the stromatal surface, and in having larger ascospores. The secondary metabolite profiles of these species were studied by HPLC–DAD–MS and these correspond to the results of the morphological and phylogenetic studies. Additionally, we also report *Amphirosellinia evansii*, *H. howeanum*, *H. cinnabarinum* and *Stilbohypoxylon quisquiliarum* from Panama for the first time and *Citrus sinensis* as a new host of *H. cinnabarinum*. With this work, the number of species of Xylariales and *Hypoxylon* reported from Panama have increased to 103 and 18, respectively.

Key words – Biodiversity – Fungi – Sordariomycetes – Hypoxylaceae

Introduction

The order Xylariales comprises 26 families that include approx. 2,500 species in 210 genera (Hongsanan et al. 2017). Species in this order are common worldwide, though the highest diversity of Xylariales is found in the tropics (see Dayarathne et al. 2017, Li et al. 2017, Sir et al. 2016b for

recent records of new tropical taxa). Of the 26 families of Xylariales (Helaly et al. 2018), the Xylariaceae and Hypoxylaceae are two of the most diverse and include the specimens studied in this work. The Xylariaceae (Tul. & C. Tul. [as “Xylariei”] *Selects fung Carpol* (Paris) 2: 3 (1863) emend M. Stadler & L. Wendt) is second to Amphispheariaceae (Xylariales) in terms of species richness, and comprises 823 species in 32 genera (Wendt et al. 2018). Species of Xylariaceae produce a geniculosporium-like asexual state, and in general, their stromata do not contain extractable pigments in 10% KOH (Wendt et al. 2018). Recently, 674 species were segregated from Xylariaceae and distributed into three families (Graphostromataceae, Hypoxylaceae, and Lopadostomataceae) while several more were segregated but are currently considered *incertae sedis* within Xylariales and Sordariomycetes, due to the lack of sufficient molecular data and information on the types of the asexual morph (Wendt et al. 2018). The Hypoxylaceae contains more than 360 species in 18 genera including recently described new taxa (Wendt et al. 2018, Daranagama et al. 2018, Lambert et al. 2019, De Long et al. 2019, Sir et al. 2019). The recent update of Sordariomycete families (Hyde et al. 2020) even listed 422 species in 19 genera.

The first records of Xylariales from Panama were based on specimens collected in Barro Colorado Island, Panama Canal Area, in the 1920s (Weston 1933, Standley 1933), and included 11 species of *Hypoxylon*, but only four of them are currently accepted in this genus: *H. haematostroma*, *H. fuscum*, *H. hypomiltum*, and *H. rubiginosum*. These records were followed by the reports of *H. subgilvum* and *H. dieckmanii* from the Panama Canal Area by Dennis in 1956, 1970 respectively. More recent collections were recorded for western Panama by Carmona et al. 2009 (*H. anthochroum*, *H. crocopeplum*, *H. investiens*, *H. fendleri*, and *H. lividipigmentum*), Piepenbring et al. 2012 (*H. pelliculosum*), and Kuhnert et al. 2015 (*H. trugodes*). Despite these studies, the reports of Xylariales from Panama are very scarce; only 95 species have been reported in the country for this highly diverse order (Piepenbring 2013). Recent surveys of Sordariomycetes in Panama conducted by our research group suggest that many species of Xylariales remain to be described. Here we present new records of Xylariales from Panama and the description of three new species of *Hypoxylon* based on comparative morphology, molecular phylogeny, and chemotaxonomy.

This study was based on specimens collected at six sites in the Republic of Panama and they are characterized based on a polyphasic approach (morphological, DNA based phylogeny and secondary metabolite profiling).

Materials & Methods

Collection of specimens and preparation of cultures

Since the scope of this study was to make an inventory of *Hypoxylon*, a genus whose stromata almost exclusively occur on woody, dicotyledonous angiosperms, the collection work focused on the forests that are known for a high diversity of such potential hosts. Two sites in the Panama Canal Area: Soberania National Park (SNP) and Camino de Cruces Park (CCP). Four sites in the western province of Chiriquí: Barú Volcano National Park (BVNP), La Concepción, Sortová, and La Amistad International Park (PILA, Chiriquí side). Four of these sites (SNP, CCP, Sortová and Concepción) are localized in lowlands at <700 meters above sea level (m.a.s.l.) and two at elevations higher than 1500 m.a.s.l. (PILA and BVNP).

Each of the sites were visited at least one time and specimens of fungi with typical morphological characters of Xylariaceae and Hypoxylaceae were collected, placed in paper bags, dried overnight at ambient temperature to remove excess humidity and transported to the laboratory for detailed observation and isolation of pure cultures. In the laboratory, perithecia were removed from the stromata using a sterile scalpel under a dissecting stereomicroscope (Nikon SMZ800, Melville, NY, USA), placed on a microscope slide with 50 µl of sterile water and crushed to release ascospores. Thereafter, 10 µl of the ascospore suspension were transferred to Petri dishes with potato dextrose agar (PDA) supplemented with rifampicin (PDA+r). After 24 hours the media were observed for sporulation and monosporic cultures were generated. In rare cases, cultures were

generated from multiple germinated spores or cultured from a small piece of the stromata. The cultures were preserved in 10% glycerol at -20°C and maintained in corn meal agar (CMA) slants at 8°C . Cultures were grown in: Potato dextrose agar (PDA), 2 % malt extract agar (2%MEA), V8 agar, oatmeal agar (OA), and potato carrot agar (PCA).

Morphological observations

Sexual structures of the collected specimens were extracted from fresh material, immersed in distilled water and 10 % KOH respectively, and size measurements were performed. Melzer's reagent was used to test the presence of an amyloid reaction of the apical apparatus. The color of the stromata and the extractable pigments were reported following the codes according to Rayner (1970). The stroma and perithecia of each specimen were photographed with a camera (ZEISS AxioCam ICc 5, Jena, Germany) connected to the microscope (ZEISS Axio Scope.A1, Germany). Asci and ascospores were observed, photographed, and measured using an Olympus BX53 microscope equipped with an Olympus DP73 camera and the CellSens Dimensions Software (Olympus, Japan). Specimens were preliminary identified morphologically using the key to species of *Hypoxylon* by Ju & Rogers (1996), Fournier et al. (2015) and Sir et al. (2016a), and in the case of *Stilbohypoxyton* by Petrini (2004). Further identification was accomplished using both, morphological and phylogenetic analyses (see below). Dried specimens were deposited in the herbarium of the Autonomous University of Chiriquí, Faculty of Natural and Exact Sciences, Chiriquí, Panama (UCH).

DNA extraction, amplification, and sequencing

DNA was extracted from 5 days old mycelial cultures grown on PDA media. Approximately a square centimeter of mycelium, taken from the growth front of the cultures was scraped off the surface of the medium and transferred to an extraction tube. The DNA was extracted using the Genra Puregene Tissue kit (QIAGEN, Germany) following the manufacturer's instructions. The TUB2 gene region was amplified using the primers T1 and T22 (O'Donnell & Cigelnik 1997). Amplifications were performed in 25 μl assays containing: 5–15 ng of genomic DNA, 200 μM of each dNTP, 2.5 units of Taq DNA Polymerase (QIAGEN, Germany), 2 μM of each primer and 10x buffer with 15 mM MgCl_2 ; using the iCycler thermal cycler ABI 2720 (Applied Biosystems, USA) under the following reaction conditions: 5 min at 94°C , followed by 32 cycles of 45 s at 94°C , 45 s at 50°C , and 1.5 min at 72°C , with a final extension period of 10 min at 72°C . The ITS gene region was amplified according to Mejía et al. (2008).

PCR products were purified using ExoSAP-IT (USB, Cleveland, USA) following the manufacturer's instructions, and the purified DNA was used for further cycle sequencing reactions with the BigDye Terminator v 3.1 kit (Applied Biosystems, Life Technologies Corporation, Carlsbad CA). The cycle sequencing reactions were performed using the same PCR primers and for TUB2 additionally, the internal primers T2 and T12 (O'Donnell & Cigelnik 1997). The reactions samples were purified with the Big Dye Xterminator kit and the PCR products were sequenced with an Applied Biosystems 3500 Genetic Analyzer (Life Technologies, Carlsbad, CA).

Phylogenetic analysis

DNA sequences of the new species were analyzed for quality and edited using the software Sequencher 5.1 (versión 5.1, Gene codes corporations, Ann Arbor, USA). The generated DNA sequences were collated against deposited sequences in the GenBank® database using the Basic Local Alignment Search Tool for Nucleotide sequences (Benson et al. 2013). Authentic ITS and TUB2 sequences of suitable *Hypoxylon* taxa from published studies were selected for the phylogenetic analyses (Table 1). Furthermore, the type species of prominent *Hypoxylon* sister genera were also included and *Xylaria hypoxyton* (Xylariaceae) was the outgroup. MAFFT alignments (G-INS-i, PAM 200/ k=2, GOP = 1.53, OV = 0.123; Katoh & Kuma 2002) of the ITS and TUB2 sequence dataset were calculated respectively, using the MAFFT v7.017 plug-in of Geneious® 7.1.9 (<http://www.geneious.com>, Kearse et al. 2012).

