# Lenormandins A—G, new azaphilones from *Hypoxylon lenormandii* and *Hypoxylon jaklitschii* sp. nov., recognised by chemotaxonomic data

Eric Kuhnert · Frank Surup · Esteban B. Sir ·
Christopher Lambert · Kevin D. Hyde ·
Adriana I. Hladki · Andrea I. Romero · Marc Stadler

**Abstract** Seven new azaphilone pigments named lenormandins A–G were isolated from stromata (fruiting bodies) of the xylariaceous fungus, *Hypoxylon lenormandii* using preparative High Performance Liquid Chromatography (HPLC) and their structures were elucidated by NMR spectroscopy, high resolution mass spectrometry and other spectral methods. Their occurrence in herbarium specimens (including various type materials collected in the 19th and early 20th century) and several fresh collections was studied by analytical HPLC with diode array and mass spectrometric detection (HPLC-DAD/MS), revealing that this group of pigments is specific for *Hypoxylon lenormandii* from various geographic regions and otherwise only occurs in closely related taxa, i.e. *H. sublenormandii* and an undescribed fungus from Sri Lanka, for which we propose the name *H. jaklitschii*. The status of the new species was established by using molecular phylogenetic data based on ITS and partial beta-tubulin sequences, and by detailed morphological studies.

**Keywords** Chemotaxonomy · *Hypoxylon* · Phylogeny · Secondary metabolites · Systematics · Xylariaceae

**Taxonomic Novelties** *Hypoxylon jaklitschii* Sir & Kuhnert, sp. nov.

E. Kuhnert · F. Surup · C. Lambert · M. Stadler
Department of Microbial Drugs, Helmholtz-Zentrum für
Infektionsforschung GmbH, Inhoffenstrasse 7, 38124 Braunschweig,
Germany

E. Kuhnert · F. Surup · C. Lambert · M. Stadler (✉)
German Centre for Infection Research (DZIF), partner site
Hannover-Braunschweig, 38124 Braunschweig, Germany
e-mail: Marc.Stadler@helmholtz-hzi.de

E. B. Sir · A. I. Hladki
Fundación Miguel Lillo, Institute of Mycology, Miguel Lillo 251,
San Miguel de Tucumán 4000, Tucumán, Argentina

E. B. Sir · A. I. Romero
Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET), Av. Rivadavia 1917, Buenos Aires C1033AAJ,
Argentina

K. D. Hyde
Institute of Excellence in Fungal Research and School of Science,
Mae Fah Luang University, Chiang Rai 57100, Thailand

A. I. Romero
Department of Biodiversity and Experimental Biology, University of
Buenos Aires, Int. Güiraldes 2620, Buenos Aires C1428EHA,
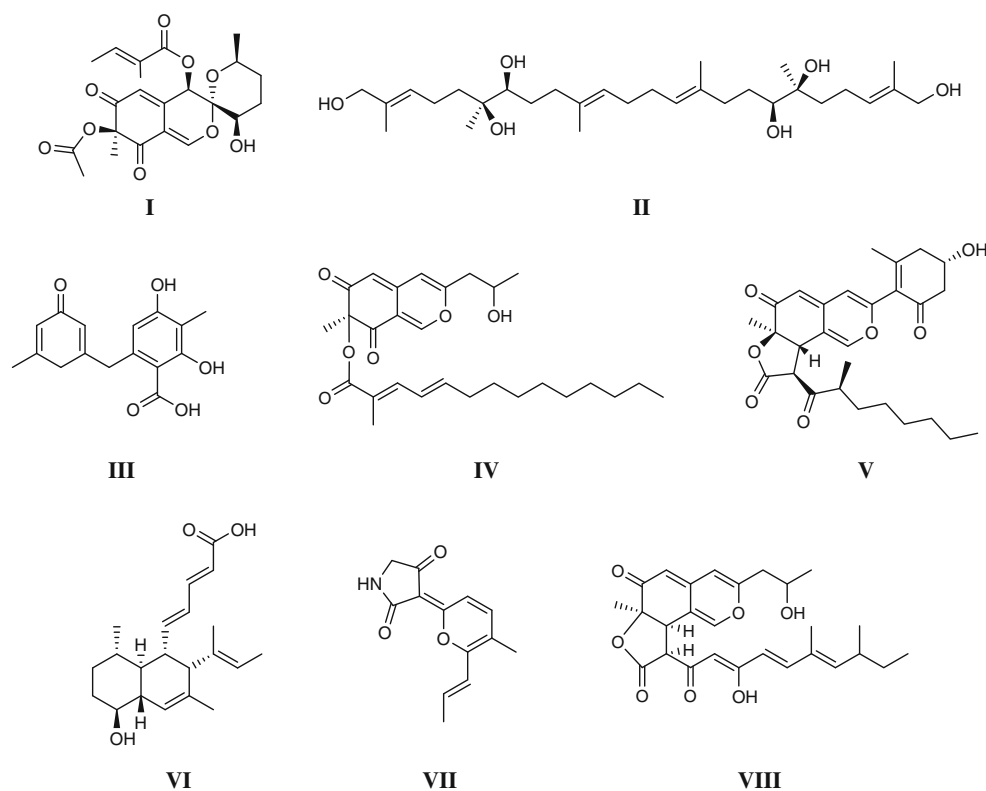Argentina

## Introduction

As one of the largest families in the Ascomycota, the Xylariaceae show an extraordinary diversity with respect to morphology and secondary metabolism. Seventy-two genera are presently accepted within the family and most of them can be assigned to the two informal "subfamilies" Xylarioideae and Hypoxyloideae (Stadler et al. 2013), which have not yet been validly erected. Within the latter "subfamily", which is characterised by a nodulisporium-like conidial state the genus *Hypoxylon* comprises by far the highest number of accepted species (more than 150). This genus is widely distributed, but shows it highest diversity in the tropics. Hsieh et al. (2005) used β-tubulin and alpha actin, rather than the more commonly employed 5.8S/ITS region of the rRNA to better resolve inter- and intrageneric relationships among the Hypoxyloideae. Based on their results, Kuhnert et al. (2014a), who also compared ITS and beta tubulin sequence data for a significant number of species for the first time, showed that an increase of the taxon sampling led to a weaker resolution of the tree. The occurring clade formation

then often lacks support from the concurrently observed morphological data. To explain this conflict, additional data are required. Ju and Rogers (1996) already established an alternative concept by using the colour of pigments, surrounding the perithecia as well as in the surface of stromata, which could easily be extracted with potassium hydroxide (KOH) solution as a valid character that helped to resolve various species complexes. However, highly similar colours of the KOH- extractable pigments might be derived from secondary metabolites with entirely different chemical structures, hence modern analytical techniques were needed to evaluate the chemical principles. During the search for novel therapeutic lead structures, the Xylariaceae were also screened for new secondary metabolites. Steglich et al. (1974) reported the mitorubrins from fruiting bodies of *Hypoxylon fragiforme* as the first stromatal pigments from the genus. Meanwhile, around 100 pigments and other secondary metabolites were identified by using analytical as well as preparative high performance liquid chromatography (HPLC). Previous studies revealed insights into the structural diversity and complexity of secondary metabolites found in species of the Hypoxyloideae. Important examples (Fig. 1) are the daldinins (I, Hashimoto et al. 1994, Quang et al. 2004a), concentricols (II, Stadler et al. 2001a, Quang et al. 2002), macrocarpones (III, Mühlbauer et al. 2002), rubiginosins (IV, Quang et al. 2004b) and cohaerins (V, Quang et al. 2005a, Quang et al. 2006a, Surup et al. 2013). With the knowledge about the structures it was now possible to correlate compounds with

species and assign them to different chemotypes (Stadler & Hellwig 2005, Stadler & Fournier 2006). This advance established chemotaxonomy as third important characteristic besides morphology and phylogeny to describe species of the Hypoxyloideae. Interestingly some compounds are limited to only one particular species like the carneic acids in *H. carneum* (VI, Quang et al. 2006b, absolute stereochemistry revised by Yamakoshi et al. 2009) or the hypoxyvermelhotins in *H. lechatii* (VII, Kuhnert et al. 2014b) allowing this species to be identified alone by chemotaxonomic data. This is especially useful when dealing with very old herbaria material, where DNA isolation becomes impossible and micromorphological features are often difficult to interpret. Most of the previous work focused on species with high quantities of easily accessible stromata collected in the Northern temperate climates. However the diversity of Xylariaceae species is much higher in the tropics and chemical data are badly needed to distinguish between species and for resolving species complexes. Ju and Rogers (1996) already mentioned various putative species complexes in their world monograph of *Hypoxylon*, e.g. *H. fuscum*, *H. rubiginosum* and *H. lenormandii*. The latter is widespread in the tropics and subtropics occurring on all respective continents (Dennis 1963 as *H. oodes*, Martin 1969 as *H. oodes*; Van der Gucht 1995 as *H. oodes*, Ju and Rogers 1996). Furthermore it has been found on a broad host range involving monocots (bamboo) and dicots.



**Fig. 1** Chemical structures of marker metabolites produced in stromata of various Xylariaceae species. **I**—Daldinin C, **II**—concentricol A, **III**—macrocarpone A, **IV**—rubiginosin C, **V**—cohaerin C, **VI**—carneic acid A, **VII**—hypoxyvermelhotin A and **VIII**—sassafrin A

Hellwig et al. (2005) already found that this species contained specific compounds in its stromata, which could then not be isolated and identified for lack of material and due to their instability. Meanwhile, more fresh material from Argentina and Thailand has become available, allowing for a new attempt to characterise these specific pigments. The present paper is thus dedicated to an in-depth study of their chemistry, but also provides new evidence on the phylogenetic position of their producer organism and its affinities to some closely related taxa.

## Materials and methods

### General

If not indicated otherwise solvents were obtained in analytical grade from J.T. Baker (Deventer, Netherlands) or Merck (Darmstadt, Germany). Bold Arabic numbering refers to the chemical structures depicted in Figs. 2 and 3. All scientific names of fungi follow the entries in Mycobank (www.mycobank.org), hence no authorities and years of publications are given. Reference specimens are housed in K (Royal Botanical Gardens, Kew, UK), LIL (Criptogamic Herbarium Miguel Lillo Foundation, San Miguel de Tucumán, Argentina), LIP (University of Lille, France), MFLU (Mae Fah Luang University Chiang Rai, Thailand),

MUCL (Louvain, Belgium), S (Swedish Museum of Natural History, Stockholm, Sweden) and corresponding reference cultures have been deposited with CBS (Utrecht, The Netherlands), BCC (BIOTEC, Pathumthani, Thailand), MFLUCC (Chiang Rai, Thailand), MUCL or STMA (personal culture collection of M. Stadler); see Kuhnert et al. (2014a) for further details. Acronyms of herbaria and culture collections are given as recommended in Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

### Fungal material

To study their morphological, chemical and molecular diversity 24 strains of *Hypoxylon lenormandii* and closely related species were selected. The origin of these strains as well as the obtained data is shown in Table 1.

