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The new genus *Rostrohypoxylon* and two new *Annulohypoxylon* species from Northern Thailand

Jacques Fournier · Marc Stadler · Kevin D. Hyde · Minh Lam Duong

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Abstract An inventory of xylariaceous pyrenomycetes of Northern Thailand resulted in the discovery of a new monotypic genus, here named *Rostrohypoxylon* as well as two new species of *Annulohypoxylon*. These new taxa are introduced, fully described and compared with similar species in this paper. The new genus is recognized based on new combinations of anamorphic and teleomorphic characters. The status of these new taxa is supported by secondary metabolite profiling using high performance liquid chromatography, coupled with diode array detection and mass spectrometry (HPLC-DAD-MS).

J. Fournier (✉)
Las Muros,
09420 Rimont, France
e-mail: jacques.fournier@club-internet.fr

K. D. Hyde
School of Science, Mae Fah Luang University,
Tasud, Muang,
Chiang Rai 57100, Thailand

M. Stadler
Faculty of Biology, Chemistry and Earth Sciences,
Department of Mycology, University of Bayreuth,
Universitätstraße 30, NW1,
95440 Bayreuth, Germany

M. Stadler
InterMed Discovery GmbH,
Otto-Hahn-Straße 15,
44227 Dortmund, Germany

M. L. Duong
Faculty of Biology, Department of Microbiology
& Biotechnology, Hanoi National University of Education,
136 Xuanthuy, Caugiay,
Hanoi, Vietnam

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Introduction

The xylariaceous genus *Annulohypoxylon* was recently erected (Hsieh et al. 2005) to accommodate species formerly included in *Hypoxylon* sect. *Annulata* J.H. Mill. emend. Y.M. Ju & J.D. Rogers (Ju and Rogers 1996). The basis for the new genus was supported by molecular and morphological data. *Annulohypoxylon* was segregated from *Hypoxylon* Bull. by three key morphological features: *a*) carbonaceous stromata that become highly melanized at maturity; *b*) papillate ostioles surrounded by an annulate disc; *c*) ascospores with a perispore bearing a thickening on the same side as the germ slit. The characteristic disc is lacking in some taxa from Europe and New Zealand, and some species have an indehiscent perispore; this makes it difficult to locate the characteristic thickening of the perispore. However, the affinities of these "aberrant" species to *Annulohypoxylon* were also clearly demonstrated by molecular data, and supported by chemotaxonomic characters.

As in other xylariaceous genera referred to as "Hypoxyloideae" (e.g., the related *Daldinia* Ces. & De Not., *Entonaema* Möller and *Hypoxylon* Bull.), secondary metabolites are present in the stromata of *Annulohypoxylon* as subsurface granules yielding colored pigments in 10% KOH. Their chemotaxonomic importance has been evaluated by Quang et al. (2005a, b, 2006) and illustrated by Stadler and Fournier (2006).

During several field expeditions in the vicinity of the Mushroom Research Centre (MRC) in Northern Thailand from mid-May to mid-June 2005, several *Annulohypoxylon* spp. were collected, of which two species appeared to be new to science. Moreover, a taxon obviously related to

Annulohypoxylon was encountered, for which the new genus *Rostrohypoxylon* is proposed. Based on morphological, cultural and chemotaxonomic data, these taxa are described and illustrated below. It is noteworthy that *Annulohypoxylon* was by far the best represented xylariaceous genus in good condition among the pyrenomycetes encountered during the above mentioned forays, which were conducted during the late dry season and early monsoon season. This period obviously favored drought-tolerant *Xylariaceae* with carbonaceous stromata, such as *Annulohypoxylon*.

Materials and methods

Measurements of asci and ascospores were made from slides mounted in water, the reaction of apical rings being tested by addition of a drop of Melzer's reagent at the edge of the cover slip. Microscopic observations and photos were made and taken through a brightfield microscope. The dehiscence of the perispore was likewise tested by addition of a drop of 10% KOH to a water mount where free ascospores released from the asci had been first observed, for two reasons. First, the dehiscence of the perispore is often difficult to see while ascospores are still in the ascus, especially in some species where the perispores are hardly dehiscent. Second, unlike in *Hypoxylon* and *Daldinia* where perispores conspicuously dehisce by transverse breaking off, those in *Annulohypoxylon* dehisce by longitudinal splitting from one end and can be mistaken for immature hyaline ascospores. Adding KOH to a water mount allows microscopic determination of the perispore dehiscence with greater reliability. Colours refer to Rayner (1970), and therefore, also to the species descriptions provided by Ju and Rogers (1996) and Ju et al. (2004). Cultures were obtained from perithecial contents plated onto YMG agar as previously described by Stadler et al. (2004) and propagated in different culture media for secondary metabolite analyses as described by Stadler et al. (2008a, b). For HPLC profiling of stromatal methanol extracts, the methodology described by Stadler et al. (2008a) was also employed, using standards of the extrolites that were obtained previously (Bitzer et al. 2007, 2008; Quang et al. 2005a, b). The chemical structures of the secondary metabolites detected are depicted in Fig. 4. The HPLC-UV data presented in Figs. 5 and 8 were also verified by HPLC-MS analyses (data not shown).

Results

Chemotaxonomic studies

HPLC profiles of the species collected (and, if available, of their cultures), were recorded and compared with previously obtained data (e.g. Quang et al. 2005a; Bitzer et al. 2008) on *Annulohypoxylon* species and other *Xylariaceae*. The HPLC profiles of representative collections are depicted for comparison in Figs. 5 and 8.

Taxonomic part

Rostrohypoxylon J. Fourn. & M. Stadler, **gen. nov.**

(Figs. 1, 2, 3)

MycoBank: MB512543

Etymology: from rostrum (= beak) for long-beaked ostiolar necks and *Hypoxylon* in reference to the affinities with this genus.

A totis generis Xylariaceis differt in stromatibus carbonaceis, ostolis compactis cum cerviculis prominentis praeditae, foraminis cylindricis profundis dispersis inter tumulis peritheciorum.

Stromata erumpent from bark, strongly carbonaceous, yielding pigments in 10% KOH, bearing stout ostiolar necks and deep cylindrical holes. Asci unitunicate, cylindrical, stipitate, lacking an apical apparatus. Ascospores brown, cylindrical with broadly rounded ends, one-celled, smooth, with a straight germ slit. Anamorph *Nodulisporium*-like (*Sporothrix* type to *Virgariella* type as defined in Ju and Rogers (1996).

Type species: *Rostrohypoxylon terebratum.*

Rostrohypoxylon terebratum J. Fourn. & M. Stadler, **sp. nov.**

(Figs. 1, 2, 3)

MycoBank: MB512544

Etymology: from Latin: *terebrare* (= to bore) in reference to the perforated stromatal surface.

Stromata effuso-pulvinata, per corticem hospitis erumpentia, tumulis peritheciorum inconspicuis vel conspicuis, 1–1.7 mm crassis, textura carbonacea, crusta 100–120 µm crassa; externe nigra. Superficie confragosa, foraminis cylindricis profundis (180–280 µm diametro × 500–700 mm profundis) irregulatiter dispersis inter tumulis peritheciorum. Granulis inconspicuis pallide olivaceis in KOH dissolutis. Perithecia obovoidea, 0.6–1 mm alta × 0.5–0.6 mm diam. Ostiolis compactis, cerviculis prominentibus praeditae, 0.25–0.5 mm alti × 0.25–0.35 mm diametro ad basae, cum aperturis minutis, umbilicatis. Ostiola deffindentes ad apicis glomeratis conicis con cerviculis nigris praeditae.