Table 1 List of strains used in the phylogenetic analyses. GenBank accession numbers for the ITS and TUB2 sequences are given. Strains derived from type specimens are labelled with T (holotype), IT (isotype), ET (epitype). Sequences derived from this study are highlighted in **bold**. Scientific names follow Index Fungorum (<http://www.indexfungorum.org/>).

Species name	Strain	ITS	TUB2	Location	Status	Reference
<i>Annulohypoxyplon truncatum</i>	CBS 140778	KY610419	KX376352	Texas (USA)	ET	Kuhnert et al. (2017)
<i>Daldinia concentrica</i>	CBS 113277	AY616683	KC977274	Germany		Triebel et al. (2005; ITS), Kuhnert et al. (2014; TUB2)
<i>Hypomontagnella monticulosa</i>	MUCL 54604	KY610404	KX271273	French Guiana	ET	Wendt et al. (2018)
<i>Hypoxylon addis</i>	MUCL 52797	KC968931	KC977287	Ethiopia	T	Kuhnert et al. (2014)
<i>Hypoxylon anthochroum</i>	BCRC 34050	JN660819	AY951703	Mexico		Hsieh et al. (2005)
<i>Hypoxylon baruense</i> sp. nov.	MC66, UCH9545	ex MN056428	MK908142	Panama		This study
<i>Hypoxylon begae</i>	BCRC 34051	JN660820	AY951704	Hawaii (USA)		Hsieh et al. (2005)
<i>Hypoxylon bellicolor</i> sp. nov.	MC36, UCH9543	ex MN056425	MK908139	Panama		This study
<i>Hypoxylon brevisporum</i>	BCRC 33809	JN660821	AY951705	Puerto Rico		Hsieh et al. (2005)
<i>Hypoxylon calileguense</i>	STMA 14059	KU604566	KU604579	Argentina		Sir et al. (2016a)
<i>Hypoxylon carneum</i>	BCRC 34052	JN660822	AY951706	France		Hsieh et al. (2005)
<i>Hypoxylon cercidicola</i>	CBS 119009	KC968908	KC977263	France		Kuhnert et al. (2014)
<i>Hypoxylon cinnabarinum</i>	BCRC 34055	JN979409	AY951708	Puerto Rico		Hsieh et al. (2005)
<i>Hypoxylon cinnabarinum</i>	MC97, UCH9546	ex MN056429	MK908143	Panama		This study
<i>Hypoxylon crocopeplum</i>	BCRC 34056	JN979410	AY951710	Indiana (USA)		Hsieh et al. (2005)
<i>Hypoxylon dieckmanii</i>	BCRC 34058	JN979412	AY951712	Mexico		Hsieh et al. (2005)
<i>Hypoxylon erythrostroma</i>	MUCL 53759	KC968910	KC977296	Martinique		Kuhnert et al. (2014)
<i>Hypoxylon fendleri</i>	BCRC 34064	JN979418	AY951718	Taiwan		Hsieh et al. (2005)
<i>Hypoxylon fendleri</i>	MC25, UCH9552	ex MN056423	MK908137	Panama		This study
<i>Hypoxylon ferrugineum</i>	CBS 141259	KX090079	KX090080	Austria		Friebes & Wendelin (2016, direct submission)
<i>Hypoxylon flavoargillaceum</i>	STMA 14062	KU604577	KU159532	Argentina		Sir et al. (2016a)
<i>Hypoxylon fragiforme</i>	MUCL 51264	KC477229	KX271282	Germany	ET	Wendt et al. (2018)
<i>Hypoxylon fraxinophilum</i>	MUCL 54176	KC968938	KC977301	France	ET	Kuhnert et al. (2014)
<i>Hypoxylon fulvosulphureum</i>	MFLUCC 13-0589	KP401576	KP401584	Thailand	T	Sir et al. (2015)
<i>Hypoxylon fuscopurpureum</i>	BCRC 34067	JN979421	AY951721	Virginia (USA)		Hsieh et al. (2005)
<i>Hypoxylon fuscum</i>	CBS 113049	KY610401	KX271271	France	ET	Wendt et al. (2018)
<i>Hypoxylon griseobrunneum</i>	CBS 331.73	KY610402	KC977303	India	T	Kuhnert et al. (2014; TUB2), Wendt et al. (2018; ITS)
<i>Hypoxylon haematostroma</i>	MC34, UCH9555	ex MN056424	MK908138	Panama		This study
<i>Hypoxylon haematostroma</i>	MUCL 47600	AM749924	KC977279	Panama		Kuhnert et al. (2014)
<i>Hypoxylon howeanum</i>	MC62, UCH9565	ex MN056427	MK908144	Panama		This study
<i>Hypoxylon howeanum</i>	MC65	MN056430	MK908141	Panama		This study
<i>Hypoxylon howeanum</i>	MUCL 47599	AM749928	KC977277	Germany		Kuhnert et al. (2014)
<i>Hypoxylon hypomiltum</i>	MUCL 53312	KC968914	KC977298	Guadeloupe		Kuhnert et al. (2014)

Table 1 Continued.

Species name	Strain	ITS	TUB2	Location	Status	Reference
<i>Hypoxylon investiens</i>	CBS 118183	KC968925	KC977270	Malaysia		Kuhnert et al. (2014)
<i>Hypoxylon jaklitschii</i>	CBS 138916	KM610290	KM610304	Sri Lanka	T	Kuhnert et al. (2015)
<i>Hypoxylon jecorinum</i>	YMJ 39	JN979429	AY951731	Mexico		Hsieh et al. (2005)
<i>Hypoxylon lateripigmentum</i>	MUCL 53304	KC968933	KC977290	Martinique	T	Kuhnert et al. (2014)
<i>Hypoxylon lenormandii</i>	STMA 14072	KM610283	KM610299	Argentina		Kuhnert et al. (2015)
<i>Hypoxylon liviae</i>	CBS 115282	KC968922	KC977265	Norway	ET	Kuhnert et al. (2014)
<i>Hypoxylon lividicolor</i>	BCRC 34076	JN979432	AY951734	Taiwan	T	Hsieh et al. (2005)
<i>Hypoxylon lividipigmentum</i>	BCRC 34077	JN979433	AY951735	Mexico	IT	Hsieh et al. (2005)
<i>Hypoxylon macrosporum</i>	BCRC 34078	JN979434	AY951736	Canada		Hsieh et al. (2005)
<i>Hypoxylon munkii</i>	MUCL 53315	KC968912	KC977294	Martinique		Kuhnert et al. (2014)
<i>Hypoxylon musceum</i>	MUCL 53765	KC968926	KC977280	Guadeloupe		Kuhnert et al. (2014)
<i>Hypoxylon ochraceum</i>	MUCL 54625	KC968937	KC977300	Martinique	ET	Kuhnert et al. (2014)
<i>Hypoxylon papillatum</i>	ATCC 58729	KC968919	KC977258	West Virginia (USA)	T	Kuhnert et al. (2014)
<i>Hypoxylon perforatum</i>	MUCL 54174	KC968936	KC977299	Japan		Kuhnert et al. (2014)
<i>Hypoxylon petriniae</i>	CBS 114746	KY610405	KX271274	France	T	Kuhnert et al. (2014; TUB2), Wendt et al. (2018; ITS)
<i>Hypoxylon pilgerianum</i>	BCRC 34985	JQ009310	AY951744	Taiwan		Hsieh et al. (2005)
<i>Hypoxylon polyporoideum</i>	BCRC 34088	JQ009311	AY951747	Taiwan		Hsieh et al. (2005)
<i>Hypoxylon porphyreum</i>	CBS 119022	KC968921	KC977264	France		Kuhnert et al. (2014)
<i>Hypoxylon pulvicicidum</i>	MUCL 49879	JX183075	JX183072	Martinique	T	Bills et al. (2012)
<i>Hypoxylon rickii</i>	MUCL 53309	KC968932	KC977288	Martinique	ET	Kuhnert et al. (2014)
<i>Hypoxylon rubiginosum</i>	MUCL 52887	KC477232	KY624311	Germany	ET	Stadler et al (2013; ITS), Wendt et al. (2018; TUB2)
<i>Hypoxylon samuelsii</i>	MUCL 51843	KC968916	KC977286	Guadeloupe	ET	Kuhnert et al. (2014)
<i>Hypoxylon shearii</i> var. <i>minor</i>	BCRC 34093	EF026142	AY951753	Mexico	IT	Hsieh et al. (2005)
<i>Hypoxylon spegazzianum</i>	STMA 14082	KU604573	KU604582	Argentina	T	Sir et al. (2016a)
<i>Hypoxylon sporistriataticum</i> sp. nov.	MC50, UCH9542	ex MN056426	MK908140	Panama		This study
<i>Hypoxylon subgilvum</i>	BCRC 34094	JQ009314	AY951754	Hawaii (USA)		Hsieh et al. (2005)
<i>Hypoxylon subrutiloides</i>	F 202416	FJ185304	FJ185281	New Zealand		Platas et al. (2009)
<i>Hypoxylon ticinense</i>	BCRC 34097	JQ009317	AY951757	France		Hsieh et al. (2005)
<i>Hypoxylon ulmophilum</i>	BCRC 34100	JQ009320	AY951760	Primorsky (Russia)		Hsieh et al. (2005)
<i>Hypoxylon vogesiacum</i>	CBS 115273	KC968920	KX271275	France		Kuhnert et al. (2014; ITS), Kuhnert et al. (2017; TUB2)
<i>Jackrogersella multiformis</i>	CBS 119016	KC477234	KX271262	Germany	ET	Kuhnert et al. (2014)
<i>Pyrenopolyporus hunteri</i>	MUCL 52673	KY610421	KU159530	Ivory Coast	ET	Kuhnert et al. (2017)
<i>Xylaria hypoxylon</i>	CBS 122620	KY610407	KX271279	Sweden	ET	Wendt et al. (2018)