### Morphological characterisation

Morphology and size of macroscopic and microscopic features of the stromata were determined as described by Fournier et al. (2010). Photos of micromorphological structures were taken through a brightfield microscope at 400–1000×magnification. Micrographs of ascospores were taken with a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany). For studies of conidiogeneous structures and sequencing, cultures of the specimens were obtained
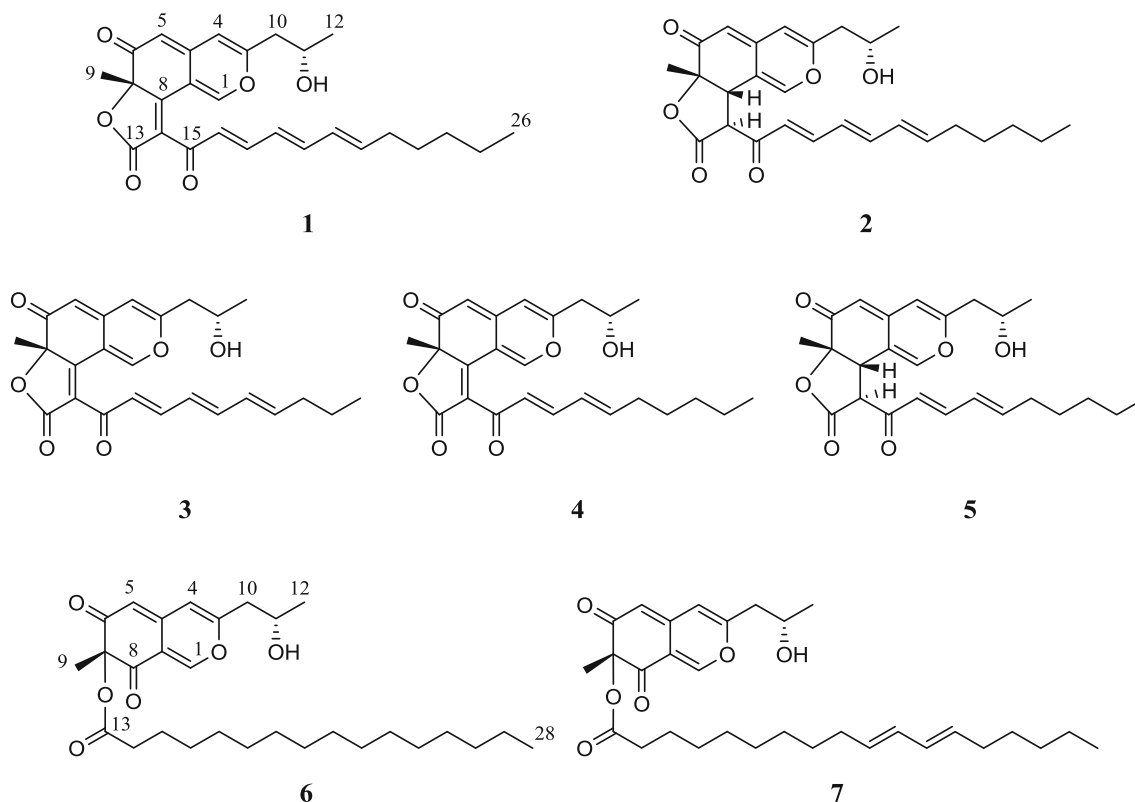


Fig. 2 Chemical structures of lenormandins A–G (**1**–**7**) isolated from *Hypoxylon lenormandii*
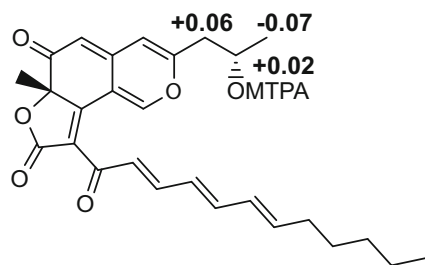
**Fig. 3** $\Delta\delta^{SR}$ values (ppm) of the MTPA derivatives of lenormandin A (**1**)

from multispore isolates according to Ju and Rogers (1996), but using yeast-malt-glucose-agar (YMG: yeast extract 4 g/l; malt extract 10 g/l; dextrose 4 g/l; cf. Bitzer et al. 2008). For examination of macro-morphology, the strains were grown on Difco Oatmeal Agar (OA), following the protocols by Ju and Rogers (1996). Conidiogeneous structures were observed by phase contrast microscopy and differential interference contrast under 400–1000×optical magnification. Anamorph types were classified according to Ju and Rogers (1996). Pigment colours were determined as in the latter monograph and, accordingly, colour codes follow Rayner (1970).

HPLC profiling

Stromatal methanol extracts were obtained according to the protocol of Stadler et al. (2001b). Samples were analyzed by using an analytical HPLC instrument (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 comprising standards of known secondary metabolites produced by Xylariaceae from previous work (Bitzer et al. 2007).

Molecular phylogenetic analyses

DNA isolation and generation of the ITS region of the ribosomal RNA (ITS) as well as the β-tubulin coding gene (TUB) was carried out as described by Kuhnert et al. (2014b). Some of the sequence data were generated at BIOTEC, Thailand, using the methodology as described by Læssøe et al. (2013). In total 15 ITS and 12 β-tubulin sequences of various *H. lenormandii* strains and closely related species were obtained. Each set of sequences was aligned with a selection of Xylariaceae sequences published in previous studies (Hsieh et al. 2005, Kuhnert et al. 2014a; for details see Table 1). The selection included 28 representatives of the genus *Hypoxylon*, seven *Annulohypoxylon* spp., four *Daldinia* spp. and one *Nemania* species. The phylogenetic analyses were performed for each gene as described by Kuhnert et al. (2014a) by using maximum likelihood (ML) as optimality criterion. Phylogenetic reconstruction of the ML tree was achieved 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.

Extraction and isolation of stromatal pigments

A large quantity of *Hypoxylon lenormandii* stromata (approx. 1 g) from the collection MFLU 13–0343 were detached from the substrate and extracted three times with 100 ml methanol in an ultrasonic bath for 30 min at 40 °C. The fungal material was removed by filtration, and then extracts were combined and evaporated. The crude extract was filtered again with methanol through a RP solid phase cartridge (Strata-X 33 mm, Polymeric Reversed Phase; Phenomenex, Aschaffenburg, Germany) to yield 250 mg crude product. The latter was divided into four portions and each subjected to preparative HPLC (PLC 2020, Gilson, Middleton, USA). A VP Nucleodur C18 ec column (125×20 mm, 7 μm; Macherey-Nagel) was used as stationary phase. The mobile phase was composed of deionised water (Milli-Q, Millipore, Schwalbach, Germany) as solvent A and acetonitrile (ACN) as solvent B. A flow rate of 15 ml min⁻¹ was used for the following gradient: 50–100 % solvent B in 35 min, afterwards isocratic conditions at 100 % for 10 min. UV detection was carried out at 210 and 254 nm and fractions were collected and combined according to the observed peaks. Most compounds proved to be unstable after processing with the exception of **2** (3.0 mg, lenormandin B) at a retention time (Rt)=19–20 min, compound **7** (1.0 mg, lenormandin G) at Rt=36–37 min and compound **6** (1.0 mg, lenormandin F) at Rt=44–45 min. Later stromata of the collection EBS 253 (0.5 g) were processed in the same way as described, but under exclusion of light to yield 100 mg crude product. Parameters of the preparative HPLC separation were similar as described for the collection MFLU 13–0343 with the following changes: linear conditions from 30 to 100 % solvent B in 40 min. Due to instability reasons only compound **5** (1.0 mg; lenormandin E) was obtained at Rt=28–29 min. To purify the instable main metabolites a mixture of stromata (1.2 g) from multiple collections (EBS 272, 202, 060, 228, 650, 722, 522, 661) was extracted three times with 100 ml acetone in an ultrasonic bath for 30 min at 40 °C. The combined extract was evaporated to yield an oily crude product. This was filtered in ACN through a RP solid phase cartridge. The resulting crude extract (100 mg) was absorbed on silica gel and eluted with ACN to separate the main compound lenormandin A from an yet unidentified secondary metabolite. The eluate was subjected to preparative HPLC under the same conditions as for MFLU 13–0343 with an extension of the isocratic condition at 100 % solvent B to 15 min. The fractionation yielded five

**Table 1** List of used taxa for chemical analysis and phylogenetic reconstruction. GenBank accession numbers, strain ID of public culture collections or herbaria (if available), origin and reference studies are given. Type specimens are labelled with **T** (holotype) or **ET** (epitype). Strains included in the chemical study are marked in bold

| Species | GenBank Acc No ß-tubulin | GenBank Acc No ITS | Specimen or strain ID | Origin | Reference |
|---|---|---|---|---|---|
| *Annulohypoxylon annulatum* | KC977276 | AM749938 | MUCL 47218 | China | Bitzer et al. (2008), Kuhnert et al. (2014a) |
| *A. bovei var. microspora* | AY951654 | EF026141 | BCRC 34012 | Taiwan | Hsieh et al. (2005) |
| *A. cohaerens* | AY951655 | – | BCRC 34013 | France | Hsieh et al. (2005) |
| | – | KC477233 | CBS 119126 | Austria | Stadler et al. (2013) |
| *A. minutellum* | AY951658 | – | BCRC 34016 | France | Hsieh et al. (2005) |
| | – | AJ390399 | ATCC 38977 | France | Sánchez-Ballesteros et al. (2000) as *Hypoxylon cohaerens* var. *microsporum* |
| *A. multiforme* | AY951661 | – | BCRC 34019 | France | Hsieh et al. (2005) |
| | – | KC477234 | CBS 119016 | Germany | Stadler et al. (2013) |
| *A. nitens* | KC977275 | KC968927 | CBS 119134 | Guadeloupe | Kuhnert et al. (2014a) |
| *A. stygium* | KC977304 | KC968940 | MUCL 54600 | French Guyana | Kuhnert et al. (2014a) |
| *Daldinia childiae* | AY951691 | – | BCRC 34044 | Hawaii (USA) | Hsieh et al. (2005) |
| | – | JX658462 | MUCL 46818 | France | Stadler et al. (2014) |
| *D. 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 aeruginosum* | KC977305 | KC968941 | MUCL 54620 | French Guiana (**ET**) | Kuhnert et al. (2014a) |
| *H. carneum* | AY951706 | JN660822 | BCRC 34052 | France | Hsieh et al. (2005) |
| *H. crocopeplum* | KC977268 | KC968907 | CBS 119004 | France | Kuhnert et al. (2014a) |
| *H. erythrostroma* | KC977296 | KC968910 | MUCL 53759 | Martinique | Kuhnert et al. (2014a) |
| *H. fendleri* | KF300547 | KF234421 | MUCL 54792 | French Guiana | Kuhnert et al. (2014a) |
| *H. fragiforme* | AY951719 | – | BCRC 34065 | France | Hsieh et al. (2005) |
| | – | AY616690 | CBS 114745 | Germany | Triebel et al. (2005) |
| *H. fraxinophilum* | KC977301 | KC968938 | MUCL 54176 | France (**ET**) | Kuhnert et al. (2014a) |
| *H. fuscum* | AY951723 | JN979423 | BCRC 34069 | Taiwan | Hsieh et al. (2005) |
| *H. griseobrunneum* | KC977281 | KC968928 | MUCL 53310, CBS 129346 | Guadeloupe | Kuhnert et al. (2014a) |
| *H. haematostroma* | KC977291 | KC968911 | MUCL 53301 | Martinique (**ET**) | Kuhnert et al. (2014a) |
| *H. hypomiltum* | KC977298 | KC968914 | MUCL 53312, CBS 129036 | Guadeloupe | Kuhnert et al. (2014a) |
| *H. investiens* | KC977293 | KC477239 | MUCL 53307, CBS 129034 | Martinique | Kuhnert et al. (2014a) |
| *H. isabellinum* | KC977295 | KC968935 | MUCL 53308, CBS 129035 | Martinique (**T**) | Kuhnert et al. (2014a) |
| **H. jaklitschii** | KM610304 | KM610290 | CBS 138916, MUCL 55383 | Sri Lanka (**T**) | This study |
| *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. lateripigmentum* | KC977290 | KC968933 | MUCL 53304, CBS 129031 | Martinique (**T**) | This study |
| **H. lenormandii** | KM610296 | KM610285 | STMA 14098 | Argentina | This study |
| **H. lenormandii** | KM610297 | KM610288 | STMA 14103 | Argentina | This study |
| **H. lenormandii** | KM610298 | KM610287 | STMA 14101 | Argentina | This study |
| **H. lenormandii** | KM610301 | KM610284 | STMA 14073 | Argentina | This study |
| **H. lenormandii** | KM610299 | KM610283 | STMA 14072 | Argentina | This study |
| **H. lenormandii** | KM610300 | KM610282 | STMA 14099 | Argentina | This study |