Asci 80–90 µm longitudine tota × 4–5 µm crassi, partibus sporiferis 60–70 µm longitudine, stipitibus 20–30 µm longitudine, sine anulis apicalis. Ascosporae 6.6–8.5 × 3–4.2 µm, brunneae, unicellularae, oblongae, apicibus latis, rima germinativa recta inconspicua abbreviata praeditae; perisporium in KOH indehiscens; episporium leve.

Stromata effused-pulvinate, 9–42 mm long × 4–19 mm broad × 1–1.7 mm thick (excluding ostiolar necks), with

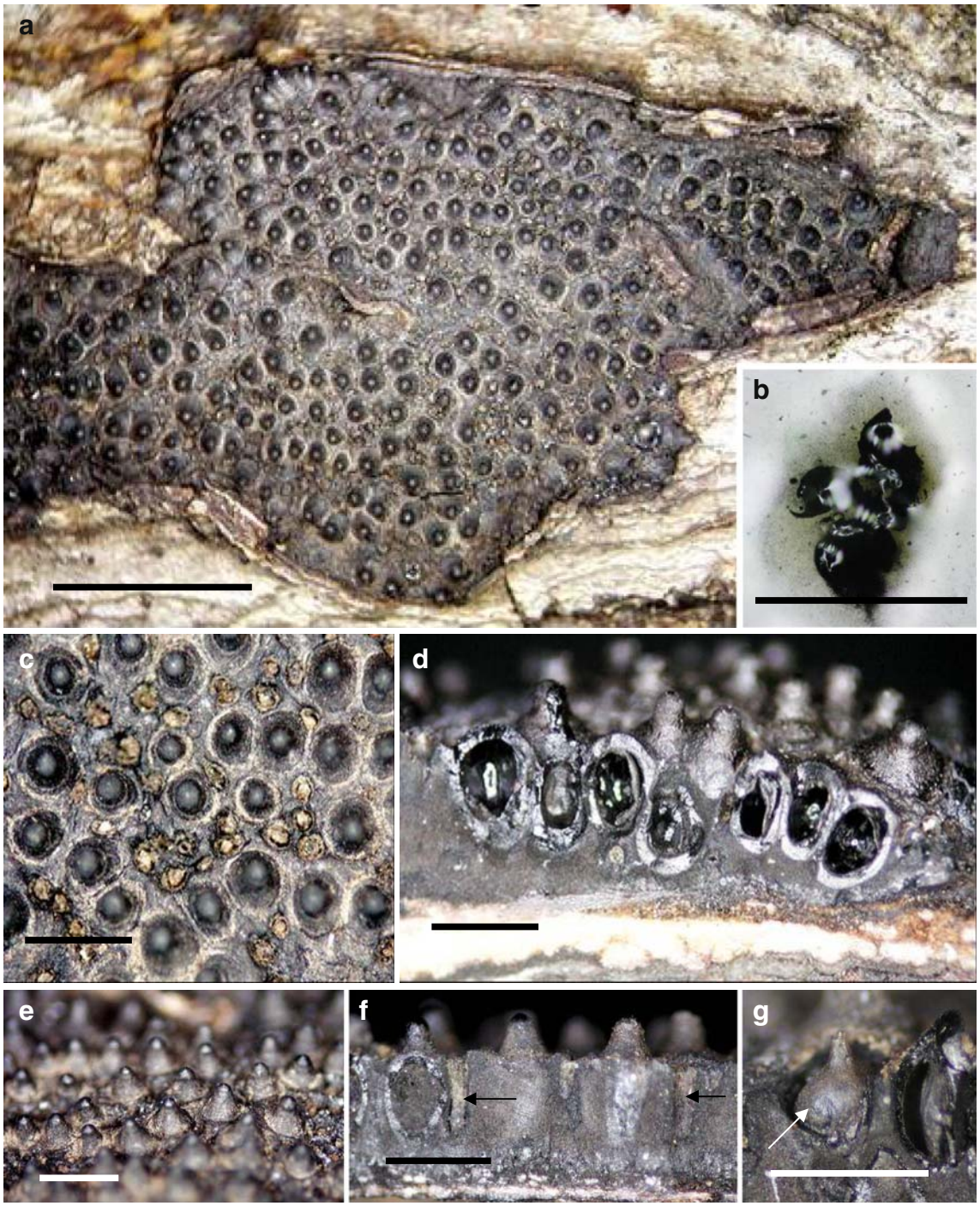


Fig. 1 Stromatal morphology of *Rostrohypoxylon terebratum*, from holotype specimen. Stromatal habit. **b** Pigments in KOH. **c** Rostrate ostioles and perforations seen from above. **d**,**f** Section through stroma, showing perithecia and perforations (*arrows*). **e** Stromatal surface in side view. **g** Section showing a perithecium (*arrow*) encased in carbonaceous tissue. Scale is indicated by *bars*. **a** 5 mm. **b**, **c**, **d**, **e**, **f**, **g** 1 mm

inconspicuous to 1/3 exposed perithecial mounds, developing within bark, erumpent through the periderm, yielding a dilute Greenish Olivaceous (90) pigment in 10% KOH; surface dull black, strongly uneven owing to stout ostiolar necks and cylindrical holes 180–280 µm diam (arrows), 500–700 µm deep irregularly scattered between perithecial mounds, usually filled with olivaceous-yellow material; stromatal crust strongly carbonaceous, 100–120 µm thick; interperithecial tissue blackish, powdery, without visible colored granules, subperithecial tissue 0.15–0.7 mm thick, black.