The obtained alignments were cured using the Castresana Lab Gblocks Server v0.91b at low stringency settings (allowance of smaller final blocks & gap positions within the final blocks; http://molevol.cmima.csic.es/castresana/Gblocks_server.html, Talavera & Castresana 2007,

Castresana 2000) and flanking positions with missing sequence data was replenished with “?”. A modeltest (N=88) was performed for both cured alignments respectively, with default settings in jModeltest 2.1.10 v20160303 (Darriba et al. 2012), BIC values were calculated for all tested substitution models and the best substitution model for a combined alignment was chosen. The cured ITS and TUB2 alignment were concatenated, and a maximum likelihood analysis (ML) was performed with RAxML7.2.8 (Stamatakis 2006, 2014, Stamatakis & Alachiotis 2010) starting with a complete random tree, GTR+G+I as substitution model and 1000 bootstrap replicates, carried out in Geneious®.

The ML tree was rooted with *Xylaria hypoxylon* as outgroup, exported as scalable vector graphic (svg) and opened in Inkscape 0.92.3 (© 1989, 1991 Free Software Foundation, Inc., <https://www.inkscape.org>) for layout implementation (Fig. 1).

HPLC profiling

Stromatal extracts were obtained by removal of small amounts of the stromata tissue, immersion in acetone and extraction in an ultrasonic bath at 40°C for 15 min. The reaction tubes were centrifuged and the supernatant was used for chemical analyses using high performance liquid chromatography coupled with diode array and electrospray mass spectrometric detection (HPLC/DAD–ESIMS) as described in Kuhnert et al. (2017), and the gradient was the same as described by Surup et al. (2018). The mass spectra, UV–visible spectra and retention times were compared with an internal database comprising standards of known compounds from Xylariales to identify correlating peaks. The results are depicted in Fig. 5. Some characteristic metabolites are shown in Fig. 6.

Results

Phylogenetic analyses

For the phylogenetic analysis the ITS and TUB2 sequences of the eight collected Panamanian species were compared with sequences of 58 taxa, including sequences of many type and authentic species of *Hypoxylon* (Kuhnert et al. 2014, Wendt et al. 2018). Sequences of the type species of sister genera to *Hypoxylon*, namely *Annulohypoxyplon truncatum*, *Daldinia concentrica*, *Hypomontagnella monticulosa*, *Jackrogersella multififormis* and *Pyrenopolyporus hunteri* were included as well.

The MAFFT alignment of ITS and TUB2 consisted, respectively of 1672 and 2024 characters prior curing and 363 and 1085 after (21% and 53% of the original alignment length). Excluded flanking regions were [54-59], [99-113], [115-119], [125-136], [545-551], [591-603], [606-610], [620-625], [638-647], [651-656], [662-828], [835-848], [850-871], [895-911], [913-945], [962-968], [970-975] and [1007-1018] for the ITS alignment; and [126-133], [197-202], [204-208], [421-431], [439-443], [450-487], [528-539], [548-561], [576-604], [674-721], [760-764], [886-893], [895-955], [1034-1042], [1047-1341], [1343-1479], [1481-1735], [1737-1849], [1898-1905] and [1919-1936] for the TUB2 alignment. The modeltest identified GTR+I+G as sixth and fifth best suited substitution model for the cured ITS ($-\ln(L) = 5553$, $K = 140$, $BIC = 11932$) and TUB2 ($\ln(L) = 13461$, $K = 140$, $BIC = 27901$) alignment, respectively and the best substitution model for the calculation of a ML tree based on the combined alignment of the cured ITS and TUB2 alignments (ITS-TUB2 alignment, length: 1448 characters). The RAxML analysis resulted in the ML tree depicted in Fig. 1 with a $-\ln(L)$ value of 19353 and a tree length of 8.2866 (model information: $\alpha = 0.947863$; $inv = 0.547936$; substitution rates: $A \leftrightarrow C = 0.765339$, $A \leftrightarrow G = 3.433984$, $A \leftrightarrow T = 1.232713$, $C \leftrightarrow G = 0.519272$, $C \leftrightarrow T = 5.779039$, $G \leftrightarrow T = 1.000000$; base frequencies: $f\pi(A) = 0.20635$, $f\pi(C) = 0.301368$, $f\pi(G) = 0.243091$, $f\pi(T) = 0.249276$).

The calculated ML tree showed strong bootstrap support for most of the terminal clades but lacked general support for the tree backbone. This issue has been observed in previous studies of Xylariales (Hsieh et al. 2005, Kuhnert et al. 2014, Wendt et al. 2018) and could possibly be overcome by including further phylogenetic markers and taxa.