**Table 1** (continued)

| Species | GenBank Acc No ß-tubulin | GenBank Acc No ITS | Specimen or strain ID | Origin | Reference |
|---|---|---|---|---|---|
| *H. lenormandii* | KM610302 | KM610286 | STMA 14100 | Argentina | This study |
| *H. lenormandii* | – | – | F 10677 | Brazil | holotype of *Kretzschmaria stilbophora* |
| *H. lenormandii* | KM610295 | KM610281 | CBS 135869 | Cameroon | This study |
| *H. lenormandii* | – | – | F 7347 | Congo | Ju & Rogers (1996), holotype of *Rosellinia pulchella* |
| *H. lenormandii* | – | – | K(M) 120982 | Cuba | Ju & Rogers (1996) |
| *H. lenormandii* | KC977273 | KC968943 | CBS 119003 | Ecuador | Kuhnert et al. (2014a) |
| *H. lenormandii* | – | – | MJF 10016 (LIP) | Martinique | This study |
| *H. lenormandii* | – | – | F 10678 | Philippines | Ju & Rogers (1996), holotype of *Hypoxylon fulvoochraceum* |
| *H. lenormandii* | AY951732 | JN979430 | N/A | Taiwan | Hsieh et al. (2005) |
| *H. lenormandii* | AY951733 | JN979431 | BCRC34075 | Taiwan | Hsieh et al. (2005) |
| *H. lenormandii* | KM610305 | KM610289 | MFLU 13–0343, MFLUCC 13–0580, BCC 71961 | Thailand | This study |
| *H. lenormandii* | – | – | MFLU 13–0359, MFLUCC 13-0596 | Thailand | This study |
| *H. lenormandii* | – | KM610292 | BCC 31564 | Thailand | This study |
| *H. lenormandii* | – | KM610293 | BCC 32658 | Thailand | This study |
| *H. lenormandii* | – | KM610294 | BCC 55917 | Thailand | This study |
| *H. lenormandii* | – | – | F 7377 | US Virgin Islands (St. John) | Ju & Rogers (1996), syntype of *Rosellinia metachroa* |
| *H. lividicolor* | AY951734 | JN979432 | BCRC 34076 | Taiwan (**T**) | Hsieh et al. (2005) |
| *H. monticulosum* | KC977302 | KC968939 | MUCL 54626 | Martinique | Kuhnert et al. (2014a) |
| *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) |
| *H. pilgerianum* | AY951744 | JQ009310 | BCRC 34985 | Taiwan | Hsieh et al. (2005) |
| *H. rickii* | KC977288 | KC968932 | MUCL 53309, CBS 129345 | Martinique (**ET**) | Kuhnert et al. (2014a) |
| *H. rubiginosum* | AY951751 | – | BCRC 34116 | UK | Hsieh et al. (2005) |
|  | – | KC968929 | MUCL 52672 | Germany | Kuhnert et al. (2014a) |
| *H. rutilum* | AY951752 | JF908084 | BCRC 34092 | France | Hsieh et al. (2005) |
| *H. samuelsii* | KC977286 | KC968916 | MUCL 51843 | Guadeloupe (**ET**) | Kuhnert et al. (2014a) |
| *H. sublenormandii* | KM610303 | KM610291 | CBS 138917, MUCL 55384 | Sri Lanka | This study |
| *H. ticinense* | AY951757 | JQ009317 | BCRC 34097 | France | Hsieh et al. (2005) |
| *H. trugodes* | KF300548 | KF234422 | MUCL 54794 | Sri Lanka (**ET**) | Kuhnert et al. (2014a) |
| *Nemania serpens* | GQ470223 | – | N/A | Canada | Hsieh et al. (2010) |
|  | – | FN428829 | CBS 533.72 | Netherlands | Stadler et al. (2010) |

stable compounds with lenormandin A **1** (5.3 mg, lenormandin A) at Rt=26–27 min, compound **3** (1.5 mg, lenormandin C) at Rt=19–20 min, compound **4** (4.0 mg, lenormandin D) at Rt=21–22 min, compound **7** (2.8 mg) at Rt=40 min and compound **6** (1.5 mg) at Rt=45 min.

Lenormandin A (**1**): Yellow oil, $[\alpha]^{25}_D$ +1367 (*c* 0.1, CH$_3$CN); CD (CH$_3$CN) λ$_{max}$ (δε): 264 nm (+18.5), 356 nm (−20.8), 421 nm (+17.2); UV (MeOH) λ$_{max}$ (log ε) 227 nm (4.19), 268 nm (4.26) 342 nm (4.37); $^1$H NMR

(700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; electospray ionisation mass spectrometry (ESIMS) *m/z* 451.20 [M+H]$^+$, 449.17 [M-H]$^-$; high resolution electospray ionisation mass spectrometry (HRESIMS) *m/z* 451.2120 [M+H]$^+$ (calcd for C$_{27}$H$_{31}$O$_6$, 451.2115).

Lenormandin B (**2**): Yellow oil, $[\alpha]^{25}_D$ +99 (*c* 0.1, CH$_3$CN); CD (CH$_3$CN) λ$_{max}$ (δε): 237 nm (+2.0), 323 nm (−10.7), 358 nm (+10.2); UV (MeOH) λ$_{max}$ (log ε) 228 nm (3.98),

**Table 2** $^{13}$C NMR data of lenormandins A–G (**1–7**) in CHCl$_3$-$d$ (175 MHz)

| | A (**1**) | B (**2**) | C (**3**) | D (**4**) | E (**5**) | F (**6**) | G (**7**) |
|---|---|---|---|---|---|---|---|
| 1 | 153.4, CH | 147.6, CH | 153.3, CH | 153.2, CH | 147.6, CH | 153.9, CH | 153.9, CH |
| 3 | 159.1, C | 159.7, C | 159.0, C | 159.1, C | 159.7, C | 159.1, C | 159.1, C |
| 4 | 109.7, CH | 108.5, CH | 109.6, CH | 109.6, CH | 108.5, CH | 110.7, CH | 110.7, CH |
| 4a | 143.8, C | 144.3, C | 143.7, C | 143.7, C | 144.2, C | 142.3, C | 142.3, C |
| 5 | 105.4, CH | 106.0, CH | 105.4, CH | 105.4, CH | 106.1, CH | 107.4, CH | 107.4, CH |
| 6 | 190.6, C | 191.7, C | 190.4, C | 190.2, C | 191.8, C | 192.9, C | 192.9, C |
| 7 | 87.8, C | 82.9, C | 87.6, C | 87.7, C | 83.0, C | 84.1, C | 84.1, C |
| 8 | 165.4, C | 43.0, CH | 165.3, C | 165.3, C | 43.0, CH | 193.4, C | 193.4, C |
| 8a | 111.7, C | 114.7, C | 111.6, C | 111.7, C | 114.8, C | 115.3, C | 115.3, C |
| 9 | 26.2, CH$_3$ | 23.3, CH$_3$ | 26.2, CH$_3$ | 26.2, CH$_3$ | 23.3, CH$_3$ | 22.2, CH$_3$ | 22.2, CH$_3$ |
| 10 | 42.7, CH$_2$ | 42.9, CH$_2$ | 42.7, CH$_2$ | 42.7, CH$_2$ | 42.9, CH$_2$ | 42.6, CH$_2$ | 42.6, CH$_2$ |
| 11 | 65.4, CH | 65.5, CH | 65.3, CH | 65.3, CH | 65.6, CH | 65.5, CH | 65.5, CH |
| 12 | 23.7, CH$_3$ | 23.6, CH$_3$ | 23.7, CH$_3$ | 23.7, CH$_3$ | 23.7, CH$_3$ | 23.6, CH$_3$ | 23.6, CH$_3$ |
| 13 | 168.3, C | 168.9, C | 168.2, C | 168.2, C | 168.9, C | 173.2, C | 173.1, C |
| 14 | 124.3, C | 54.7, CH | 124.3, C | 124.3, C | 54.6, CH | 33.2, CH$_2$ | 33.2, CH$_2$ |
| 15 | 184.4, C | 190.1, C | 184.3, C | 184.8, C | 190.6, C | 24.7, CH$_2$ | 24.6, CH$_2$ |
| 16 | 125.7, CH | 126.6, CH | 125.7, CH | 124.9, CH | 126.1, CH | 28.8, CH$_2$ | 28.9, CH$_2$ |
| 17 | 146.4, CH | 146.6, CH | 146.3, CH | 146.6, CH | 147.0, CH | | |
| 18 | 128.6, CH | 127.9, CH | 128.6, CH | 129.2, CH | 128.8, CH | | 29.2–29.7, |
| 19 | 144.9, CH | 144.8, CH | 144.8, CH | 149.1, CH | 149.1, CH | | 3xCH$_2$ |
| 20 | 130.2, CH | 129.9, CH | 130.3, CH | 33.3, CH$_2$ | 33.3, CH$_2$ | 29.1- | 27.3, CH$_2$ |
| 21 | 142.9, CH | 142.9, CH | 142.4, CH | 28.2, CH$_2$ | 28.2, CH$_2$ | 29.8, | 25.7, CH$_2$ |
| 22 | 33.1, CH$_2$ | 33.1, CH$_2$ | 35.2, CH$_2$ | 31.3, CH$_2$ | 31.4, CH$_2$ | 9xCH$_2$ | 130.24, CH |
| 23 | 28.6, CH$_2$ | 28.5, CH$_2$ | 22.1, CH$_2$ | 22.4, CH$_2$ | 22.5, CH$_2$ | | 128.0, CH |
| 24 | 31.4, CH$_2$ | 31.4, CH$_2$ | 13.7, CH$_3$ | 14.0, CH$_3$ | 14.0, CH$_3$ | | 128.0, CH |
| 25 | 22.5, CH$_2$ | 22.5, CH$_2$ | | | | | 130.18, CH |
| 26 | 14.0, CH$_3$ | 14.0, CH$_3$ | | | | 32.0, CH$_2$ | 27.2, CH$_2$ |
| 27 | | | | | | 29.1, CH$_2$ | 29.1, CH$_2$ |
| 28 | | | | | | 14.1, CH$_3$ | 31.6, CH$_2$ |
| 29 | | | | | | | 22.6, CH$_2$ |
| 30 | | | | | | | 14.1, CH$_3$ |

238 nm (3.98) 334 nm (4.51); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; ESIMS $m/z$ 453.31 [M+H]$^+$, 451.23 [M-H]$^-$; HRESIMS $m/z$ 453.2295 [M+H]$^+$ (calcd for C$_{27}$H$_{33}$O$_6$, 453.2272).

Lenormandin C (**3**): Yellow oil, $[\alpha]^{25}_D$ +1414 ($c$ 0.1, CH$_3$CN); CD (CH$_3$CN) $\lambda_{max}$ ($\delta\varepsilon$): 264 nm (+13.6), 356 nm (−15.4), 422 nm (+12.1); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 228 nm (4.25), 266 nm (4.31) 338 nm (4.37); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; ESIMS $m/z$ 423.15 [M+H]$^+$, 421.11 [M-H]$^-$; HRESIMS $m/z$ 423.1826 [M+H]$^+$ (calcd for C$_{25}$H$_{27}$O$_6$, 423.1802).

Lenormandin D (**4**): Yellow oil, $[\alpha]^{25}_D$ +893 ($c$ 0.1, CH$_3$CN); CD (CH$_3$CN) $\lambda_{max}$ ($\delta\varepsilon$): 258 nm (+19.3), 298 nm (−9.5), 356 nm (−11.5), 421 nm (+11.7); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 264 nm (4.29) 330 nm (4.41); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3;

ESIMS $m/z$ 425.18 [M+H]$^+$, 423.12 [M-H]$^-$; HRESIMS $m/z$ 425.1966 [M+H]$^+$ (calcd for C$_{25}$H$_{29}$O$_6$, 425.1959).

Lenormandin E (**5**): Yellow oil, $[\alpha]^{25}_D$ +24 ($c$ 0.1, CH$_3$CN); CD (CH$_3$CN) $\lambda_{max}$ ($\delta\varepsilon$): 241 nm (+1.2), 274 nm (−1.6), 301 nm (−0.5), 327 nm (−2.1), 367 nm (+1.8); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 287 nm (3.70) 344 nm (3.59); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; ESIMS $m/z$ 427.27 [M+H]$^+$, 425.19 [M-H]$^-$; HRESIMS $m/z$ 427.2123 [M+H]$^+$ (calcd for C$_{25}$H$_{31}$O$_6$, 427.2115).

Lenormandin F (**6**): Yellow oil, $[\alpha]^{25}_D$ -38 ($c$ 0.1, CH$_3$CN); CD (CH$_3$CN) $\lambda_{max}$ ($\delta\varepsilon$): 231 nm (+2.0), 251 nm (−0.0), 274 nm (+2.1), 325 nm (−2.2); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 222 nm (4.00) 327 nm (4.04); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; ESIMS $m/z$ 489.35 [M+H]$^+$, 487.29 [M-H]$^-$; HRESIMS $m/z$ 489.3228 [M+H]$^+$ (calcd for C$_{29}$H$_{45}$O$_6$, 489.3211).