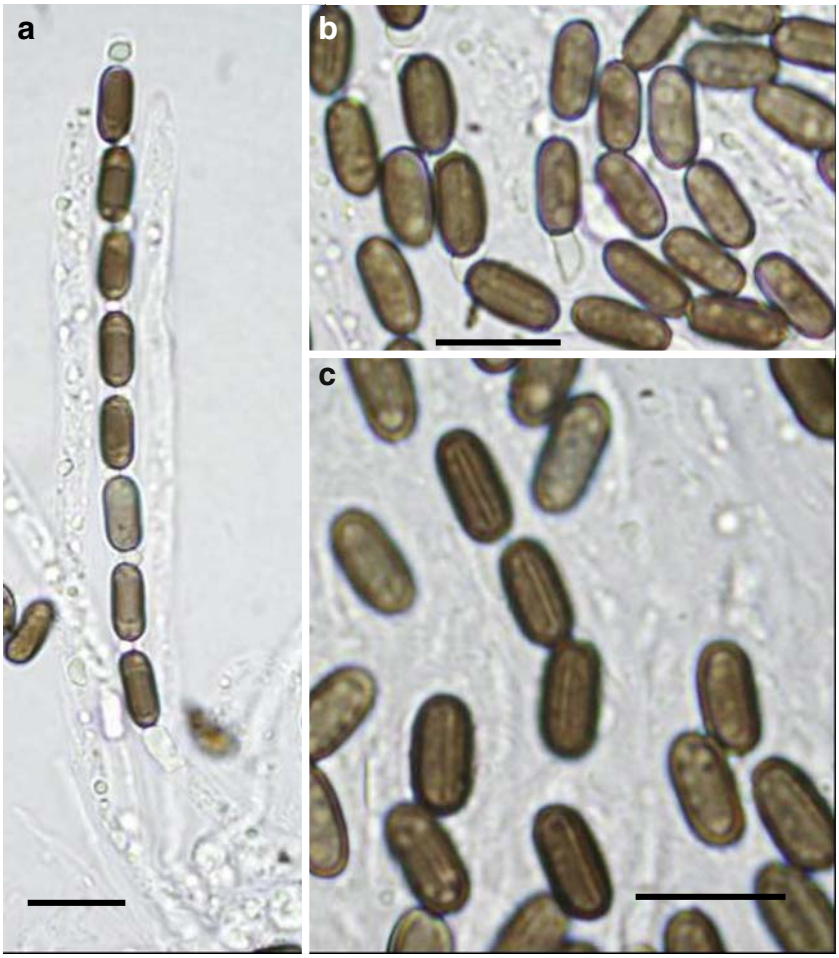


Fig. 2 Asci (**a**) and ascospores (**b**, **c**) of *Rostrohypoxylon terebratum*, from holotype specimen, Fig. 2c clearly showing the ascospore germ slits. Scale bars: 10 µm

Perithecia obovoid to flask-shaped, 0.6–1 mm high×0.5–0.6 mm diam, completely encased in thick carbonaceous tissue.

Ostioles opening as minute, umbilicate pores at the broadly rounded top of stout, conical black necks 0.25–0.5 mm high×0.25–0.35 mm diam at the base.

Asci unitunicate, cylindrical, eight-spored, fragile and readily deliquescing, 80–90 µm total length×4–5 µm broad, the spore-bearing parts 60–70 µm long, the stipes 20–30 µm long, without apparent apical apparatus, not bluing in Melzer's reagent. Paraphyses deliquescing, much longer than asci, hyaline, septate, 3 µm to 4 µm broad at the base, tapering above.

Ascospores 6.6–8.5×3–4.2 µm (M=7.4×3.7 µm, n=60), brown, one-celled, cylindrical with broadly rounded ends, with a faint straight germ slit 4/5 to almost spore length, lacking cellular appendages or gelatinous sheath; perispore not dehiscent in 10% KOH; epispore smooth

Anamorph: *Sporothrix*-like to *Virgariella*-like in culture

Cultural characteristics: Colonies on OA and YMG covering Petri dish in 8–10 days, at first whitish, becoming olivaceous green (90), felty, azonate, with diffuse margins. Reverse dark brick (60).on YMG, remaining uncolored on OA. Reddish exsudates are released from the aerial mycelia on OA after 2 wk. Melanized mycelia not containing conidiogenous structures, but rather comprising a network of hyphae of up to 4.5 µm diam, showing brownish incrustations, but not differentiating further. Sporulating regions only observed on YMG, arising first from the center of colonies, later scattered over entire surface of colony, olivaceous buff (89). Conidiogenous structure *Sporothrix*- to *Virgariella*-like as defined in Ju and Rogers (1996), yellowish, becoming finely roughened. Conidiophores up to 80 µm long, mostly simple occasionally branched. Conidiogenous cells hyaline, smooth, 13–25×3–3.5 µm. Conidia hyaline, smooth to finely roughened, subglobose to ellipsoid, 3.5–5.5×2.5–3 µm, normally arising from the tips of the conidiogenous cells,. but sometimes also arising from lateral parts of undifferentiated vegetative hyphae.

Habitat: Stromata grow on dead bark (so far only found on *Lithocarpus*).

Known distribution: Northern Thailand.

Material examined: Thailand: Chiang Mai Province, Mae Teang District, Bahn Pha Deng, Mushroom Research