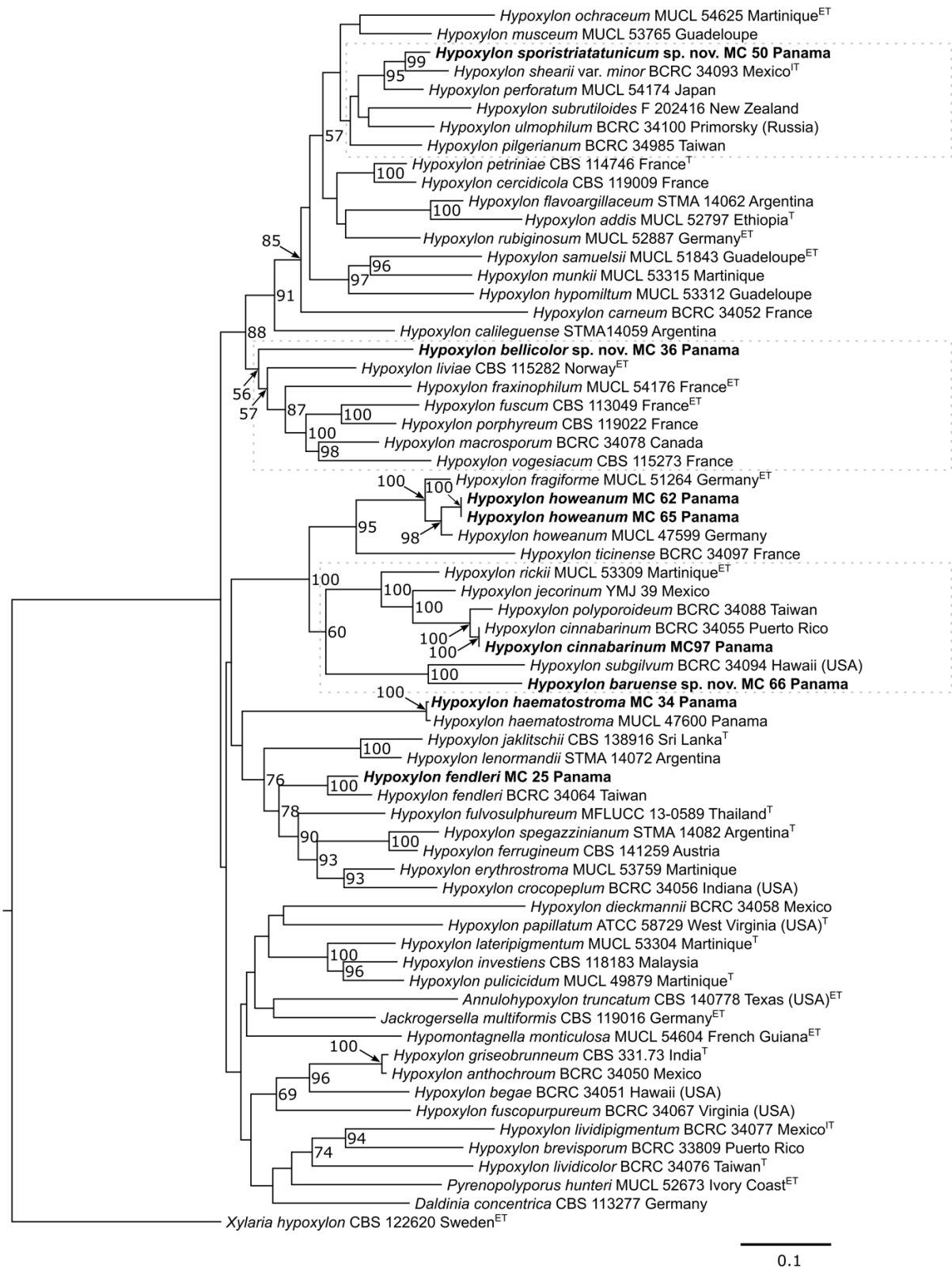


Figure 1 – Phylogenetic tree of *Hypoxylon*. Maximum Likelihood analysis (RaxML) inferred from a cured & combined MAFFT alignment of ITS and TUB2 DNA sequences. Bootstrap support values from 1000 replicates were indicated at the respective nodes. The tree was rooted with *Xylaria hypoxylon* as outgroup and taxa derived from type material are indicated with superscripts ^T for holotype, ^{ET} for epitype, ^{IT} for isotype. Sequences highlighted in bold were derived from this study.

The postulated new species of *Hypoxylon* clustered predominantly in fully supported clades. *Hypoxylon baruense* sp. nov. clustered with *Hypoxylon subgilvum* (BS 100%) while *H. sporistriatatunicum* sp. nov. appears closely related to *H. shearii* var. *minor* (BS 99%). *Hypoxylon bellicolor* sp. nov. occurred in a separate clade with only 56% BS support. Hence, it is likely that closer relatives have still not been found as yet and further collections of new *Hypoxylon* species are needed for a better phylogenetic resolution. However, our DNA based analysis herein support our morphological observations and analysis of the secondary metabolites by HPLC and justify the erection as new species of *Hypoxylon*. Furthermore, the collected specimen of *H. cinnabarinum* (MC 97), *H. fendleri* (MC 25), *H. haematostroma* (MC 34) and *H. howeanum* (MC 62 & 65), clustered respectively in fully supported clades with their reference taxon, which confirms their identification

Description of new species of *Hypoxylon*

Hypoxylon baruense M. Cedeño–Sanchez, L. Wendt & L.C. Mejía sp. nov.

Fig. 2

Mycobank number: MB 832717; Facesoffungi number: FoF 07186

Etymology – Named after the Barú Volcano National Park, where the species was collected for the first time.

Stromata – effused–pulvinate, sessile, with inconspicuous perithecial mounds, 0.3 – 1.7 cm long x 0.2 – 1.3 cm broad x 0.2 cm thick (n=1); surface Rust (39), Blood Colour (3); with orange granules beneath the surface and between the perithecia; with KOH–extractable pigments Greenish Yellow (16) or Luteous (12). *Perithecia*: obovoid to tubular, 0.3 mm broad x 0.5 –0.6 mm high (n=6). *Ostioles*: lower than the stromatal surface. *Asci*: 120 – 157 µm x 5 – 7 µm (n=6), with apical apparatus bluing in Melzer’s reagent, 2 – 3.6 µm long x 1–1.4 µm broad (n=5). *Ascospores*: dark brown, unicellular, ellipsoid–inequilateral, 10–15 x 5–7.6 µm (n=50), with straight conspicuous germ slit less than spore–length; perispore indehiscent in 10% KOH, smooth.

Culture characteristics – Colonies on PDA covering a 60 mm Petri dish in 3 weeks, mycelium white above, reverse dark green, with concentric zones. No asexual morph observed in the culture media used i.e. PDA, 2% MEA, V8 agar, oatmeal agar and potato carrot agar.

Secondary metabolites: Binaphthalene tetrol (BNT) as major component, mitorubrinol acetate as minor component and various unknown lipophilic components.

Distribution – Panama

Holotype – Panama, Chiriquí, Barú Volcano National Park, Quetzales trail, latitude 8°50'47.5" longitude 82°31'47.9", on dead trunk, 23 April 2016, M. Cedeño–Sanchez (UCH9545). Ex type: MC66 deposited at HZI.

Notes – *Hypoxylon baruense* sp. nov. is closely related to *H. subgilvum*, but can be differentiated as it has a smooth perispore indehiscent in 10% KOH and larger ascospores. There is only one other species of *Hypoxylon* with indehiscent perispore reported in Panama, *H. cinnabarinum*, but *H. baruense* differs from this species by having rust and blood colored stromata surface and KOH– extractable pigments with greenish yellow or luteous. In addition, *H. subgilvum* contains mitorubrin as major components but is lacking BNT (Hellwig et al. 2005).

Hypoxylon bellicolor M. Cedeño–Sanchez, L. Wendt & L.C. Mejía, sp. nov.

Fig. 3

Mycobank number: MB 832716; Facesoffungi number: FoF 07188

Etymology – Lat. “*bellus*”: pretty, color (referring to the color of the stromata).

Stromata – effused–pulvinate, sessile, with conspicuous perithecial mounds, 0.4 – 1.4 cm x 0.4 – 0.7 cm (n=4); surface Fulvous (43), Rust (39); with orange granules beneath the surface; with KOH–extractable pigments Sienna (8), Luteous (12). *Perithecia*: obovoid, 0.1 – 0.2 mm broad x 0.2 – 0.3 mm high (n=6). *Ostioles*: lower than the stromatal surface. *Asci*: not seen. *Ascospores*: dark brown, unicellular, ellipsoid, 10–12 x 4–6 µm (n=13), with germ slit less than spore–length; perispore dehiscent in 10% KOH, smooth.

Culture characteristics – on PDA covering a 60 mm Petri dish in 4 weeks, mycelium white above, reverse dark brown, with concentric zones. No asexual morph observed in the different culture media used i.e. PDA, 2% MEA, V8 agar, Oatmeal agar and potato carrot agar.