**Table 3**  $^1$H NMR data of lenormandin A–G (**1**–**7**) in CHCl$_3$-*d* (700 MHz)

| | A (**1**) | B (**2**) | C (**3**) | D (**4**) | E (**5**) | F (**6**) | G (**7**) |
|---|---|---|---|---|---|---|---|
| 1 | 8.88, d (0.9) | 7.41, d (0.9) | 8.88, brs | 8.85, d (0.9) | 7.40, d (0.9) | 7.88, d (0.9) | 7.88, d (0.9) |
| 4 | 6.18, s | 6.09, s | 6.18, s | 6.18, s | 6.08, s | 6.20, s | 6.20, s |
| 5 | 5.31, d (0.9) | 5.40, d (0.9) | 5.32, brs | 5.31, d (0.9) | 5.40, d (0.9) | 5.55, d (0.9) | 5.55, d (0.9) |
| 8 | | 3.97, m | | | 3.97, m | | |
| 9 | 1.71, s | 1.60, s | 1.71, s | 1.71, s | 1.60, s | 1.53, s | 1.53, s |
| 10 | 2.59, dd (14.8, 4.0)  2.54, dd (14.8, 8.2) | 2.54, dd (14.8, 4.0)  2.49, dd (14.8, 8.2) | 2.59, dd (14.8, 4.0)  2.53, dd (14.8, 8.2) | 2.59, dd (14.8, 4.0)  2.53, dd (14.8, 8.2) | 2.54, dd (14.6, 8.2)  2.49, dd (14.6, 4.2) | 2.56, dd (14.7, 4.7)  2.52, dd (14.7, 7.7) | 2.56, dd (14.7, 4.5)  2.52, dd (14.7, 8.0) |
| 11 | | 4.16, dqd (8.2, 6.3, 4.3) | 4.21, m | 4.21, m | 4.15, m | 4.17, dqd (7.7, 6.5, 4.7) | 4.17, dqd (8.0, 6.5, 4.5) |
| 12 | 1.34, d (6.3) | 1.30, d (6.3) | 1.34, d (6.0) | 1.34, d (6.0) | 1.30, d (6.5) | 1.32, d (6.5) | 1.32, d (6.5) |
| 14 | | 3.97, m | | | 3.97, m | 2.45, t (7.5) | 2.45, t (7.5) |
| 15 | | | | | | 1.63, m | 1.63, quin (7.5) |
| 16 | 7.05, d (15.1) | 6.43, d (15.3) | 7.05, d (15.1) | 6.97, d (15.1) | 6.38, d (15.5) | 1.32, m | 1.31, m |
| 17 | 7.42, dd (15.1, 11.6) | 7.31, dd (15.3, 11.4) | 7.42, dd (15.1, 11.6) | 7.36, dd (15.1, 10.2) | 7.25, m | | 1.26 |
| 18 | 6.35, dd (14.8, 11.6) | 6.28, dd (14.6, 11.4) | 6.35, dd (14.6, 11.6) | 6.31, m | 6.25, dd (14.8, 11.0) | | – |
| 19 | 6.67, dd (14.8, 10.8) | 6.66, dd (14.6, 10.8) | 6.68, dd (14.6, 10.8) | 6.31, m | 6.31, dt (14.8, 7.0) | | 1.38, m |
| 20 | 6.20, dd (14.8, 10.8) | 6.18, dd (15.1, 10.8) | 6.20, dd (14.8, 10.8) | 2.21, m | 2.20, q (7.0) | 1.26 | 1.35, m |
| 21 | 6.03, dt (14.8, 7.3) | 6.03, dt (15.1, 7.2) | 6.03, dt (14.8, 7.3) | 1.45, quin (7.4) | 1.44, m | – | 2.77, brt (7.1) |
| 22 | 2.17, q (7.3) | 2.16, q (7.3) | 2.16, q (7.3) | 1.27, m | 1.28, m | 1.39, m | 5.39, m |
| 23 | 1.43, tt (7.3, 7.2) | 1.43, tt (7.3, 7.2) | 1.46, m | 1.30, m | 1.31, m | | 5.35, m |
| 24 | 1.29, m | 1.29, m | 0.93, t (7.4) | 0.90, t (7.0) | 0.89, m | | 5.35, m |
| 25 | 1.30, m | 1.31, m | | | | | 5.40, m |
| 26 | 0.90, t (7.2) | 0.90, t (7.2) | | | | 1.26, m | 2.05, m |
| 27 | | | | | | 1.29, m | 1.31, m |
| 28 | | | | | | 0.89, t (7.1) | 1.29, m |
| 29 | | | | | | | 1.31, m |
| 30 | | | | | | | 0.90, t (7.1) |

Lenormandin G (**7**): Yellow oil, $[\alpha]^{25}_{D}$ -53 (*c* 0.1, CH$_3$CN); CD (CH$_3$CN) $\lambda_{max}$ ($\delta\varepsilon$): 233 nm (+3.8), 273 nm (+4.9), 323 nm (−4.8); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 223 nm (4.28) 328 nm (4.35); $^1$H NMR (700 MHz, CDCl$_3$) see Table 2, $^{13}$C NMR (175 MHz, CDCl$_3$) see Table 3; ESIMS *m/z* 513.36 [M+H]$^+$, 511.30 [M-H]$^-$; HRESIMS *m/z* 513.3226 [M+H]$^+$ (calcd for C$_{31}$H$_{45}$O$_6$, 513.3211).

## Structure elucidation

Optical rotations were determined with a PerkinElmer 241 polarimeter, UV–vis spectra were recorded with a Shimadzu (Kyoto, Japan) UV–vis spectrophotometer UV-2450. NMR spectra were recorded with a Bruker (Bremen, Germany) Ascend 700 spectrometer with a 5 mm TXI cryoprobe ($^1$H 700 MHz, $^{13}$C 175 MHz). ESIMS spectra were obtained with an ion trap MS (Amazon, Bruker), HRESIMS spectra were obtained with a time-of-flight MS (Maxis, Bruker) as described by Pažoutová et al. (2013).

## Synthesis of (*R*)- and (*S*)-MPTA esters of **1**

For the preparation of the (*S*)-MPTA ester 0.5 mg of **1** was dissolved in 600 μL pyridine-*d$_5$* and 5 μL (*R*)-MPTA chloride was added. The mixture was kept at 25 °C for 15 min before measurement of proton and TOCSY NMR spectra; $^1$H NMR (pyridine-*d$_5$*, 700 MHz, only signals for derivatized 2-hydroxypropyl moiety assigned): δ 1.35 (d, 6.5, H$_3$-12), 2.84 (m, H-10b), 2.88 (m. H-10a), 5.60 (m, H-11). The (*R*)-MPTA ester was prepared in the same manner by the addition of 7 μL

(S)-MPTA chloride; $^1$H NMR (pyridine-$d_5$, 700 MHz, only signals for derivatized 2-hydroxypropyl moiety assigned): δ 1.42 (d, 6.5 Hz, H$_3$-12), 2.78 (m, H-10b), 2.82 (m. H-10a), 5.58 (m, H-11).

Bioactivity assay

Minimum inhibitory concentrations (MIC) were recorded in a serial dilution assay with pure substances dissolved in acetonitrile using various test organisms for antibacterial and antifungal activity (Halecker et al. 2014). The *in vitro* cytotoxicity assay with the established mouse fibroblast cell line L929 was performed as reported by Okanya et al. (2011). As a minor change the solvent was replaced by acetonitrile.

# Results

Taxonomic part

In the following, one new species of *Hypoxylon* is described and illustrated (Fig. 4) based on morphological and chemotaxonomic data. Furthermore an updated description of *H. lenormandii sensu lato* is given (Fig. 5). A comparison of stromatal HPLC profiles of the new taxon and closely related species is depicted in Fig. 6.

**Hypoxylon jaklitschii** Sir & Kuhnert, **sp. nov.**
MycoBank MB810265. Fig. 4

*Holotype:* **Sri Lanka**, Central Province, Sigiriya, on wood of broad-leaved tree, 16 Jan. 2013, leg. W. Jaklitsch JF13037 (MUCL, ex-type cultures CBS 138916 & MUCL 55383; GenBank Acc. No: ITS – KM610290, ß-tubulin – KM610304).

*Etymology*: In honour of our colleague, the Austrian mycologist Walter Jaklitsch, who collected the type specimen.

*Known distribution/host preference of stromata*: Only known from the holotype.

*A Hypoxylo lenormandii differt superficiem stromatibus rufobrunneis, peritheciis parviores (0.3–0.4 mm diametro), ascis brevistipitatis, partibus sporiferis parviores (75–92 μm×5.5–7 μm), usque at ascosporibus 9.5–11.5(−12)× 4–5.5 μm.*

*Sexual morph: Stromata* glomerate to effused-pulvinate, with the tendency to be perithecioid, with very conspicuous perithecial mounds, 1–15 mm long×0.7–8 mm broad×0.5–1 mm thick, surface Sepia (63) to Dark Brick (66), pruinose, with orange brown or dark brown granules immediately beneath surface and between perithecia, with KOH extractable pigment Umber (9) or Dark Brick (66), tissue bellow the perithecial layer inconspicuous, brown, 0.2–0.4 mm thick. *Perithecia* spherical, 0.3–0.4 mm diam, ostioles lower than the stromatal surface, umbilicate or with very small black

papilla. *Asci* 8-spored, cylindrical, 120–175 μm total length, the spore-bearing parts 75–92 μm×5.5–7 μm broad, stipes 40–100 μm long, with amyloid, discoid apical apparatus 1 μm high×2(−2.5) μm broad. *Ascospores* brown to dark brown, ellipsoid-inequilateral, with narrowly rounded ends, slightly curved, 9.5–11.5(−12)×4–5.5 μm, with slightly sigmoid germ slit spore length on convex side, perispore dehiscent in KOH, conspicuous coil-like ornamentation, epispore smooth.

*Cultures and asexual morph*: Colonies on OA covering Petri dish in 4 weeks, at first whitish, becoming Olivaceous (48) velvety to felty, faintly zonated, with entire margins; reverse Ochraceous (44) to Olivaceous (107). Sporulating regions scattered over entire surface of the colony, Hazel (88). *Conidiogenous structures* nodulisporium-like, around or close to the young stromata. *Conidiophores* brown vinaceous to pale brown vinaceous, finely roughened. *Conidiogenous cells* hyaline to pale brown vinaceous, finely roughened, 13–22× 2.5–3 μm. *Conidia* ellipsoid, hyaline, smooth to finely roughened, 4.5–5×2.5–3 μm.

*Secondary metabolites*: Stromata contain a lenormandin-like compound as major metabolite with a *m/z* of 453.2276 [M+H]+(calcd for $C_{27}H_{33}O_6$, 453.2272) identical to lenormandin B but with a different retention time. Lenormandin A (**1**) can be detected in traces. In addition multiple unknown lenormandin derivatives besides another unknown compound class are present as minor metabolites [e.g. *m/z* of 374.1964 [M+H]+(calcd for $C_{21}H_{28}NO_5$, 374.1962), *m/z* of 360.1807 [M+H]+(calcd for $C_{20}H_{26}NO_5$, 360.1805), *m/z* of 346.2016 [M+H]+(calcd for $C_{20}H_{28}NO_4$, 346.2013)].

*Notes*: *Hypoxylon jaklitschii* is morphologically similar to *H. lenormandii*, but the stromatal surface colour of the new taxon is Sepia (63) to Dark Brick (66) rather than Grayish Sepia (102), Fuscous (103) or Brown Vinaceous (84) as stated by Ju and Rogers (1996) for *H. lenormandii*. *Hypoxylon jaklitschii* also has slightly smaller perithecia (0.3–0.4 *vs* 0.3–0.5 mm diam), and the dimensions of the spore-bearing parts of its asci are larger (70–140×6–9 *vs* 75–92×5.5–7 μm). Consequently, the ascospores of *H. lenormandii* are also larger (9.5–12×4–5.5 *vs* 11–17×4–6.5; cf. updated description of *H. lenormandii* below). In addition, the ornamentation of the perispore appears more conspicuous in *H. jaklitschii* under the light microscope. No known secondary metabolites besides BNT where detected in the stromata except for the lenormandins A (**1**), F (**6**) & G (**7**) which were only found in traces. Instead a yet unidentified lenormandin derivative was present in large amounts, which was not found in any other specimens of *H. lenormandii* (or, for that matter, in any other species of *Hypoxylon* that we have studied previously). Furthermore, all studied specimens of *H. lenormandii* contain lenormandin A (**1**) as major metabolite and were devoid of BNT. The unique stromatal HPLC profile as well as spore and