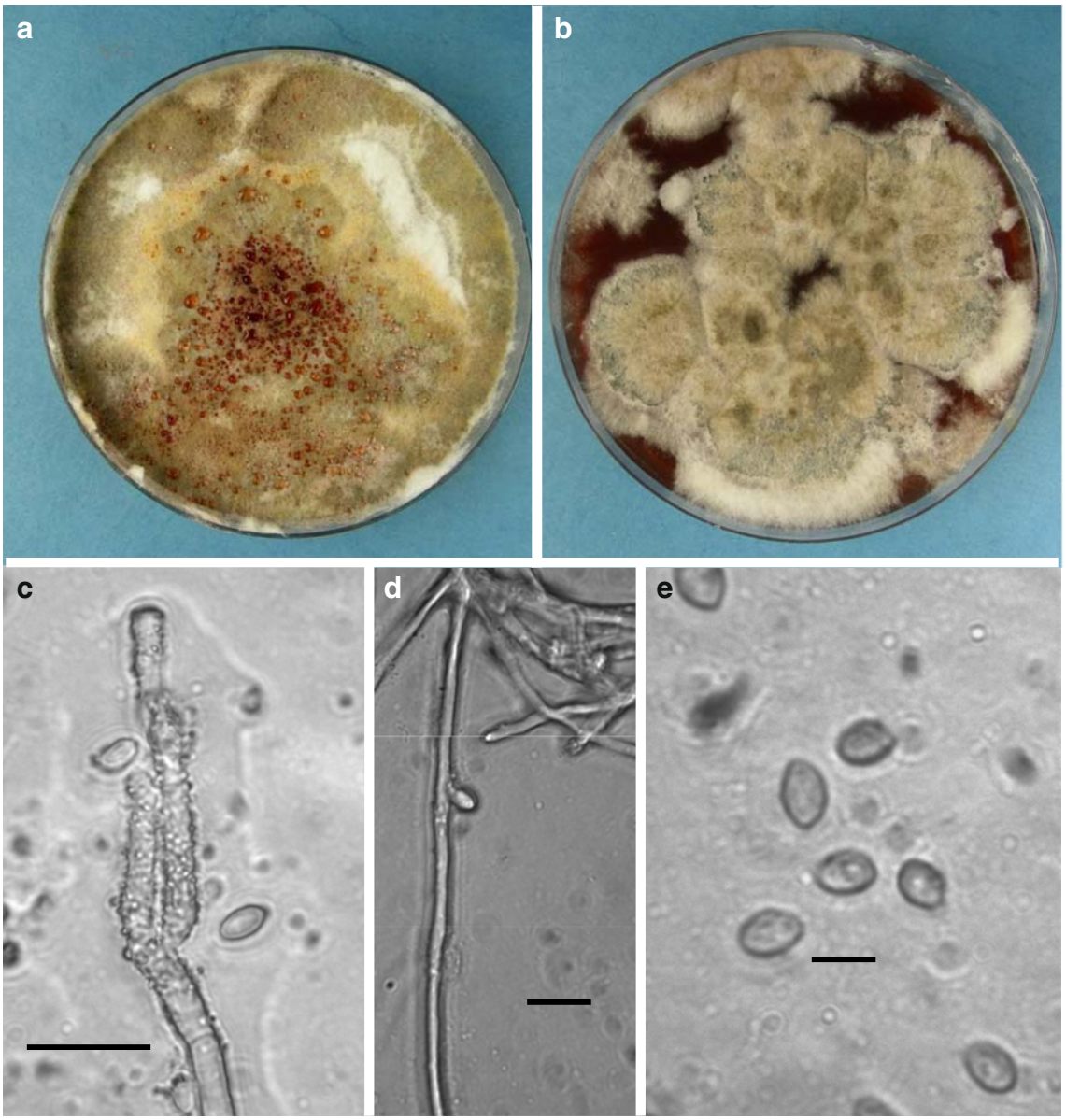


Fig. 3 *Rostrohypoxylon terebratum*, ex type culture. **a**, **b** Cultures after 2 weeks on 9 cm agar slants. **a** Difco Oatmeal agar, showing characteristic red exsudates that are released from aerial mycelium **b** YMG medium. **c**–**e** Phase contrast micrographs (1000×) from YMG culture. **c** Tip of conidiophore, showing finely roughened conidiogenous cells and condidia. **d** Conidium arising laterally from undifferentiated hyphae. **e** Conidia. Scale is indicated by *bars* in **c**–**e**. **c**, **d** 10 μm; **e** 5 μm

Centre, N 19° 01′ 615″ E 98° 41′ 884″, 900 m, on bark of *Lithocarpus* sp., 6 Jun. 2005, J. Fournier JF-TH 06-04 (MFLU **holotype** of *Rostrohypoxylon terebratum*, extype cultures BCC and CBS 119137; Genbank Acc. No for DNA sequences: DQ631943, DQ840069, DQ631954, DQ840097, cf. Tang et al. 2009); Chiang Mai Province, Mae Teang District, track to Tung Joaw Village, N 19°807″ E 98° 389″, 1,350 m, on bark of *Lithocarpus* sp., 7 Jun. 2005, J. Fournier JF-TH 24-02 (MFLU 08-0521).

Notes: Owing to its erumpent effused stromata featuring stout, strongly protruding ostiolar necks, the present fungus first recalls a member of *Diatrypaceae*, such as *Eutypa* or *Diatrype*. A more exhaustive study shows that, unlike in *Diatrypaceae* the stroma is strongly carbonaceous and yields pigments in 10% KOH, ostiolar necks are smooth and broadly rounded at the top, asci are cylindrical and ascospores are brown, not allantoid and have a germ slit.

Based on stromatal shape, ascospore morphology and unitunicate asci in a paraphysate hamathecium, and despite the lack of apical apparatus, *R. terebratum* appears best placed in the *Xylariaceae*. The presence of an apical apparatus, usually bluing in iodine reagents, is considered a key character for the *Xylariaceae*, but exceptions are known for several genera and species currently accepted in this

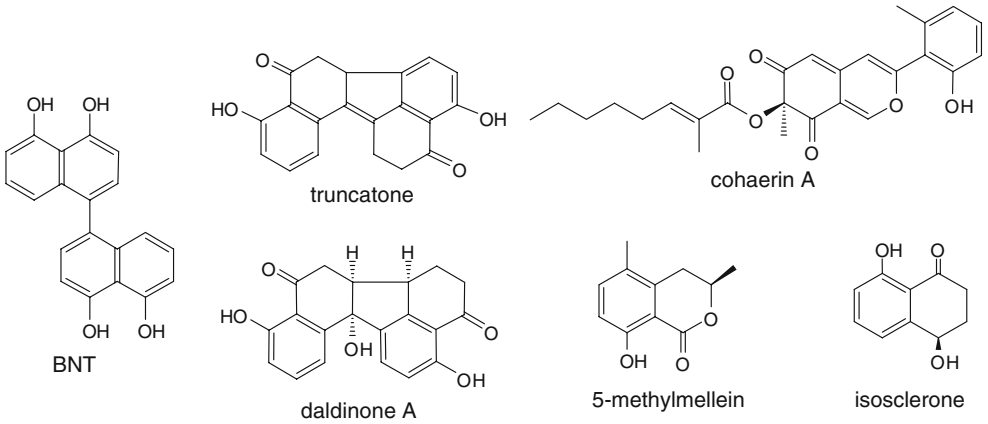


Fig. 4 Chemical structures of some chemotaxonomically significant secondary metabolites detected in this study (BNT = **B**i-**N**aphthalene **T**etrol = 4,4′-Dihydroxy-5,5′-dimethoxy-1,1′-binaphthyl). See further Stadler and Fournier (2006)

family, including *Leprieuria* Læssøe, J.D. Rogers & Whalley, *Obolarina* Pouzar, *Phylacia* Lév., *Poroleprieuria* M.C. González et al.,, *Pyrenomyxa* Morgan, *Rhopalostroma* D. Hawksw., *Theissenia* Maubl., *Thamnomyces* Ehrenb., *Wawelia* Namysl., and some species in *Anthostomella* Sacc. and *Hypoxylon* (Rogers 1994; Ju and Rogers 1996; Stadler et al. 2005). Anamorphic structures in stromata and cultures have not yet been observed in many species of the *Xylariaceae*; others are known to sporulate only sporadically.

The strongly carbonaceous stromata yielding pigments in KOH points toward close affinities with *Annulohypoxylon* (Hsieh et al. 2005), which primarily differs in having low papillate ostioles encircled with a disc and ellipsoid-inequilateral ascospores. Some *Thamnomyces* spp. resemble *R. terebratum* in featuring flask-shaped, separate perithecia in a carbonaceous stroma, asci lacking an apical ring and similar ascospores with indehiscent perispore. *Thamnomyces* is strikingly different in that its stromata are wiry vs. effused, and ascospores reniform to ellipsoid-inequilateral vs. cylindrical.

Arguably, the carbonaceous, erumpent stroma with conical ostioles also recall *Entoleuca* Syd. *sensu* Rogers and Ju (1996). However, no *Geniculosporium*-like anamorph, which is produced readily by all *Entoleuca* cultures we so far studied, was observed. Furthermore, molecular phylogenetic and chemotaxonomic data (see "Discussion") preclude its inclusion in the xylarioid *Xylariaceae* in any case. As pointed out to us by Yu-Ming Ju (pers. comm.). *Hypoxylon tormentosum* Ces. shows certain morphological similarities to *R. terebratum*. However, the types of this name were studied by Ju and Rogers (1996), who found it depauperate and neither observed asci nor the characteristic stromatal surface. They could not even safely determine whether or not the stromata are bipartite. We therefore refrain from speculations on the possible synonymy of this taxon.

The deep cylindrical holes penetrating the stroma are not known from any other xylariaceous genus. Their function, if they have any, remains unclear. One could suppose they are associated with anamorphic structures, but this needs further observations.

Both hitherto obtained records of *R. terebratum* were encountered in dry, sun-exposed locations, on branches not in contact with the soil, at the end of the dry and hot season. This indicates that *R. terebratum*, like several other xylariaceous genera with carbonaceous stromata (e.g., *Annulohypoxylon, Biscogniauxia*, *Camillea*), is xerophilic. Further collections are needed to confirm its ecological requirements and its possible host specificity for *Lithocarpus*.