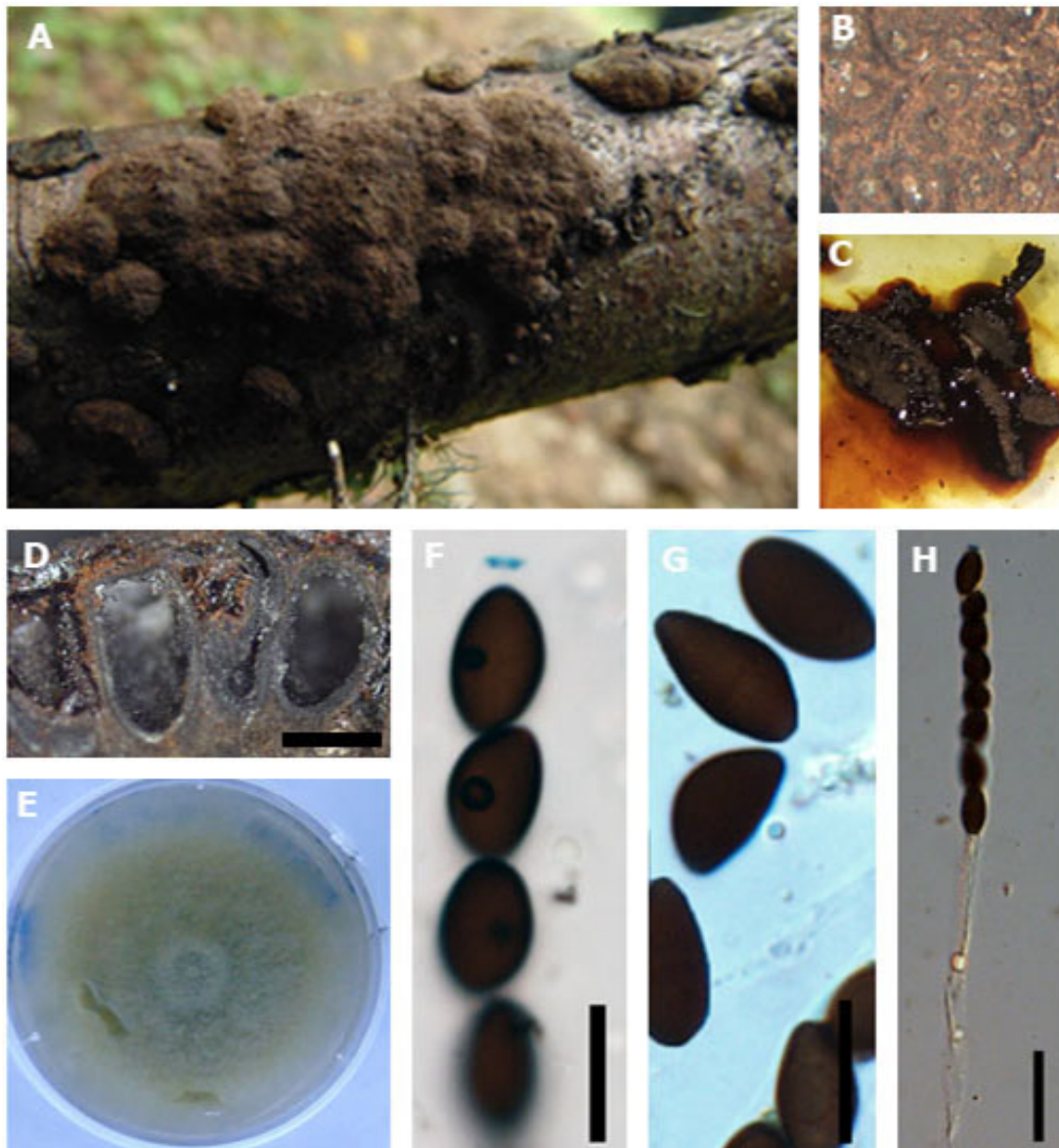


Figure 2 – *Hypoxylon baruense* sp. nov. (Holotype UCH9545). a Stroma. b Ostioles. c KOH-extractable stromatal pigments. d Perithecia (cross section). e PDA culture. f Amyloid apical apparatus. g Ascospores. h Ascus. Scale bars: d = 0.3 mm, e = 10 μ m, g = 10 μ m, f = 20 μ m.

Secondary metabolites – Stromata contain BNT in small amounts and otherwise yield little pigments.

Distribution – Panama.

Holotype – Panama, Chiriquí, Bugaba, latitude 09°14'59.2" longitude 79°41'32.8", on dead trunk, 9 February 2016, M. Cedeño (UCH9543). No Ex type culture exists as the culture obtained and used for phylogenetic analyses (MC36) did not survive.

Notes – This species can be distinguished by having the following combination of morphological characters: effused–pulvinate stromata, umbilicate ostioles, and ascospore size 10–12 x 4–6 μ m. *Hypoxylon bellicolor*, *H. macrocarpum* and *H. brevisporum* have effused–pulvinate stromata, ostioles lower than the stromatal surface and dehiscent perispore in 10% KOH. However,

H. bellicolor has much larger ascospores (size) than *H. brevisporum*; and *H. bellicolor* has fulvous stromata and obovoid perithecia while *H. macrocarpum* has violet–purple or vinaceous reddish–purple stromata and ovoid larger perithecia.



Figure 3 – *Hypoxylon bellicolor* sp. nov. (Holotype UCH9543). a Stroma. b Ostioles. c Perithecia (cross section). d PDA culture. e KOH–extractable stromatal pigments. f Ascospores with dehiscent perispore in 10% KOH. Scale bars: a = 5 mm, b = 2 mm, c = 0.5 mm, f = 10 μ m.

Hypoxylon sporistriataticum M. Cedeño–Sanchez, L. Wendt & L.C. Mejía, sp. nov. Fig. 4

Mycobank number: MB 832718; Facesoffungi number: FoF 07187

Etymology: Referring to the striated perispore.

Stromata – glomerate, sessile, with conspicuous perithecial mounds, 0.2 – 0.3 cm long x 0.2 – 0.4 cm broad (n=15); surface Fawn (87); with orange granules beneath the surface; with KOH–extractable pigments Luteous (12), Sienna (8), Greenish Yellow (18). **Perithecia**: obovoid, 0.3 – 0.4 mm broad x 0.4 – 0.5 mm high (n=8). **Ostioles**: slightly higher than the stromatal surface. **Asci**: 92–140 x 7–9 μ m (n=18), with apical apparatus bluing in Melzer’s reagent, 2.7–3 μ m long x 0.5–1.1 μ m broad (n=10). **Ascospores**: dark brown, unicellular, ellipsoid, with rounded ends, 10.4–13.2 x 5.4–7.3 μ m (n=50), with germ slit almost the length of the spore; perispore dehiscent in 10% KOH, with conspicuous coil–like ornamentation.

Culture characteristics: on PDA covering a 60 mm Petri dish in 4 weeks, mycelium white above, reverse dark brown, with concentric zones. No asexual morph observed in the different culture media used i.e. PDA, 2% MEA, V8 agar, Oatmeal agar and Potato carrot agar.

Secondary metabolites – Stromata contain hypomiltin as major component and other azaphilones similar to rubiginosin C as well as BNT as minor components. Macrocarpone A was also detected tentatively.

Distribution – Panama

Holotype – Panama, Chiriquí, International Park La Amistad, latitude 8°53'39.4" longitude 82°36'55", on dead trunk, 27 March 2016, M. Cedeño (UCH9542). Ex type culture (MC50), deposited at HZI.

Additional specimen examined – Panama, Chiriquí, Barú Volcano National Park Park, Quetzales trail, latitude 8°50'50.3" longitude 82°31'39.6", on dead trunk, 23 April 2016, M. Cedeño (UCH9551).

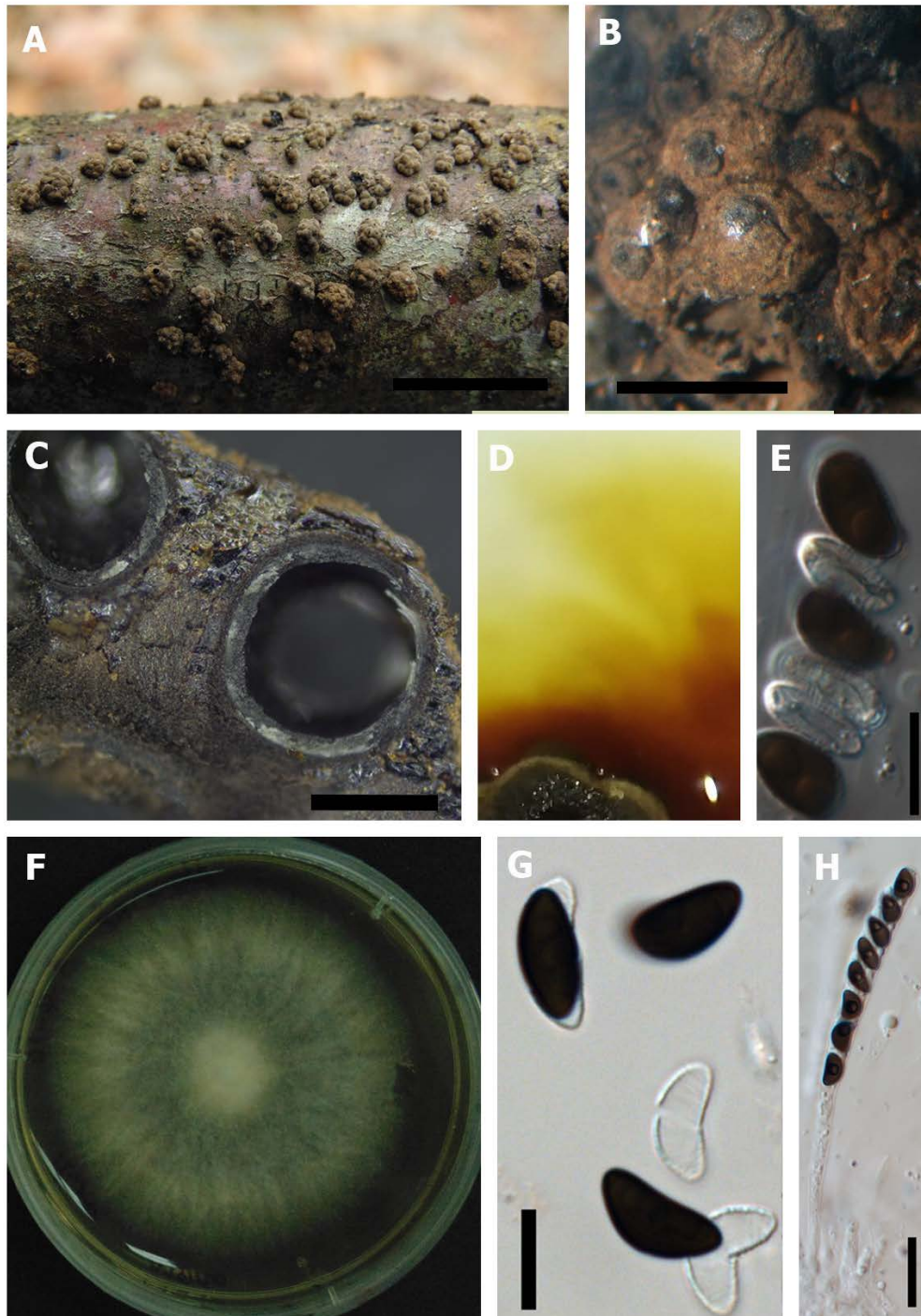


Figure 4 – *Hypoxylon sporistriatatunicum* sp. nov. (Holotype UCH9542). a Stroma. b Ostioles. c Perithecium cross section. d KOH-extractable stromal pigments. e Ascospores with striated perispore. f PDA culture. g Dehiscent perispore in KOH 10%. h Asci. Scale bar: a = 1 cm, b = 1 mm, c = 0.2 mm, e = 10 μ m, g = 10 μ m, H = 20 μ m.