**Fig. 4** *Hypoxylon jaklitschii*, from holotype specimen JF13037. **a** Stromatal habit. **b** Extractable pigments in KOH. **c**: Close-up view of stromatal surface. **d**: Close-up view of stromatal surface showing small ostioles. **e**: Section through stroma showing perithecia. **f**: Conidiophores from natural substrate. **g**: Asci. **h**: Apical ring bluing in Melzer's iodine reagents. **i**: Ascospores showing sigmoid germ slit (black arrow). **j**: Ascospores with perispore dehiscent in KOH (black arrow). **k**–**m**: SEM microphotographs of the conspicuous ornamented perispore. Scale is indicated by bars (**a**, **b**: 2 mm. **c**, **d**, **e**: 1 mm. **f**, 20 μm. **g**, **h**, **i**, **j**, : 10 μm. **k**, **l**: 4 μm. **m**: 200 nm)
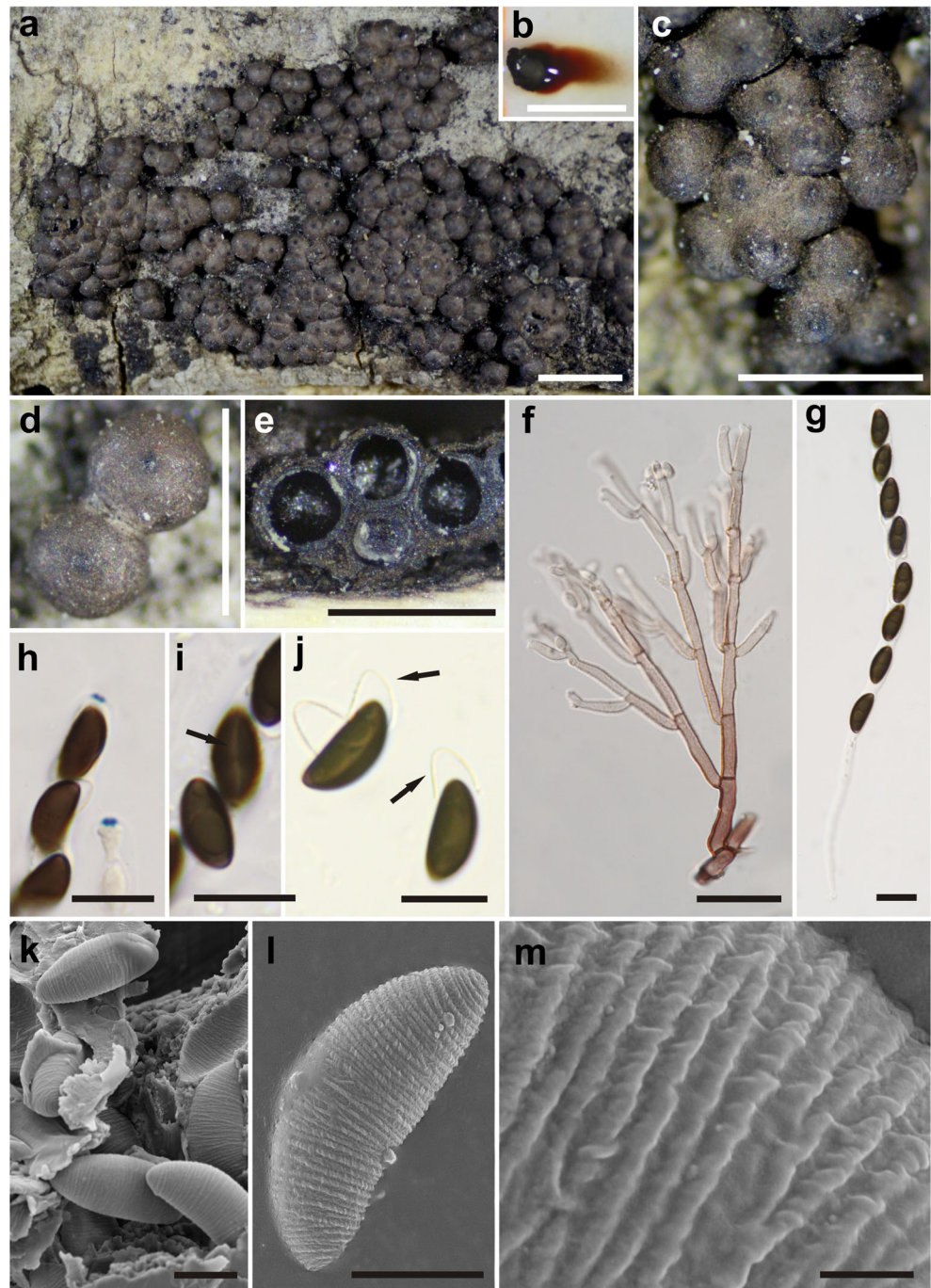
asci size ranges set *H. jaklitschii* apart from its closest relatives, which justifies the erection of a new species.

Notably, we studied the type material of *H. oodes* from Sri Lanka, which is also a synonym of *H. lenormandii* fide Ju and Rogers (1996). The type material is depauperate. We did not see any intact ascospores and could not even obtain conclusive results by HPLC-MS on its secondary metabolite profile.

Albeit the possibility exists that *H. oodes* might be an earlier name eligible for the taxon that is here newly introduced,

we prefer to follow Ju and Rogers (1996), who have reported the ascospore size range to be in accordance with their concept of *H. lenormandii*.

An additional species that apparently belongs to this group of *Hypoxylon*, *H. olliforme*, was erected by San Martin et al. (1999), based on a specimen collected in Mexico. The habit of the ascomata is reminiscent to that of *H. lenormandii*, but it was also reported to have rather small ascospores (10.5–12× 4.5–5 μm). It varies from *H. jaklitschii* in the surface colour

**Fig. 5** *Hypoxylon lenormandii*, from specimen EBS272. **a** and **b** Stromatal habit. **c** Close-up view of stromatal surface **d** Sections through stromata showing perithecia. **e** Extractable pigments in KOH. **f** Asci. **g** Ascus tip in Melzer's reagent. **h** Perispore. **i** Ascospores. **j** Ascospores showing germ slit. **k** SEM microphotographs of the inconspicuous ornamented perispore. **l** Anamorph. Scale is indicated by bars (**a**: 3 mm. **b**, **c**, **d**: 1 mm. **e**, **f**, **g**, **h**, **i**, **j**: 10 μm. **k**: 2 μm. **l**: 20 μm)
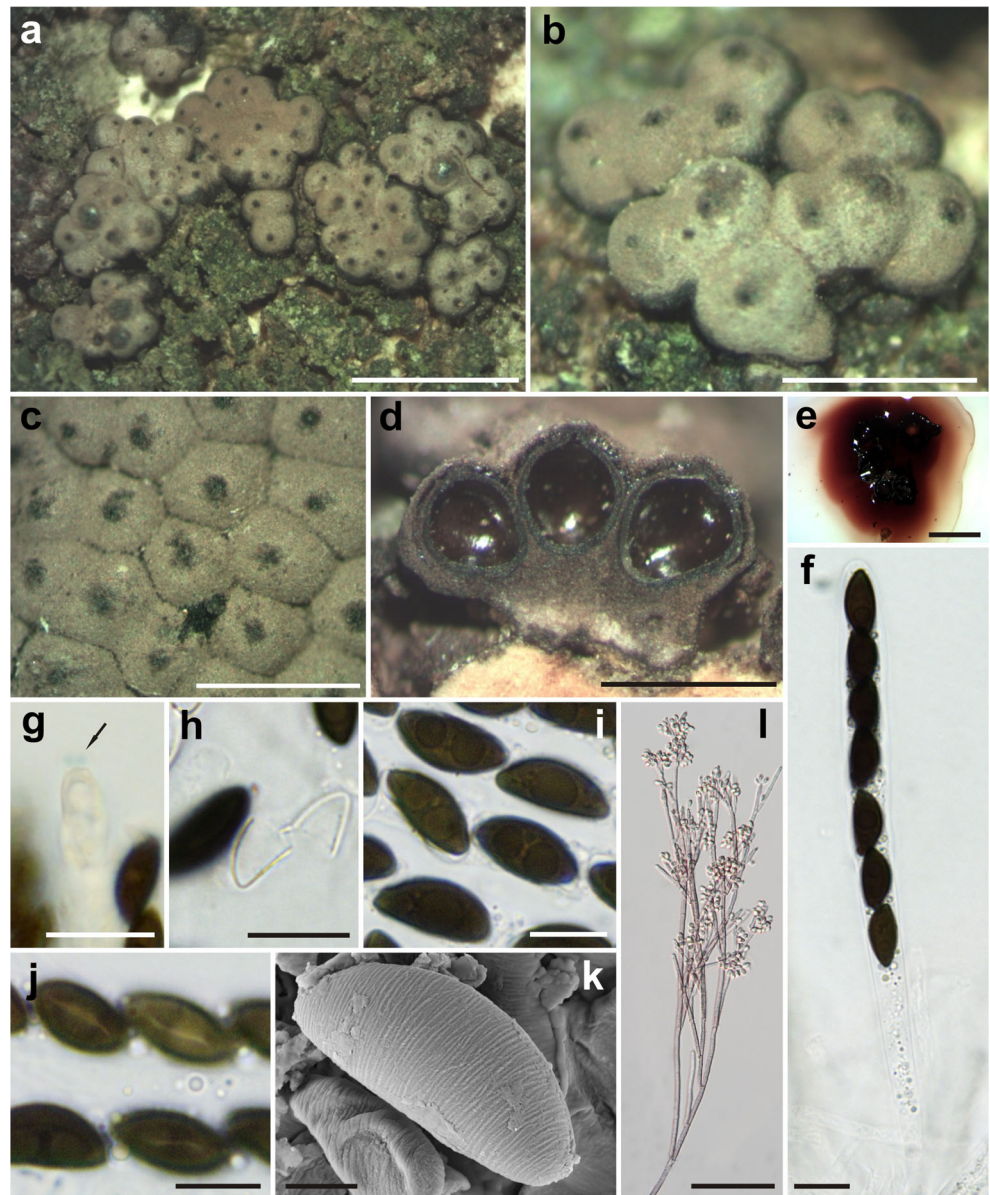
(brownish red to reddish orange in *H. olliforme*) as well as in the colour of the granules (red vs. orange brown to dark brown). Furthermore there is no report about conspicuously ornamented ascospores, which is a salient feature of *H. jaklitschii*.

Moreover, the "holotype specimen" of *H. olliforme*, was deposited in the personal herbarium of the senior author of the cited publication according to the protologue (which raises some doubt whether it was validly described). The material does not seem to be available in the public domain because we did not receive it on request from the ICTV herbarium, where other specimens of F. San Martín are kept. Therefore, no chemical and molecular phylogenetic data are available on this species for now.

An emended description of *H. lenormandii* is given below.

***Hypoxylon lenormandii*** Berk. & M.A. Curtis [as 'lenormandi'], in Berkeley, J. Linn. Soc., Bot. 10 (no. 46): 385 (1868) [1869] emend. Sir & Kuhnert

MycoBank MB 152747 Fig. 5

=*Sphaeria subaenea* Berk. & M. A. Curtis apud Berk., J. Linn. Soc., Bot. 10: 387. 1869

≡*Rosellinia subaenea* (Berk. & M. A. Curtis) Sacc. Syll. Fung. I, p. 256. 1882

≡*Hypoxylon subaeneum* (Berk. & M. A. Curtis) Speg., Anales Soc. Ci. Argent. 26: 32. 1888

=*Hypoxylon oodes* Berk. & Broome, J. Linn. Soc. Bot. 14: 122. 1873

=*Hypoxylon subvinosum* Speg., Anales Soc. Ci. Argent. 18: 269. 1884.

=*Rosellinia melaleuca* Ellis & Everh. apud C. L. Smith, Bull. Iowa Univ. Lab. Nat. Hist. 2: 402. 1893

=*Rosellinia metachroa* Ferd. & Winge, Bot. Tidsskr. 29: 16. 1908

=*Hypoxylon riograndense* Rehm, Ann. Mycol. 7: 154. 1909

=*Rosellinia pulchella* Syd. & P. Syd. apud Wildeman, Fl. Bas-Moyen-Congo III, p.7. 1909

=*Hypoxylon fulvoochraceum* Rehm, Philipp. J. Sci. 8. 188. 1913.

*Type:* **Cuba**, decorticated wood, C. Wright 486 (K(M) 120982, isotype).

*Known distribution/host preference of stromata:* Widely distributed in warmer climates. Occurs on monocots and dicots.

*Sexual morph: Stromata* glomerate to effused-pulvinate; with the tendency to be perithecioid (approaching rosellinioid) but united by very thin stromatal tissue, usually confluent, 13–85 mm long×6–27 mm broad×0.5–1 mm tick; with very conspicuous perithecial mounds; surface Grayish Sepia (106), Fuscous (103) or Brown Vinaceous (84); pruinose; dull orange brown or dark brown granules immediately beneath surface and between perithecia; with KOH-extractable pigments Blood Colour (3), Umber (9), or Dark Vinaceous (82); the tissue below the perithecial layer brown or dark brown, 0.2–0.8 mm. *Perithecia* spherical or obovoid 0.4–0.7 mm high× 0.3–0.6 mm diam; ostioles slightly higher than the stromatal surface or with small papilate. *Asci* 8-spored, cylindrical, 126–189 μm total length, the spore-bearing parts 68–106 μm long×5.5–8 μm broad, the stipes 42–105 μm long, with amyloid, discoid apical apparatus 0.7–1.5 high×2–3.5 μm broad. *Ascospores* brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 11–15(−17)×4–6 (−6.5) μm; with slightly sigmoid to sigmoid germ slit spore-length on convex side; perispore dehiscent in KOH, with inconspicuous coil-like ornamentation; epispore smooth.

*Cultures and asexual morph*: Colonies in OA covering Petri dish in 3 weeks, at first white, becoming Hazel (88) to Grayish Sepia (106) velvety felty, slightly zonate, with entire margins; reverse Fuscous (103) eventually with Dull Green (70) tones. Sporulating regions scattered over entire surface of colony, Hazel (88). *Conidiogenous structure* nodulisporium-like, arranged in palisades. *Conidiophores* reddish-brown, finely roughened. *Conidiogenous cells* reddish-brown colour, finely roughened, 10–60×2–3.5(−4) μm. *Conidia* ellipsoid, hyaline, generally smooth, (3.5–)4–6×2–3.5 μm.