Chemotaxonomic data from HPLC profiling also revealed significant differences of *R. terebratum* as compared to the species of *Annulohypoxylon* and many other *Xylariaceae* that were hitherto examined by us. The stromatal HPLC profile of both extant specimens (see Fig. 5 for the holotype) revealed various unknown compounds that did not correspond with any other known components of *Annulohypoxylon*, hence it can be assumed with certainty that the weak greenish stromatal pigments observed are not caused by daldinone A, truncatone, or azaphilones of the cohaerin/multiformin type. BNT, a rather ubiquitous component in the stromata of *Annulohypoxylon*, was only detected in traces and would have been overlooked if mass spectrometric detection and a standard had not been available to confirm its occurrence. Still, its occurrence provided corroborating evidence to regard the new genus as a member of the hypoxyloid *Xylariaceae*. The culture of *R. terebratum* also showed a rather specific HPLC profile. Besides some apparently specific compounds, 5-methylmellein was present in traces. Such dihydroisocoumarins were already detected in many species of the *Xylariaceae* by Whalley and Edwards (1995). Recently, Bitzer et al. (2008) refined these results for a larger number of taxa, with representative strains being available in public collections. They also showed that 5-methylmellein itself is not a genus specific marker metabolite but the compound occurs in many species of *Annulohypoxylon*, *Biscogniauxia*, *Camillea,*

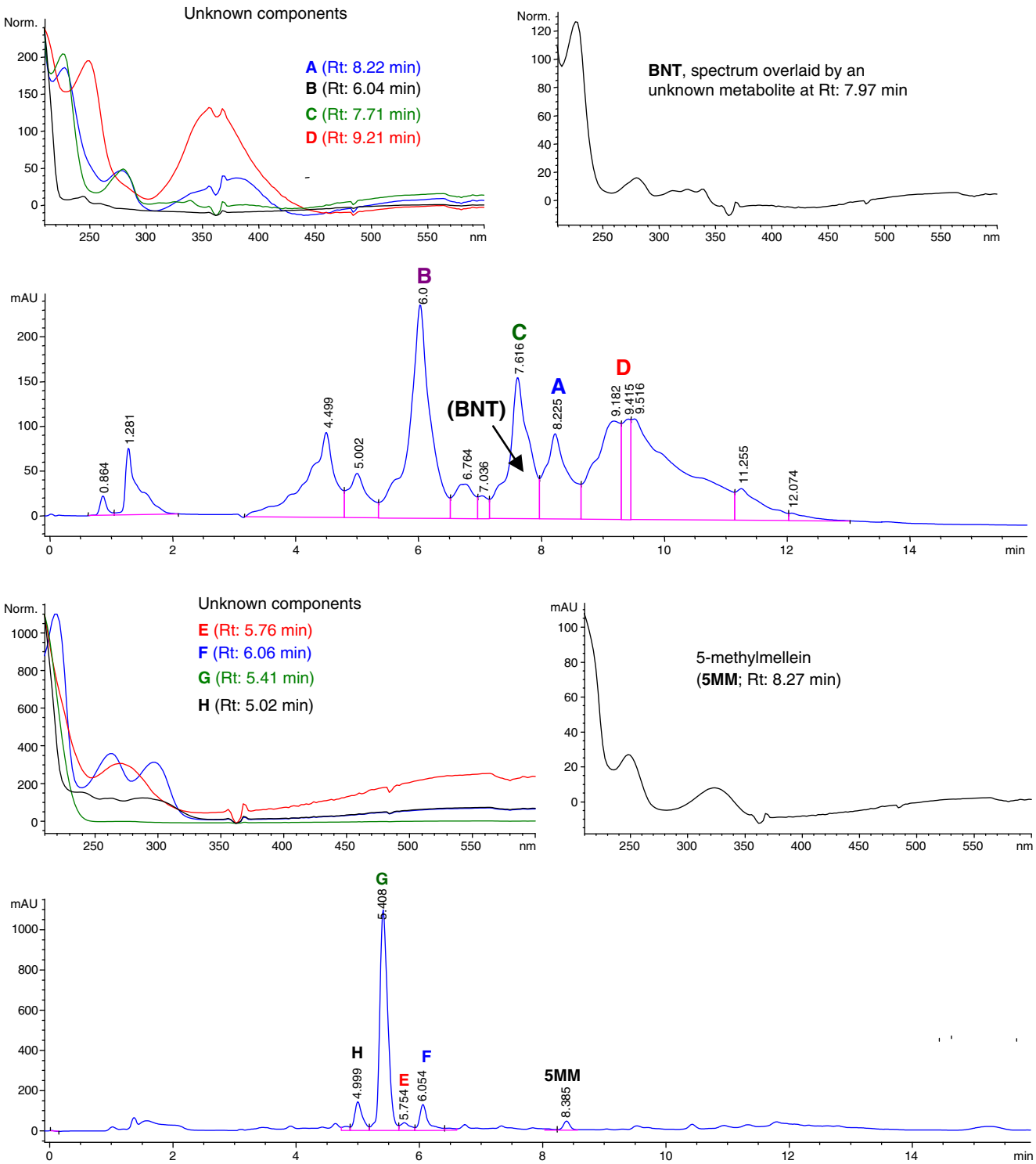


Fig. 5 *Rostrohypoxylon terebratum*, HPLC-UV chromatograms (210 nm) of the stromatal methanol extract of holotype specimen (*above*) and ethyl acetate extract of submerged YMG culture after 168 h of fermentation (*below*). In both cases, the UV-vis spectra of some of the major, yet unknown components (**a**–**d** in the stromatal extract and **e**–**h** in the extract from YMG culture) were included. In the stromatal extract, the binaphthalene BNT (Fig. 4) was detected in traces, overlaid by one of the unknown major components, as indicated

Lopadostoma, and *Hypoxylon*. While even being present in some of the *Annulohypoxylon* spp. treated here, 5-methylmellein was so far not found in *Entoleuca* and other xylarioid *Xylariaceae* with *Geniculosporium*-like anamorphs. It was also detected in the cleistocarpous *Pyrenomyxa*, but not in *Daldinia* and its immediate allies (i.e. *Entonaema*, *Phylacia*).

As another significant difference to *Annulohypoxylon* spp., the *Rostrohypoxylon* cultures were apparently devoid of isosclerones, a second marker metabolite class, found by Bitzer et al. (2008) in several species of *Annulohypoxylon*. Even though the specific compounds of *R. terebratum* remain to be isolated and identified, the above chemotaxonomic results agree well with the proposed status of *Rostrohypoxylon* as being derived from *Annulohypoxylon*, or that it constitutes a separate, yet unknown lineage of the hypoxyloid *Xylariaceae* that evolved in parallel to *Annulohypoxylon* from biscogniauxioid forms. The molecular study by Tang et al. (2009) supports this hypothesis (see "Discussion")

Annulohypoxylon bahnphadengense J. Fournier & M. Stadler, **sp. nov.**

(Fig. 6)

MycoBank: MB512545

Etymology: In reference to Bahn Pha Deng (Thailand), the locality where the fungus was collected.

Stromata effuso-applanata, tumulis peritheciorum inconspicuis vel conspicuis, 0.7–1.1 mm crassa, textura carbonacea; externe atrovinosa vel nigra, con granulis brunneis vel pallide olivaceis in KOH dissolutis. Perithecia globosa vel obovoidea, 0.5–0.65 mm alta×0.5–0.6 mm diam. Ostiola papillata ab disco truncatum simili 0.25–0.3 mm diam.