Notes – *Hypoxylon sporistriatatunicum* sp. nov. is closely related to *H. shearii* var. *minor*, but differs from this species by presenting hemispherical to spherical stroma, ostioles higher than the surface, and larger ascospores. Additionally, *H. sporistriatatunicum* has a combination of features that make it different to other species of *Hypoxylon* including glomerate stromata, ostioles slightly higher than the stromatal surface, dehiscent perispore in 10% KOH, and conspicuous coil-like ornamentation of the perispore. From a chemotaxonomic point of view, it is interesting that the prevailing component in the stromatal extract (hypomiltin) is also the main metabolite of *H. perforatum*, which also clusters in the same major subclade. *H. shearii* var. *minor*, the next relative in the phylogenetic tree, was so far not studied by HPLC because the type specimen could not be obtained from the Mexican herbarium (ITCV) on our request and may actually have been lost. However, the typical variety *H. shearii* var. *shearii* was previously shown to contain mitorubrins (Stadler et al. 2008), and the two varieties share orange pigments, hence it is possible that var. *minor* also contains similar stromatal metabolites.

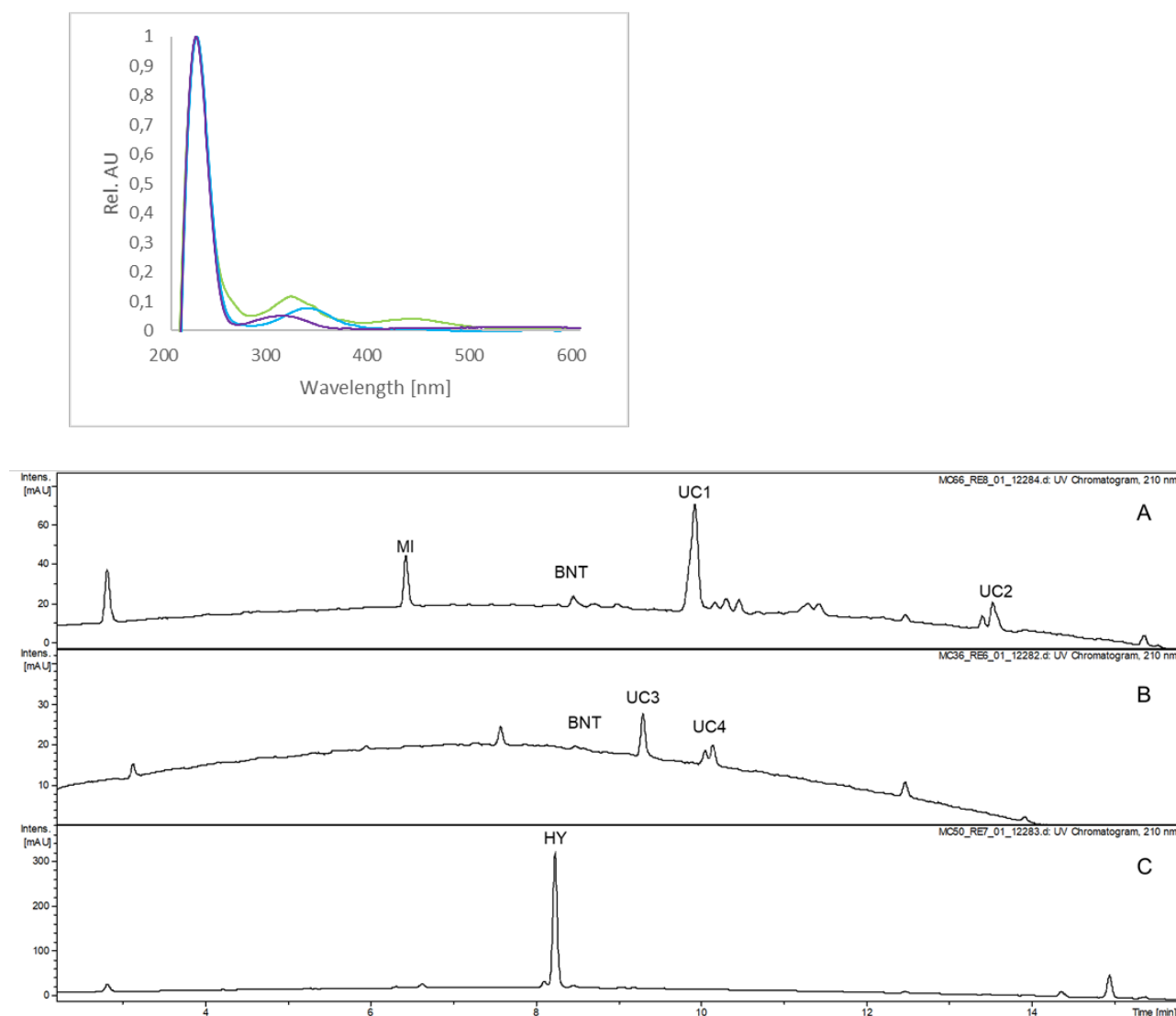


Figure 5 – Above: Normalized UV–Vis chromatograms of unknown compounds UC1, UC2, UC 3 & 4 from stroma of *Hypoxylon baruense* sp. nov. (Holotype UCH9545 ex-type MC66) and *Hypoxylon bellicolor* sp. nov. (Holotype UCH9543 ex-type MC36). Below: HPLC–UV chromatograms (210nm) of stromatal methanolic extracts of the holotypes of *Hypoxylon baruense* (A), *H. bellicolor* (B) and *H. sporistriatatunicum* (C). Identifiable compounds are BNT (only detectable in traces by HPLC–MS in *H. bellicolor*), mitorubrin (MI) and hypomiltin (HY). Unknown compounds detected are labelled with “UC1–4”.

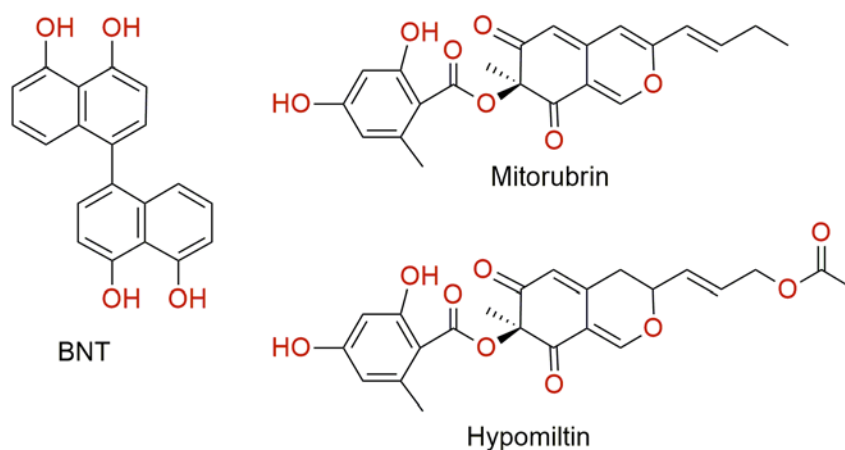


Figure 6 – Chemical structures of secondary metabolites detected in the new *Hypoxylon* species from Panama, mentioned in Fig. 5.

New reports of Xylariaceae and Hypoxylaceae from Panama

Amphirosellinia evansii (Læssøe & Spooner) Y.M. Ju, J.D. Rogers & H.M. Hsieh, in Ju, Rogers, Hsieh & Vasilyeva

Stromata: hemispherical, sessile, 1–1.3 mm long x 1–1.3 mm broad (n=10); surface brown; the tissue (fungal and host) around the stroma have a stellate pattern. *Perithecia*: round to obovoid, 0.7–0.9 mm broad x 0.7–0.9 mm high (n=10). *Ostioles*: higher than the stromatal surface. *Asci*: 158–247 x 9–10 μm (n=4), with apical apparatus bluing in Melzer's reagent, 9–10 μm high x 6–7 μm broad (n=11). *Ascospores*: dark brown, unicellular, ellipsoid, 20–22 x 8–10 μm (n=14), with spiral germ slit running over the whole spore-length; perispore indehiscent in 10% KOH, smooth.