*Secondary metabolites:* Stromata contain lenormandin A (**1**) as major metabolite and lenormandin F (**6**) and G (**7**) as minor lipophilic compounds. Depending on the origin and the age of stromata lenormandins B–E can be found in varying quantities. Fresh specimens showed in addition a characteristic metabolite with a *m/z* of 386.1970 [M+H]+(calcd for $C_{22}H_{28}NO_5$, 386.1962) overlaid by lenormandin A (**1**). In

**Fig. 6** Stromatal HPLC-UV profiles (200–600 nm) of various specimens ▶ of *Hypoxylon lenormandii* (**a–d**), *H. sublenormandii* (**e**) and the holotype of *H. jaklitschii* (**f**). The DAD spectra of lenormandin A (**1**), lenormandin G (**7**) and two characteristic unknown compounds of *H. jaklitschii* (UC 1 & UC 2) are displayed at the top. The major metabolite of the latter is an unknown lenormandin derivative (ULD). **2**—lenormandin B, **3**—lenormandin C, **4**—lenormandin D, **6**—lenormandin F, **PA**—preservative agent

some specimens (in particular in material from Thailand), the binaphthalene tetrol was also found, but never constituted the major metabolite.

*Specimens examined*: ASIA: **Indonesia,** Java, Bogor, Hortus Bogorensis. Aug. 1898. E. Nyman (S, Type of *Rosellinia bogorensis*). **Philippines**, Los Baños, 14 Sep. 1912, C.F. Baker 50, dead culms of *Bambusa* (S, Holotype of *Hypoxylon fulvoochraceum*). **Thailand**, Chiang Rai Province, Chiang Rai, Mae Fah Luang University, on dead wood of *Albizia chinensis*, 14 Aug. 2013, E. Kuhnert EK13001 (MFLU 13–0343, cultures MFLUCC 13–0580 and BCC 71961; GenBank Acc. No: ITS – KM610289, ß-tubulin – KM610305); same locality, on dead wood, 16 Aug. 2013, leg. J. Baht, EK13017 (MFLU 13–0359, culture MFLUCC 13–0596). AFRICA: **Democratic Republic of the Congo**, Kisantu. 31 Jan. 1907, H. Vanderyst (S, Type of *Rosellinia pulchella*). AMERICA: **Argentina**, Jujuy, Depto. Ledesma, National Park Calilegua, 11 May 2012, Sir & Hladki 017 (LIL, cultured; GenBank Acc. No: ITS – KM610285, ß-tubulin – KM610296); same locality, El Negrito, 11 May 2012, Sir & Hladki 060 (LIL, cultured; GenBank Acc. No: ITS – KM610288, ß-tubulin – KM610297); same locality, on the path "La Junta", 27 Apr. 2014, Sir & Hladki 650, 661, 666 (LIL, 650: cultured; GenBank Acc. No: ITS – KM610286, ß-tubulin – KM610302). Jujuy, Depto. Santa Barbara, Provincial Reserve "Las Lancitas", 13 May 2012, Sir & Hladki 253, 272 (LIL, 253: cultured, GenBank Acc. No: ITS – KM610284, ß-tubulin – KM610301; 272: cultured, GenBank Acc. No: ITS – KM610283, ß-tubulin – KM610299). Salta, Depto. Anta, National Park El Rey, on the path "El chorro de los Loros", 14 May 2012, Sir & Hladki 202 (LIL); same locality, Laguna de los Patitos, 15 May 2012, Sir & Hladki 228 (LIL, cultured; GenBank Acc. No: ITS – KM610287, ß-tubulin – KM610298); same locality, on the path "El chorro de los loros", 29 Apr. 2014, Sir & Hladki 703, 722 (LIL). Salta, Depto. General José de San Martin, Provincial Reserve Flora and Fauna Acambuco, 22 Apr. 2014, Sir & Hladki 522 (LIL); same locality, 23 Apr. 2014, Sir & Hladki 539 (LIL). Salta, Depto. La Candelaria, 03 May 2013, Sir & Hladki 402 (LIL, cultured; GenBank Acc. No: ITS – KM610282, ß-tubulin – KM610300). Salta, Depto. Orán, on the path to Isla de Cañas, 24 Apr. 2014, Sir & Hladki 596 (LIL). Tucumán, Depto. Capital, garden of Miguel Lillo Foundation, 8 Oct. 2007, Hladki 4011 (LIL). **Brazil**, Rio Grande do Sul, São Leopoldo, 23 May 1906, Rick (S,
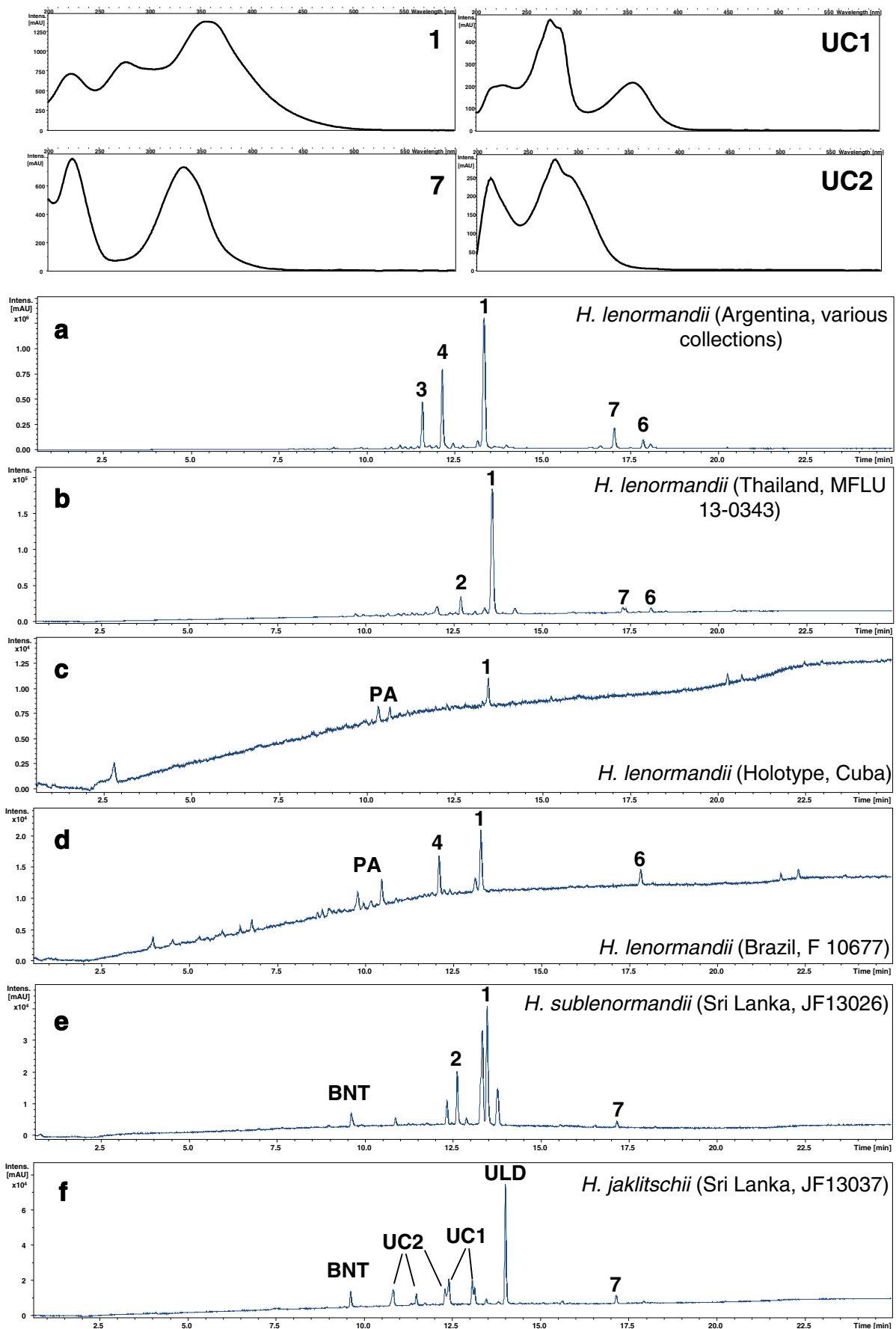
Holotype of *Kretzschmaria stilbophora*). **Cuba**, on wood, J. Wright 485, K(M) 120982, isotype of *H. lenormandii*; immature specimen containing lenormandins but no ascospores). **Martinique**: Prêcheur, Anse Couleuvre, on dicot bark, 27 Aug. 2010, J. Fournier MJF10016 (LIP). **Paraguay**, Guarapí, on logs, Nov. 1881, Speg. (LPS; Holotype of *Hypoxylon subvinosum*). **US Virgin Islands**, St. John, American Hill, 17 Mar. 1906. C. Raunkiaer (S, Type of *Rosellinia metachroa*).

*Notes:* The studied collections show the typical characteristics of *H. lenormandii*, as reported by Ju and Rogers (1996). However, according to our own observations, the perithecia are often spherical, rather than obovoid, and in some specimens, small ostiolar papilla were observed. In addition they have Blood Colour (3) and Dark Vinaceous (84) pigments in KOH, which was not reported by Ju and Rogers (1996) for this species, and the ascospores we observed were slightly longer than the dimensions given in the aforementioned monograph (9.5–16×4–7 vs 11–17×4–6.5). Hladki and Romero (2009) observed the nodulisporium-like anamorphs on the surface of specimens collected in Tucuman province, Argentina. Similar structures were found in the collections Sir and Hladki 255, 253 from Jujuy province. However, in culture and natural substrate the conidiophores were reddish-brown and arranged in palisades (see Fig. 5). In contrast, Ju and Rogers (1996) stated that the conidiogenous structures they observed in culture are hyaline to yellowish. A problem could arise from the fact that the isotype specimen of *H. lenormandii,* which was collected from Cuba, contains only largely immature stromata, and we did not observe any ascospores in the material. It remains unclear whether the holotype is in better condition, but Ju and Rogers (1996) cited a specimen from Kew as "holotype", whereas the material we were sent on our request was labelled "isotype". We trust that these authors had reported if the specimen they studied was immature.

In any case, *H. sublenormandii* as well as the new taxon *H. jaklitschii* seem to be different from all specimens we studied of *H. lenormandii* s. Ju and Rogers (1996).

We propose that in the near future, a diligent search for material from the Carribbean should be carried out and specimens from various locality compared. Finally, an epitype should be selected for this name, based on suitable material from Cuba or another part of the Caribbean that can be cultured, studied for secondary metabolites and sequenced.

Molecular phylogeny

In total the phylogenetic study contained 57 sequences of various Xylariaceae sequences derived from the ITS rRNA region and 54 sequences from the β-tubulin coding gene (Figs. 7 and 8). The majority of sequences belonged to the genus *Hypoxylon* (45 and 42, respectively). The overall support of the backbone of both phylogenetic trees is weak. In the TUB calculation the genus *Daldinia* forms a highly BS supported monophyletic clade (92 %), which is, however, not confirmed by the ITS reconstruction. The genus *Annulohypoxylon* appears paraphyletic in the ITS tree and monophyletic in the TUB tree with moderate BS support (52 %). All sequences of *H. lenormandii* together with their close relatives *H. jaklitschii* and *H. sublenormandii* form well supported clades in both trees (78 % for ITS and 97 % for TUB). Within these clades *Hypoxylon sublenormandii* emerges as a basal lineage. Both genealogies are in accordance with each other in respect to the substructure of the *H. lenormandii* clade by dividing the latter into three groups, which is also confirmed by high BS support values. The first subclade is exclusively formed by sequences derived from Asian specimens including *H. jaklitschii.* The second one is build up by sequences from two Argentine and one African specimens. The remaining sequences derived from material collected in South America cluster together in a third subclade.