Asci 100–130 µm longitudine tota, partibus sporiferis 60–70 µm longitudine×4.5–5.5 µm crassi, stipitibus 37–60 µm longitudine, annulo apicali in liquore iodato Melzeri cyanescente, discoideo, 0.8–1 µm alto×1.5–2 µm lato. Ascosporae 6.5–8.4×3–3.6 µm, brunneolae, unicellulares, ellipsoideo-inequilaterales vel equilaterales, apicibus angustatis vel latis, rima germinativa recta inconspicua longa praeditae; perisporium in KOH dehiscens; episporium leve.

Stromata effused-applanate, 6–70 mm long×4–18 mm broad×0.7–1.1 mm thick, with inconspicuous to 1/3 exposed perithecial mounds, hard-textured; surface dull black to shiny black, with a Dark Vinaceous (82) outermost tomentose layer progressively worn off at upper half but remaining between perithecial mounds; interperithecial tissue blackish-brown without visible colored granules. Dull olivaceous granules can be detected by microscopic examination in water, yielding Isabelline (65) to Grayish Sepia (106) pigments in 10% KOH; subperithecial tissue inconspicuous to 0.4 mm thick, woody, blackish.

Perithecia spherical to obovoid, 0.5–0.65 mm high× 0.5–0.6 mm diam, encased in carbonaceous tissue. Ostioles conical-papillate, encircled with a flattened *truncatum*-type disc 0.25–0.3 mm diam.

Asci cylindrical, eight-spored, short-stipitate, 100–130 µm total length, the spore-bearing parts 60–70 µm long×4.5–5.5 µm broad, the stipes 37–60 µm long, with apical ring bluing in Melzer's reagent, discoid, 0.8–1 µm high×1.5–2 µm broad.

Ascospores 6.5–8.4×3–3.6 µm (M=7.5×3.5 µm, n=90), medium brown, one-celled, ellipsoid-inequilateral to nearly equilateral with broadly to narrowly rounded ends, uniseriate in the ascus, with a faint straight germ slit spore-length on the more convex side; perispore dehiscent in 10% KOH but not readily, with a thickening on the more convex side; epispore smooth.

Anamorph on natural substrate (JF-TH 07-03): Olivaceous (48), downy, on bark around young stromata. Conidiogenous structure showing a *Nodulisporium*-like branching pattern as defined in Ju and Rogers (1996), with erect conidiophores up to 320 µm high, brown to pale olivaceous brown, finely roughened. Conidiogenous cells pale brown, smooth to finely roughened, 10–18×2.5–3.5 µm. Conidia subhyaline, smooth, ellipsoid, 4–5×2.5–3 µm.

Known distribution: Northern Thailand.

Habitat: Stromata on dead bark or wood.

Material examined: Thailand: Chiang Mai Province, Mae Teang District, Bahn Pha Deng, Mushroom Research Centre, N 19° 01′ 615″ E 98° 41′ 884″, 900 m, on wood, 29 May 2005, J. Fournier JF-TH 29-02 (MFLU-**holotype**); same location, on bark, 7 Jun. 2005, JF-TH 07-03 (MFU08-1552); same location, on bark, 8 Jun. 2005, JF-TH 08-01 (MFU08-1523).

Notes: *Annulohypoxylon bahnphadengense* clearly belongs to the genus *Annulohypoxylon* based on stromatal morphology (carbonaceous stroma with conic papillate ostioles encircled with a discoid ring, ascospores with perispores bearing a thickening on the same side as the germ slit) and typical secondary metabolites yielding pigments in 10% KOH. It is characterized by its stromatal surface becoming shiny by fading of the Dark Vinaceous outermost layer, small ostiolar discs, isabelline to greyish sepia KOH-extractable pigments and small ascospores.

Although its mature stromata display a more or less shiny surface like in *A. nitens* (Ces.) Y.M. Ju, J.D. Rogers & H.M. Hsieh and *A. purpureonitens* (Ju & Rogers) Y.M. Ju, J.D. Rogers & H.M. Hsieh, *A. bahnphadengense* is different in having *truncatum*-type ostiolar discs *sensu* Ju and Rogers (1996), while they are of *bovei*-type in the two above species. Assessing the morphological type of ostiolar discs in *H. bahnphadengense* proved difficult in absence of clear observations on their dehiscence at early stage.

Although they somewhat recall the *bovei*-type in gross morphology, they are herein referred to the *truncatum*-type based on their slightly notched rims. In addition, it yields brownish pigments in KOH while these pigments are Greenish Olivaceous (90) and Vinaceous Purple (101), respectively, in the aforementioned relatives.

The stromatal HPLC profile (Fig. 8) reveals additional differences to the above taxa as exclusively binaphthalene derivatives were detected in the stromata of the specimens examined. A series of such derivatives was detected in the specimens examined. By contrast, *A. purpureonitens* was found to yield only BNT as major component, while different compounds of the daldinone/truncatone type were previously encountered in *A. nitens* (cf. Quang et al. 2005a).

In the key provided by Ju and Rogers (1996), the present fungus keys out to *A. moriforme* (Henn.)Y.M. Ju, J.D. Rogers & H.M. Hsieh, with which it is likely to be closely