Culture characteristics: no cultures obtained.

Distribution: Australia, Great Britain, Panama, USA.

Specimens examined: Panama, Chiriquí, La Amistad International Park, latitude 8°53'46.9" longitude 82°37'1.7", on dead trunk, 27 March 2016, M. Cedeño (UCH9559); latitude 8°53'39.3" longitude 82°36'57.7", on dead trunk, 27 March 2016, Specimen MC54.

Notes: This species is distinguished by having a spiral-shaped germ slit in the ascospores and forming a star-shaped pattern around the stroma, with host tissue and fungus. These characteristics were observed in the identified specimens.

Hypoxylon cinnabarinum (Henn.) Y.M. Ju & J.D. Rogers

Stromata: effused-pulvinate, sessile, 1–3 cm long x 0.7–1.8 cm broad (n=3), with conspicuous perithecial mounds; surface Orange (7) on the ostiole side and Saffron (10) in the sterile edges; orange granules beneath the surface and surrounding the perithecia, a line of white granules beneath the perithecia, the tissue below the perithecia black; with KOH-extractable pigments Orange (7). *Perithecia*: obovoid to tubular, 0.3–0.4 mm broad x 0.4–0.5 mm high (n=8). *Ostioles*: lower than the stromatal surface. *Asci*: 144–160 x 7–10 μm broad (n=6), with apical apparatus bluing in Melzer's reagent, 1 μm high x 3–4 μm broad (n=7). *Ascospores*: brown to dark brown, unicellular, ellipsoid nearly equilateral, 11–13 x 6–7 μm (n=17), with germ slit of spore-length; perispore indehiscent in 10% KOH, smooth.

Colonies: on PDA, white mycelium above, reverse green.

Distribution: Brazil, Mexico, New Zealand, Panama, Puerto Rico, Taiwan, Venezuela. Lectotype is from Brazil and was designated by Ju & Rogers (1996). However, the culture used in the phylogenetic studies by Hsieh et al. 2005 and the description of the asexual morph by Ju & Rogers (1996) originated from Mexico. The culture from Mexico was included in the phylogeny presented here and is conspecific with the specimen from Panama (Fig. 1).

Specimens examined: Panama, Chiriquí, Sortová, latitude 08°34'08.9" longitude 82°39'25.9", on dead trunk of *Citrus sinensis*, 5 August 2016, M. Cedeño (UCH9563, consisting of a dried

specimen); Divalá, on dead branches of a live tree of *Citrus sinensis*, 9 November 2016, M. Cedeño (UCH9546).

Notes: This species has nearly equilateral ascospores with perispore frequently indehiscent in 10% KOH. Based on analyses of morphological characters, Fournier & Lechat (2015) and Fournier et al. (2015) suggested the possibility of two other species of *Hypoxyton* that resemble *H. cinnabarinum* i.e. *H. cf. cinnabarinum* sp. 1 and *H. cf. cinnabarinum* sp. 2. However, they suggested that in the absence of complete cultural, molecular, and chemotaxonomic work for all the specimens that they studied, it was better not to segregate *H. cinnabarinum* into different species (Fournier et al. 2015). The specimens here studied fits the description of *H. cinnabarinum* according to Ju & Rogers (1996) and also the one that Fournier et al. (2015) identified as *H. cf. cinnabarinum* sp. 1, so we decided to keep the specimens collected in Panama as part of this study identified as *H. cinnabarinum* in the broad sense.

***Hypoxyton howeanum* Peck**

Stromata: hemispherical to spherical, sessile, 5.5 mm long x 4 mm broad x 2.7 mm high (n=6), with inconspicuous perithecial mounds; surface color Fulvous (43), Rust (39), with orange granules beneath the surface; the tissue below the perithecia black, homogeneous; with KOH-extractable pigments Orange (7) or Rust (39). *Perithecia*: obovoid, 0.2–0.3 mm broad x 0.4 mm high (n=10). *Ostioles*: lower than the stromatal surface. *Asci*: 103–150 x 4.5–6 µm (n=5), with apical apparatus bluing in Melzer's reagent, µm 0.2.8 – 0.3 high x 1.5 – 1.8 µm broad (n=8). *Ascospores*: brown, unicellular, ellipsoid, with narrowly rounded ends, 8–9 x 4–5 µm (n=17), with germ slit spore-length; perispore dehiscent in 10% KOH, smooth.

Culture characteristics: on PDA, mycelium white above, reverse greenish pigmentation in the center.

Distribution: Australia, Czech Republic, France, Germany, India, Italy, Iran, Japan, Mexico, New Zealand, Norway, Panama, Poland, Spain, South Africa, Turkey, United Kingdom, USA. *Hypoxyton howeanum* occurs on a wide variety of angiosperm hosts in Europe, but almost never on European beech, *Fagus sylvatica*, where the sister species *H. fragiforme* is predominant.

Specimens examined: Panama, Chiriquí, Barú Volcano National Park, Quetzales Trail, latitude 8°50'50.4" longitude 82°31'39.5", on dead branch of *Alnus acuminata*., 23 April 2016, M. Cedeño (UCH9565); latitude 8°50'46.7" longitude 82°31'50.9", on dead branch of *Alnus acuminata*., 23 April 2016, Cedeño MC65.

Notes: This species is characterized by hemispherical to spherical stromata, and ostioles lower than the stromatal surface. *Hypoxyton howeanum* is similar to *H. fragiforme*, the type species of the genus, but differs in having smaller ascospores. In addition, its stromata can colonize a broad variety of hosts (including *Acer* and various species of Salicaceae and Betulaceae), while *H. fragiforme* is mainly restricted to *Fagus* in the temperate Northern hemisphere. The species was found growing on *Alnus acuminata* at 2,500 m.a.s.l. and which is native to Panama.

***Stilbohypoxyton quisquiliarum* (Mont.) J. D. Rogers & Y.–M. Ju**

Stromata: subglobose to globose, sessile, 0.8 – 1 mm high x 1 – 1.3 mm broad, black surface, with yellow scales (no too much visible). *Ostioles*: higher than the stromatal surface, papillate *Asci*: not found. *Ascospores*: dark brown, unicellular, 26–31 x 12–15 µm (n=8), inequilaterally ellipsoidal, with spiral germ slit running over the whole spore length; perispore indehiscent in 10% KOH, smooth.

Culture characteristics: no culture obtained.

Distribution: Brazil, Costa Rica, Panama, Singapore.

Specimen examined: Panama, Panama, Soberania National Park, pipeline rout trail, on dead trunk, 8 August 2015, M. Cedeño (UCH9547).

Notes: This species is distinguished by the presence of yellow scales in mature stromata, and germinal line of the length of the spore and in spiral (Petrini 2004). The germ slit was clearly

observed in the specimen, but the scales were not clear. It differs from *S. macrosporum* by having smaller ascospores.

Additional specimens of *Hypoxylon* collected in this study

Hypoxylon fendleri Berk. ex. Cooke

Stromata: effused–pulvinate, sessile, 3 cm long x 0.7 cm broad (n=1), with inconspicuous perithecial mounds; surface color Fulvous (43), Rust (39), orange granules beneath surface; with KOH–extractable pigments Orange (7) or Rust (39). *Ostioles*: lower than the stromatal surface. *Asci*: with apical apparatus bluing in Melzer’s iodine reagent, 0.6 – 1 µm high x 1.8 – 2.2 µm broad (n=9). *Ascospores*: brown, unicellular, ellipsoid, smooth, 8.9–10.2 x 3.9–4.7 µm (n=10), with straight germ slit spore–length; perispore dehiscent in 10% KOH, smooth.

Culture characteristics: on PDA white mycelium, reverse cream color, darkness in the center and light in the edge.

Distribution: Pantropical.

Specimen examined: Panama, Chiriquí, Sortová, on dead trunk, 1 January 2016, M. Cedeño (UCH9552).

Notes: This fungus is very common throughout the tropics. In Ju & Rogers (1996) it is mentioned that *Hypoxylon fendleri* differs from *H. trugodes* and *H. crocopeplum* by the color of stromal pigments extracted with 10% KOH. The specimen treated in this work showed the typical morphological characteristics of *H. fendleri* and this was also supported by the phylogenetic analysis.

Hypoxylon haematostroma Mont.