Structure elucidation of lenormandins A-G

The major compound lenormandin A (**1**) was obtained as yellow oil. Its molecular formula was deduced as $C_{27}H_{30}O_6$ by HRESIMS, indicating 13 degrees of unsaturation. The UV spectrum with absorption maxima at 226, 268 and 342 nm gave an early evidence for an azaphilone backbone (Gao et al. 2013), which is a common scaffold for pigments of related *Hypoxylon* species. The $^1H$ and $^1H,^{13}C$ HSQC spectra prominently showed signals of three methyls (as a singulet, a doublet, and a triplet), nine olefinic protons, an oxygenated methine, and five methylene groups. In addition, the $^{13}C$ NMR spectrum revealed the presence of two conjugated ketones, an ester carbonyl, one oxygenated $sp^3$ hybridized quaternary and five quaternary olefinic carbons. $^1H,^{13}C$ HMBC correlations from H-1 to C-3, C-4a, C-8; from H-4 to C-3, C-5, C-8a, C-10; from H-5 to C-4, C-6, C-8a and from $H_3$-9 to C-6, C-7, C-8 identified an azaphilone skeleton joined with a nonlinear five-membered ring lactone. Analysis of $^1H,^{13}C$ HMBC and $^1H,^1H$ COSY spectra moreover indicated the presence of two partial structures of **1** connected to the azaphilone core at C-3 and C-18. The COSY spectrum established the substructures from C-10 to C-12 as 2-hydroxypropyl- and from C-15 to C-26 as dodeca-2,4,6-trienoyl- substituents. Large vicinal coupling constants of $J_{16,17}=15.1$ Hz, $J_{18,19}=14.8$ Hz , and $J_{20,21}=14.8$ Hz revealed an all-*trans* configuration of the trien bonds. The assignment of the absolute configuration of C-7 of azaphilones by circular dichroism has been firmly established by X-ray analysis (Steyn and Vleggaar 1976, Whalley et al. 1976) and total synthesis (Clark et al. 2008). The CD spectrum of
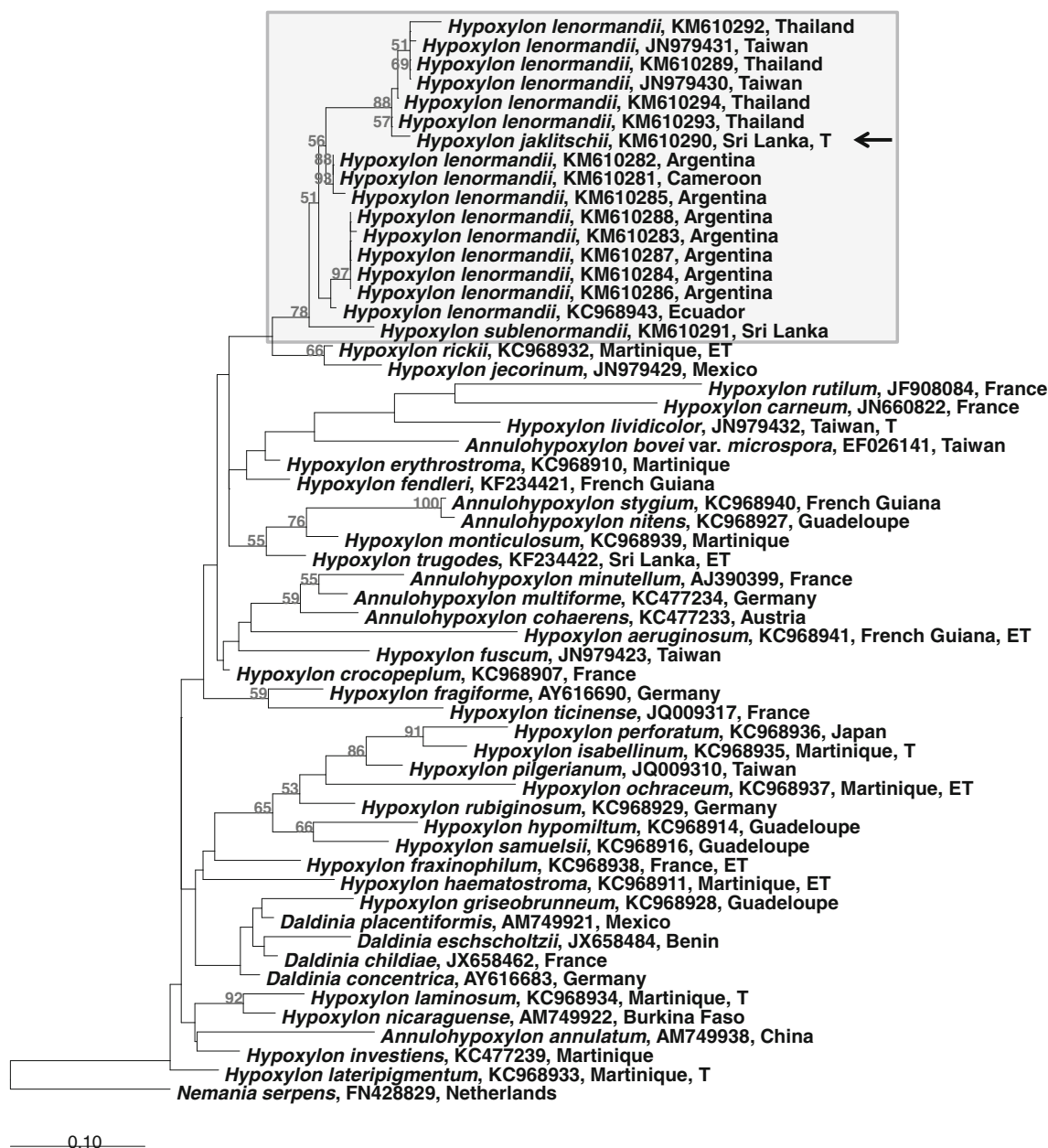
**Fig. 7** Phylogenetic relationships among *Hypoxylon lenormandii* and related Xylariaceae as inferred from internal transcribed spacer (ITS) rRNA gene sequences. The highlighted clade indicates the *H. lenormandii* group. Likelihood (ML) bootstrap support values above 50 %, from 1000 RAxML replicates 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. Sequence of the newly described species is indicated by an arrow. Type species are indicated by T (holotype) and ET (epitype)

lenormandin A (**1**) ($\Delta\varepsilon_{356}$ -20.8) clearly showed *S* configuration of C-7. Finally, we addressed the absolute configuration of C-11 by the synthesis of Mosher's esters. Because of the instability of azaphilone type compounds we performed the reaction of lenormandin A (**1**) with trifluoromethylphenyl chlorides (MPTA-Cl) in pyridine-$d_5$ and recorded $^1$H and $^1$H,$^1$H TOCSY NMR spectra without prior purification. The observed $\Delta\delta^{SR}$ values (Fig. 3) indicated the absolute configuration of C-11 is *S*. From the above evidence, the structure of

lenormandin A (**1**) was established as (6a*S*)-9-[(2*E*,4*E*,6*E*)-dodeca-2,4,6-trienoyl]-3-[(2*S*)-2-hydroxypropyl]-6a-methyl-6*H*-furo[2,3-*h*]isochromene-6,8(6a*H*)-dione.

With lenormandin B (**2**) a by-product was isolated as yellow oil. Its molecular formula $C_{27}H_{32}O_6$ was determined by HRESIMS, implying the formal addition of two protons compared to **1**. The UV spectrum showed maxima at 224, 250 and 334 nm; the hypsochromic shift (−4 nm) of the longest absorption maximum indicated a shorter conjugated π-system.
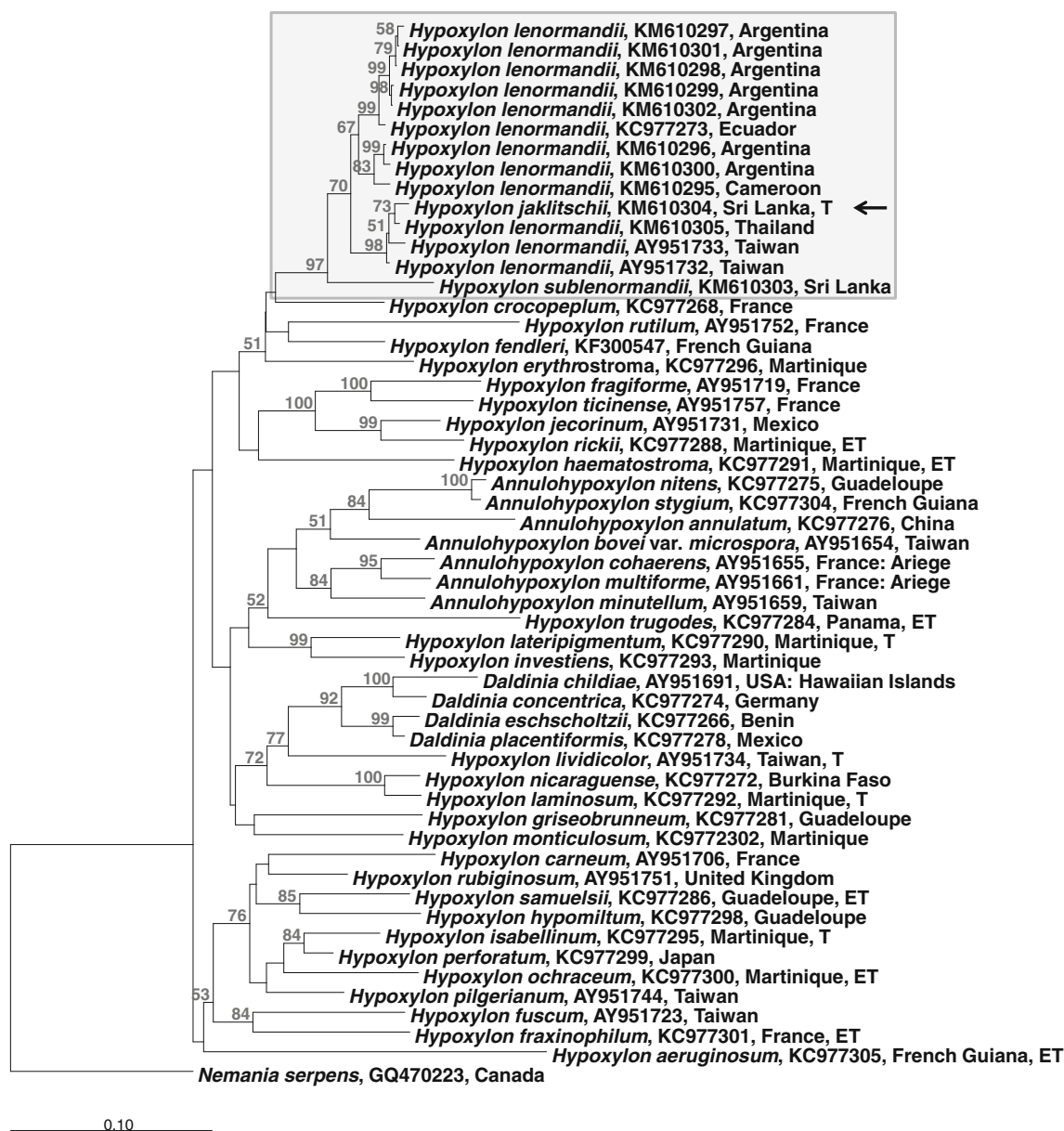
**Fig. 8** Phylogenetic relationships among *Hypoxylon lenormandii* and related Xylariaceae as inferred from β-tubulin gene sequences. The highlighted clade indicates the *H. lenormandii* group. Likelihood (ML) bootstrap support values above 50 %, from 1000 RAxML replicates are assigned to the tree topology of the most likely tree found by RAxML. Species names are followed by the Genbank accession number and countries of origin. Sequence of the newly described species is indicated by an arrow. Type species are indicated by T (holotype) and ET (epitype)

The key difference in proton and $^1$H,$^{13}$C HSQC NMR spectra of **2** compared to **1** was the replacement of two quaternary carbons by methine groups ($\delta_{H/C}$ 3.97/43.0 and 3.97/54.7). $^1$H,$^{13}$C HMBC correlations from H-1 to C-8 and H$_3$-9 to C-8 identified the position of methine C-8. HMBC correlations from C-14 to C-13 and C-15 indicated the position of methine C-14 and confirmed the azaphilone skeleton joined with a nonlinear five-membered ring lactone. The assigment of the relative configuration was hampered by the overlap of H-8 and H-14 signals in chloroform. However, their $^1$H signals were clearly separated in acetone. A $^1$H,$^1$H ROESY

correlation was observed between H$_3$-9 and H-8 ($\delta_H$ 3.97), but not between H$_3$-9 and H-14 ($\delta_H$ H 4.41), thus a 7*S*,14*S* configuration was concluded. This was confirmed by the large coupling constant of $J_{8,14}$=12.2 Hz characterizing a *trans* configuration of the two protons. Because the CD spectrum also indicated 7*S* configuration with positive (237 and 358 nm) and negative (323 nm) Cotton effects, , the structure of **2** was established as (6a*S*,9*S*,9a*S*)-9-[(2*E*, 4*E*,6*E*)-dodeca-2,4,6-trienoyl]-3-[(2*S*)-2-hydroxypropyl]-6a-methyl-9,9a-dihydro-6*H*-furo[2,3-h]isochromene-6, 8(6a*H*)-dione.

The molecular formula of lenormandin C (**3**) was determined as $C_{25}H_{26}O_6$ by HRESIMS indicating the loss of $C_2H_4$ compared to **1**. The NMR data of **3** were basically identical with that of **1**. However, only two instead of four methylene groups were observed in the $^1H,^{13}C$ HSQC spectrum. Therefore, lenormandin C (**3**) was characterized as (6a*S*)-9-[(2*E*,4*E*,6*E*)-deca-2,4,6-trienoyl]-3-[(2*S*)-2-hydroxypropyl]-6a-methyl-6*H*-furo[2,3-*h*]isochromene-6,8(6a*H*)-dione.