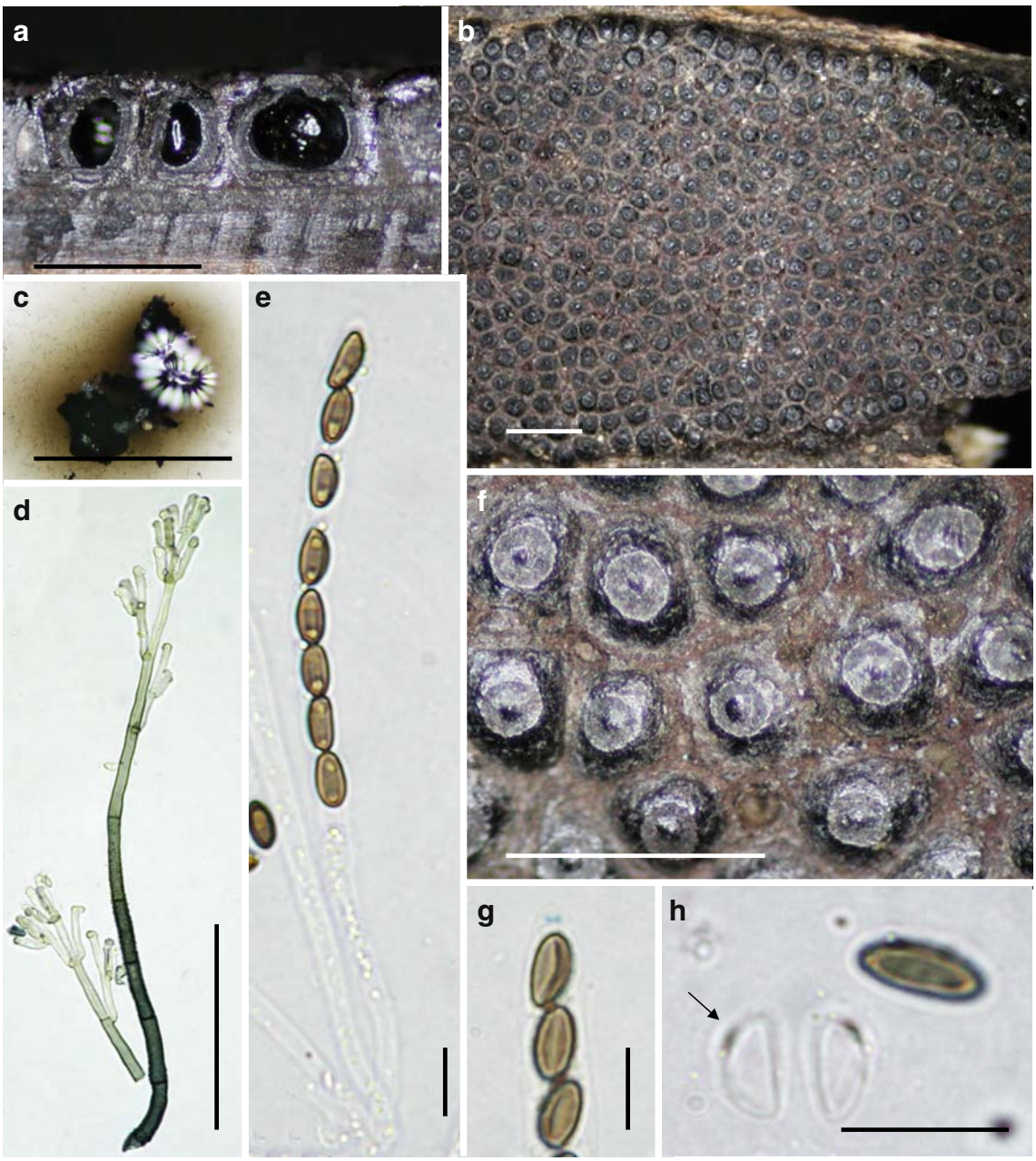


Fig. 6 *Annulohypoxylon bahnphadengense*, from holotype specimen. **a** Vertical section of a stroma. **b** Stromatal habit. **c** Pigments in KOH. D Conidiophore from natural substrate, in 3% KOH. **e** Ascus in water. **f** Stromatal surface with ostiolar discs. **g** Ascus tip in Melzer's reagent. **h** Ascospore in 10% KOH with dehiscent perispores showing the dorsal thickening (*arrow*) Scale is indicated by *bars*. **a**, **b**, **f** 1 mm. C, D: 100 μm, **e**, **g**, **h** 10 μm

related. Judging from three collections, it differs mainly from *A. moriforme* in having effused-applanate stromata with purplish-brown tone on surface vs effused-pulvinate to glomerate with olivaceous-brown tone, and Isabelline to Grayish Sepia pigments in KOH vs Greenish Olivaceous to Dull Green. Moreover, it has slightly smaller and more slender ascospores 6.6–8.4×3–3.6 µm than typical *A. moriforme* collected at the same place [8.5–9(−9.5)×3.8–4.5 µm]. In addition, its stromatal HPLC profiles revealed that the colours of its stromatal pigments are mainly due to the presence of naphthalene derivatives. Truncatone was only detected in minor quantities and daldinone A was not detected at all, but both compounds are present in fairly large amounts in *A. moriforme*.

Annulohypoxylon bahnphadengense should be likewise compared with *A. elevatidiscum* Y.M. Ju, J.D. Rogers & H. M. Hsieh, a recently described species (Ju et al. 2004, as *Hypoxylon*) that is morphologically similar but differs in having convex ostiolar discs raised above the rims and Greenish Olivaceous pigments in KOH. We have not yet studied the latter species by HPLC, but the colour of stromatal pigments suggests that either daldinone A or truncatone, which are widespread in all *Annulohypoxylon* spp. that show this characteristic colour, are probably contained in its stromata as well.

A culture was obtained from the holotype specimen but unfortunately it was soon lost due to contamination by mites carrying moulds. We failed to observe anamorphic structures, but a fermentation of the strain in YMG medium revealed 5-methylmellein as in other members of *Annulohypoxylon* that are deemed related to the new species.

Annulohypoxylon maeteangense J. Fourn. & M. Stadler, **sp. nov.**

(Fig. 7)

MycoBank: MB512546

Etymology: from the Mae Teang district in Chiang Mai Province where the collections were made.

Stromata effuso-applanata, tumulis peritheciorum conspicuis, 0.6–0.75 mm crassa, textura carbonacea; externe olivacea tomentosa vel nigra, granulis inconspicuis viridis in KOH dissolutis. Perithecia globosa, 0.35–0.55 mm diam. Ostiola papillata con discis Hypoxylo, truncato simili 0.15–0.25 mm diam.

Asci 95–115 µm longitudine tota, partibus sporiferis 45–62 µm longitudine×4–4.5 µm crassi, stipitibus 50–65 µm longitudine, annulo apicali in liquore iodato Melzeri cyanescente, discoideo, 0.8–1 µm alto×1.5–1.8 µm lato. Ascosporae 6.5–8.5×3–3.5 µm, brunneolae, unicellulares, ellipsoideo-inequilaterales, apicibus angustatis vel latis, rima germinativa recta inconspicua longa praeditae; perisporium in KOH dehiscens; episporium leve.

Stromata irregularly effused-applanate, 5–60 mm long× 2–28 mm broad×0.6–0.75 mm thick, with conspicuous perithecial mounds, often nearly rosellinioid at the effused margins, carbonaceous and hard-textured; surface brown to dull black with an Olivaceous (48) tone, matt, coated with a long-persistent tomentum of coiled, pale brown to brown, smooth to granulose hyphae 2 µm to 3 µm broad mixed with remnants of conidiophores and dull olivaceous-brown granules; interperithecial tissue entirely carbonaceous, without visible colored granules, yielding a Dull Green (70) pigment in 10% KOH; subperithecial tissue entirely inconspicuous to 0.2 mm thick, woody, black.

Perithecia spherical, 0.35–0.55 mm diam, encased in thick carbonaceous tissue.

Ostioles broadly conical-papillate, shiny black, encircled with a slightly concave *truncatum*-type disc 0.15–0.25 mm diam, sometimes overlain with white material.

Asci cylindrical, eight-spored, long-stipitate, 95–115 µm total length, the spore-bearing parts 45–62 µm long×4–4.5 µm broad, the stipes 50–65 µm long, with apical ring blueing in Melzer's reagent, discoid, 0.8–1 µm high×1.5–1.8 µm broad.

Ascospores 6.5–8.5×3–3.5 µm (M=7.3×3.3, n=90), medium brown, one-celled, ellipsoid-inequilateral with narrowly to broadly rounded ends, with a faint, straight germ slit spore-length on the more convex side; perispore dehiscent in 10% KOH, with a thickening on the more convex side; epispore smooth.

Anamorph on natural substrate (JF-TH 26-01): Olivaceous (48), downy, on bark around young stromata. Conidiogenous structure *Nodulisporium*-like with erect conidiophores 120–300 µm high, brown to pale olivaceous brown, septate finely roughened. Conidiogenous cells pale olivaceous brown, smooth, 12–18×2.5–3 µm. Conidia pale brown to subhyaline, smooth, ellipsoid, 4–5×3–3.5 µm.