Stromata: plane to effused–pulvinate, sessile, 0.3–1.7 cm long x 0.2–1.3 cm broad x 0.2 cm thick, with inconspicuous perithecial mounds; surface Sienna (8); with KOH–extractable pigments Orange (7), Scarlet (5). *Perithecia*: long tubular, 0.5 mm broad x 2 mm high (n=11). *Ostioles*: lower than the stromatal surface. *Asci*: not found. *Ascospores*: brown, unicellular, ellipsoid, smooth, 15–18 x 6–7 µm (n=11), with straight germ slit spore–length; perispore dehiscent in 10% KOH, smooth.

Culture characteristics: on PDA white mycelium, reverse greenish color, with zones with concentric.

Distribution: Pantropical

Specimen examined: Panama, Panama, Soberania National Park, El Charco trail, on dead dicotyledonous trunk, 30 January 2016, M. Cedeño (UCH9555).

Notes: This species is distinctive among species of *Hypoxylon* by having tubular perithecia and red or orange granules below the stromatal surface (Ju & Rogers 1996). Both morphological characteristics were present in the specimen examined. Additionally, the species have brown to dark brown ascospores with a perispore dehiscent in 10% KOH, nevertheless, the size of ascospores is variable according to Fournier et al. (2015) ranging from 12.5 – 18 x 5.9 – 9 µm.

Key to *Hypoxylon* species from Panama

- 1a. Perispore dehiscent in 10% KOH5
- 1b. Perispore indehiscent in 10% KOH2
- 2a. Ascospores averaging more than 10 µm in length3
- 2b. Ascospores averaging less than 10 µm in length4
- 3a. Ascospores 10–15 x 5–7.6 µm, with KOH–extractable pigments (greenish yellow or luteous)*H. baruense*
- 3b. Ascospores 11–13 x 6–7 µm, with KOH–extractable pigments orange*H. cinnabarinum*

4a. Perithecia obovoid; KOH-extractable pigments grayish sepia; with faint germ slit	<i>H. dieckmannii</i>
4b. Perithecia tubular to long tubular; KOH-extractable pigments greenish yellow, dull green or dark green; and straight germ slit	<i>H. investiens</i>
5a. Apical apparatus bluing to lightly bluing in Melzer's iodine reagent	6
5b. Apical apparatus not bluing in Melzer's iodine reagent; ascospores 9–11 x 4.5–5.5 µm with perispore with very conspicuous coil-like ornamentation; perithecia spherical	<i>H. pelliculosum</i>
6a. KOH-extractable pigments greenish yellow, orange, scarlet, rust, luteous, sienna, bay	7
6b. KOH-extractable pigments with other than above colors	13
7a. Stromata hemispherical to spherical, sometimes effused-pulvinate, KOH-extractable pigments orange, scarlet, rust	11
7b. Stromata effused-pulvinate or plane with KOH-extractable pigments orange	8
8a. Sigmoid germ slit, ascospores 9–12 x 4–5.5 µm; perithecia spherical, obovoid, ostioles lower than stromatal surface	<i>H. fendleri</i>
8b. Straight to slightly sigmoid germ slit; perithecia spherical, obovoid, tubular to long tubular ...	9
9a. Straight germ slit; perithecia spherical to obovoid; stromata effused-pulvinate, pulvinate or even semispherical; ascospores 9–12 x 4–5.5 µm	<i>H. rubiginosum</i>
9b. Straight to slightly sigmoid germ slit; perithecia obovoid, tubular to long tubular	10
10a. Straight to slightly sigmoid germ slit; ascospores 9.5–15 x 4–7 µm; perithecia spherical, obovoid to long tubular, ostioles lower than or same level as the stromatal surface	<i>H. crocopeplum</i>
10b. Straight to slightly sigmoid germ slit; ascospores 7–11 x 3.5–5 µm; perithecia obovoid to tubular, ostioles lower than the stromatal surface	<i>H. subgilvum</i>
11a. Perithecia long tubular; stromata semispherical to effused-pulvinate, KOH-extractable pigments orange, scarlet; ascospores 13.5–18 x 7–8 µm	<i>H. haematostroma</i>
11b. Perispore obovoid, stromata semispherical to spherical, ascospores less than 13 µm	12
12a. Ascospores 8–9 x 4–5 µm, with smooth perispore, apical apparatus bluing in Melzer's iodine reagent 0.28–0.3x1.5–1.8 µm; KOH-extractable pigments rust, sienna	<i>H. howeanum</i>
12b. Ascospores 11–13 x 6–7µm, with conspicuous coil-like ornamentation perispore, with apical apparatus bluing in Melzer's iodine reagent 2.7–3x0.5–1.1 µm; KOH-extractable pigments luteous, sienna, greenish yellow	<i>H. sporistriatatunicum</i>
13a. Ascospores light brown to brown, perispore smooth, ascospores 7–9 x 3–4 µm with slightly oblique to sigmoid germ slit	<i>H. hypomiltum</i>
13b. Ascospores brown to dark brown, perispore smooth or with inconspicuous coil-like ornamentation	14
14a. Straight to slightly sigmoid germ slit, stromata effused-pulvinate	15
14b. Sigmoid germ slit, stromata usually hemispherical to pulvinate, sometimes effused-pulvinate, ascospores 8–20 x 4–8 µm	<i>H. fuscum</i>
15a. Conspicuous perithecial mounds, perithecia obovoid; ascospores 10–12 x 4–6 µm	<i>H. bellicolor</i>
15b. Inconspicuous perithecial mounds, perithecia obovoid, tubular, long tubular	16
16a. Ascospores brown, straight to slightly sigmoid germ slit, KOH-extractable pigments dark livid	<i>H. lividipigmentum</i>
16b. Ascospores brown to dark brown, slightly sigmoid germ slit, KOH-extractable pigments of different colors	17
17a. KOH-extractable pigments amber, isabelline, olivaceous, gray alivaceous, greenish olivaceous; ascospores 8.5–13.5 x 4–6 µm	<i>H. anthochroum</i>
17b. KOH-extractable pigments amber, yellow; ascospores 8–11 x 3.5–4.5 µm	<i>H. trugodes</i>

Discussion

Morphological observations and analyses of DNA sequence data of Xylariales specimens collected in Panama allowed us to identify three new species of *Hypoxylon* as well as to document four new records of this genus and three new records of Xylariaceae for the country. The phylogeny

of *Hypoxylon* obtained in this study based on the concatenated analysis of β -tubulin and ITS loci (Fig. 1) is in general agreement with a recently published phylogeny of *Hypoxylon* based on multi-locus data (Wendt et al. 2018).

The species treated in this study showed a restricted altitudinal distribution, for example, *H. bellicolor* sp. nov., *H. cinnabarinum* and *S. quisquiliarum* were collected only at lowland (700 m.a.s.l. or lower). In contrast *Amphirosellinia evansii*, *H. sporistriataticum* sp. nov., *H. baruense* sp. nov., and *H. howeanum* were observed in the highlands (1500 m.a.s.l. or higher) of Chiriquí Province. In general, the species observed in this study were found only in one site, except *H. sporistriataticum* sp. nov., which was collected in La Amistad International Park and in the Barú Volcano National Park, both parks located in the province of Chiriquí. Our data suggest that the distribution of *Hypoxylon* species in Panama is restricted either by locality or elevation, but further studies are necessary to confirm these observations. The distribution of the species could be also related to the type of forest or life zone, for example, La Amistad International Park and the Barú Volcano National Park have six of the twelve life zones of Panama. The Barú Volcano National Park presents two unique life zones in the country: montane very wet forest and low montane wet forest (MiAMBIENTE 2016), unlike Soberania National Park that is predominantly tropical wet forest, and Sortová and Concepción which are premontane very wet forests.

Specimens treated in this study were collected on decomposing dead trunks or dead branches and *H. cinnabarinum* was collected growing on a dead branch but still attached to a living tree of *Citrus sinensis*, a new host of this species. This species has been found on the bark of dead branches and trunks of unidentified hosts (Ju & Rogers 1996, Fournier et al. 2015, Fournier & Lechat 2015), as well as on *Beilschmiedia tawa* and an unidentified *Nothofagus* species (Ju & Rogers 1996).

This study increased the numbers of known species of Hypoxylaceae of Panama from 30 to 35 and Xylariaceae from 45 to 47. Given the facts that *i*) several new species were recently reported from this geographic area and *ii*) many other taxa have been known from the neighbour countries of Panama during the past century, additional studies on the systematics and ecology of these families are called for. Considering the reported biological activities of fungi of the Xylariales (Helaly et al. 2018), including antimicrobials or virulence factors such as the azaphilones and mitorubrinol acetate to be produced by species described here, it is possible that new species from this order might be potential sources of new compounds for biotechnological applications. This information can be of value to promote more studies and *in situ* conservation efforts of the habitats and diversity of these fungi.

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