Lenormandin D (**4**) was obtained as yellow oil, and its molecular formula $C_{25}H_{28}O_6$ was assigned by HRESIMS. The proton and $^1H,^{13}C$ HSQC NMR spectra of **4** were very similar to that of **3**, except for the replacement of the olefinic methine signals C-20 and C-21 by two additional methylenes. Therefore, lenormandin D (**4**) was determined as (6a*S*)-9-[(2*E*,4*E*)-deca-2,4-dienoyl]-3-[(2*S*)-2-hydroxypropyl]-6a-methyl-6*H*-furo[2,3-*h*]isochromene-6,8(6a*H*)-dione.

Lenormandin E (**5**) was shown to have a molecular formula of $C_{25}H_{30}O_6$ by means of HRESIMS. The NMR spectra of **5** were similar to that of **2**, but differed in the presence of seven instead of nine olefinic protons. $^1H,^1H$ COSY and $^1H,^{13}C$ HMBC spectra showed that the difference is only a dien instead of a trien in the fatty acid side chain and identified **5** as (6a*S*,9*S*,9a*S*)-9-[(2*E*,4*E*)-deca-2,4-dienoyl]-3-[(2*S*)-2-hydroxypropyl]-6a-methyl-9,9a-dihydro-6*H*-furo[2,3-*h*]isochromene-6,8(6a*H*)-dione.

The more lipophilic metabolite lenormandin F (**6**) was isolated as a yellow solid; its molecular formula was determined as $C_{29}H_{44}O_6$. Proton and $^1H,^{13}C$ HSQC spectra revealed the presence of three methyls, three olefinic methines and 15 methenes. The chemical shifts of C-6 and C-8 of 192.9 and 193.4 ppm in the carbon spectrum indicated the presence of two conjugated ketones. $^1H,^{13}C$ HMBC correlations from H-1 to C-3, C-4a, C-8a, and ketone C-8, and of H₃-9 to C-7, C-8, and ketone C-6 indicated a rubigenosin C-like azaphilone core structure (Quang et al. 2004a); whose C-7 stereochemistry was assigned as *R* by the negative Cotton effect at 325 nm (Clark et al. 2008). $^1H,^1H$ COSY correlations between H₂-10, H-11 and H₃-12 established the 2-hydroxy-propyl side chain connected to C-3. The saturated palmityl side chain was deduced by $^1H,^1H$ TOCSY correlations from H-16 to C-28, complementing the structure of lenormandin F (**6**) as (7*R*)-3-[(2*S*)-2-hydroxypropyl]-7-methyl-6,8-dioxo-7,8-dihydro-6*H*-isochromen-7-yl hexadecanoate.

Lenormandin G (**7**) possessed the molecular formula $C_{31}H_{44}O_6$ as established from the HRESIMS spectrum. In general, the $^1H$ and $^{13}C$ spectra of **7** were similar to those of lenormandin F (**6**), except the four additional olefinic signals observed in the spectra of **7** along with a reduced number of methylenes. $^1H,^{13}C$ HMBC correlations of both H₃-30 and H₂-26 to C-28 determined the position of the new diene. The stereo chemical analysis was hampered by an overlap between protons H-22 to H-25. However, the overlap of the signals for the two outer and two inner carbons of the diene

system together with a small difference of the chemical shifts between inner and outer carbons is indicative for an all-*trans* configuration (Davis et al. 1999). Therefore, lenormandin G (**7**) was established as (7*R*)-3-[(2*S*)-2-hydroxypropyl]-7-methyl-6,8-dioxo-7,8-dihydro-6*H*-isochromen-7-yl (10*E*,12*E*)-octadecan-10,12-dienoate.

## Bioactivity of lenormandins A–G

The activity of the new compounds was evaluated with a set of gram-positive and gram-negative bacteria as well as various yeasts and one strain of a filamentous fungus (Table 4). Only lenormandin A (**1**) and D (**4**) showed weak activity against the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. In addition, weak activity of lenormandin B (**2**) against a *Mycobacterium* sp. was observed. None of the tested substances had an effect on the growth of gram-negative bacteria. All lenormandins were weakly active against the yeast *Rhodotorula glutinis*, except for lenormandin B (**2**). No activity against other fungi was detected. A cytotoxicity assay was carried out using mouse fibroblast cell line L929 (Table 2). Only lenormandins B (**2**), C (**3**) and E (**5**) showed moderate to weak effects.

## Lenormandins as marker metabolites

To study the distribution patterns of the lenormandins, 15 specimens of *Hypoxylon lenormandii* and one each of *H. jaklitschii* and *H. sublenormandii* were analysed. A comparison of representative chemical profiles is pictured in Fig. 6. Five specimens originated from old herbaria collections deposited in the Royal Botanical Gardens, Kew (K) or in the Swedish Museum of Natural History, Stockholm (S), including type specimens of *H. lenormandii* and its synonyms *H. fulvoochraceum*, *Kretzschmaria stilbophora*, *Rosellinia pulchella* and *R. metachroa*. All specimens, except for *H. jaklitschii*, contained lenormandin A as major component, but no further lenormandins could be detected in the holotype. Lenormandin B (**2**) was present in most of the materials, but not in F 7347 and *H. jaklitschii*. The highest amounts of this compound were found in *H. sublenormandii*. Lenormandin C (**3**) is a minor metabolite which was found in the Argentine and Caribbean specimens, but lacked in the Asian and African material. The Asian specimens were apparently devoid of lenormandin D (**4**), which was present in high amounts in the South American specimens and in minor quantities in the Caribbean specimens. Lenormandin E (**5**) was exclusively found in the Argentine material and *H. jaklitschii*. Most of the collections contained lenormandin F (**6**) in small amounts, except for *H. lenormandii* from Martinique and *H. sublenormandii*. Lenormandin G (**7**) could not be detected in the old herbarium material, the specimen from Martinique

**Table 4** In vitro antibacterial, antifungal & cytotoxic activity of Lenormandins A–G (1–7), all solved in acetonitrile, and three control drugs. 20 μl of acetonitrile showed no effect on the tested organisms. MIC: Minimum inhibitory concentration. A total of 2 and 20 μl were tested. [a] Oxytetracyclin hydrochloride; [b] Gentamycin; [c] Nystatin, n. i.: no inhibition. The cell density was adjusted to $8 \times 10^6$ cells/ml. * spores from agar-plate were applied without justification; DSMZ: German Collection of Microorganisms and Cell Cultures, Braunschweig

| Test organisms | MIC [μg/ml] | | | | | | | Ref [a,b,c] |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Gram-positive bacteria | | | | | | | | |
| *Bacillus subtilis* DSM 10 | 67.0 | n.i | n.i | 67.0 | n.i | n.i | n.i | 8.3 [a] |
| *Micrococcus luteus* DSM 1790 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 0.42–0.83 [a] |
| *Mycobacterium* sp. DSM 43270 | n.i | 67.0 | n.i | n.i | n.i | n.i | n.i | 3.3 [a] |
| *Staphylococcus aureus* DSM 346 | 67.0 | n.i | n.i | 67.0 | n.i | n.i | n.i | 0.42–0.83 [a] |
| Gram-negative bacteria | | | | | | | | |
| *Chromobacterium violaceum* DSM 30191 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 0.83 [a] |
| *Escherichia coli* DSM 1116 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 3.3–6.7 [a] |
| *Pseudomonas aeruginosa* DSM 50071 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 4.2 [b] |
| Yeasts | | | | | | | | |
| *Candida albicans* DSM 1665 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 8.3 [c] |
| *Wickerhamomyces anomalus* DSM 6766 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 16.6 [c] |
| *Rhodotorula glutinis* DSM 10134 | 33.3 | 67.0 | 67.0 | n.i | 67.0 | 67.0 | 33.3 | 2.1 [c] |
| *Schizosaccharomyces pombe* DSM 70572 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 16.6 [c] |
| Filamentous fungi | | | | | | | | |
| *Mucor hiemalis* DSM 2656 | n.i | n.i | n.i | n.i | n.i | n.i | n.i | 8.3 [c] |
| Cell lines | | | | | | | | |
| Mouse fibroblast cell line L929 | n.i | 18.0 | 32.0 | n.i | 22.0 | n.i | n.i | – |

All numbers bold, refers to lenormandins 1–7

and *H. sublenormandii,* but was abundant in the Thai and Argentine material.

## Discussion

The new secondary metabolites, lenormandins A–G (1–7) are further representatives of the well-known azaphilones. This structural class is characterised by a highly oxygenated pyranoquinone bicyclic core with a quaternary carbon centre and the affinity of its pyran part to make substitutions with primary amines. A huge variety of fungal organisms are capable of producing these polyketides. Many of them are pigments and therefore responsible for the colours of stromata of the Xylariaceae (Gao et al. 2013). Lenormandins A–E (1–5) show structural similarities to the sassafrins A (VIII) – C while lenormandins F and G (6 and 7) bear structural resemblance to rubiginosin C (IV, Quang et al. 2005b, Quang et al. 2004b). The sassafrins are specific metabolites of the xylariaceous fungus *Creosphaeria sassafras* with strong antifungal and moderate antibacterial activity, whereas rubiginosin C is a typical stromatal pigment of *H. rubiginosum* and other species of the *H. rubiginosum* complex. Similar to the lenormandins, the latter compound showed only weak antimicrobial activities (Stadler et al.

2007). The azaphilones of *Hypoxylon* and allied species are thought to play an important role in the protection against parasites. They strongly accumulate in the stromata, and their production affords substantial energy for the fungal organism. The high concentration of these compounds in the stromata might be crucial for their function and therefore might explain the lack of activity in our assays, where the starting concentration is set to 67 μg/ml. There is also the possibility that the lenormandins function as insect repellents rather than fungicides or antimicrobial agents, which we did not test. It is noteworthy that the new compounds seem to isomerise under light due to their highly conjugate double bounds in the fatty acid side chain.

*Hypoxylon jaklitschii* is the second species after *H. sublenormandii* being segregated from the *H. lenormandii* complex (Suwannasai et al. 2006). Notably, morphological and molecular data were insufficient to clearly segregate these taxa. However, the chemical data in comparison with type material of *H. lenormandii* pointed out conspicuous differences, which can barely be explained by environmental variability. The production of the specific lenormandin-like main compound in the stromata of *H. jaklitschii*, which we could not detect in the studied *H. lenormandii* specimens, would afford a genetic change in the underlying lenormandin gene cluster or its regulation.

Interestingly, *H. sublenormandii*, which can be well delimited from the *H. lenormandii* complex on a phylogenetic and morphological level, appears more closely related to the latter species than to *H. jaklitschii* from a chemotaxonomic viewpoint. Due to the fact that the type material of *H. oodes* from Sri Lanka was depauperate (we neither found intact ascospores nor did we obtain conclusive results by HPLC on the prevailing secondary metabolites), we had no additional collections of *H. lenormandii* from this country at our disposal. This leaves an open question, whether *H. jaklitschii* completely replaced *H. lenormandii* in Sri Lanka or whether both species coexist. To answer this matter, an extensive survey of hypoxyloid Xylariaceae in the area should be conducted in the future.

Within the phylogenetic trees *H. lenormandii* strains from Asia form a separate clade. This is in accordance with the secondary metabolite profiles which show the presence of lenormandin B (**2**), F (**6**) and G (**7**) besides A (**1**) in all examined specimens. In contrast the material from South America (including the Caribbean specimens) always contained lenormandin D (**4**) and sometimes lenormandin C (**3**), both of which typically lack in the Asian material. It is obvious that there is a characteristic distribution pattern of the lenormandins being supported by molecular data. This raises the question whether it make sense to further separate the species complex and erect new taxa or subspecies, respectively. This might even be supported by morphological features; however we had only the possibility to study four collections from Asia in detail. A broader sampling from various Asian countries is needed to further address the question.

Nowadays the combination of morphology and phylogeny to describe new species of the Xylariaceae is state of the art. However the present study points out that a polythetic approach using chemotaxonomy as additional criteria will become mandatory for a complete description of species in the "subfamily" Hypoxyloideae. Owing to the fact that chemical analyses need expensive equipment, it is important to facilitate collaborations with local chemistry departments or institutes. This will be an advantage for both sides, because tropical *Hypoxylon* species have proven to be a prolific source of new and often easy accessible secondary metabolites from stromata but also cultures. We studied about 20 different taxa from Argentina with more than half of them containing unknown compounds in their stromata alone. In the course of the ongoing search for new antibiotics to fight emerging resistance of pathogens, there is a good chance to find new lead structures within the family.

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