Anamorph in culture: Colonies on Difco OA covering Petri dish in 9 days, at first white, becoming Hazel (88), floccose, azonate, with diffuse margins, with scattered black patches; reverse Dull Green (70). Sporulating regions scattered over entire surface of colony, Olivaceous (48) to Olivaceous Buff (89). Conidiogenous structure *Nodulisporium*-like, yellowish to pale brown, roughened. Conidiogenous cells hyaline, smooth, 10–20×2.5–3 mm. Conidia hyaline, smooth to finely roughened, ellipsoid, 3.5–6×2.5–3 mm.

Notes: *Annulohypoxylon maeteangense* is another member of the genus *Annulohypoxylon* (Hsieh et al. 2005), as revealed from its carbonaceous stromata yielding pigments in 10% KOH, papillate ostioles encircled with a disc and the presence of a thickening on the more convex side of the perispores.

Among other species with greenish KOH-extractable pigments and similar ascospore size range that can be confused with the present taxon, *A. moriforme* (Henn.) Y. M. Ju, J.D. Rogers & H.M. Hsieh differs in having larger perithecia 0.4–0.8 mm diam and ostiolar discs 0.2–0.4 mm

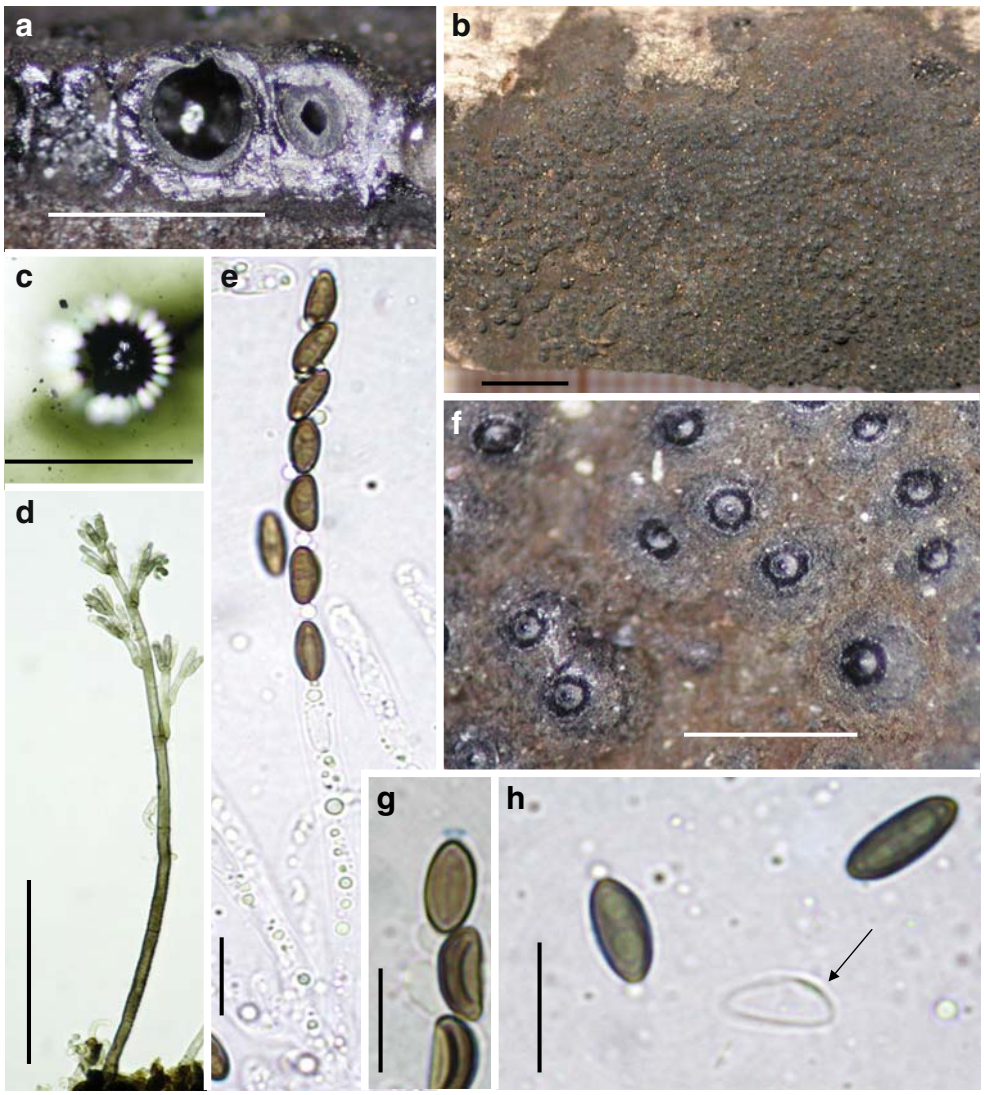


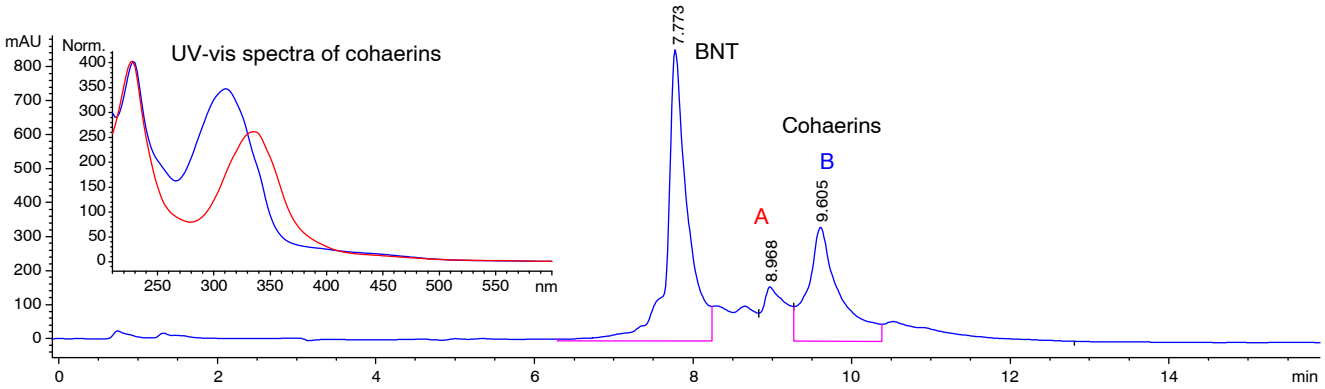
Fig. 7 *Annulohypoxylon maeteangense* , from holotype specimen. **a** Vertical section of a stroma. **b** Stromatal habit. **c** Pigments in KOH. **d** Conidiophore from natural substrate, in 3% KOH. **e** Ascus in water. **f** Stromatal surface with ostiolar discs. **g** Ascus tip in Melzer's reagent. H. Ascospores in 10% KOH with dehiscent perispore showing the dorsal thickening (*arrow*). Scale is indicated by *bars*. **a**, **f** 1 mm. **b** 5 mm. **c**, **d** 100 µm. **e**, **g**, **h** 10 µm

diam and frequently pulvinate to glomerate stromata, and *A. microcarpum* (Penz. & Sacc.) Y.M. Ju, J.D. Rogers & H.M. Hsieh differs in having smaller perithecia 0.15–0.2 mm diam. and ostiolar discs 0.1 mm diam.

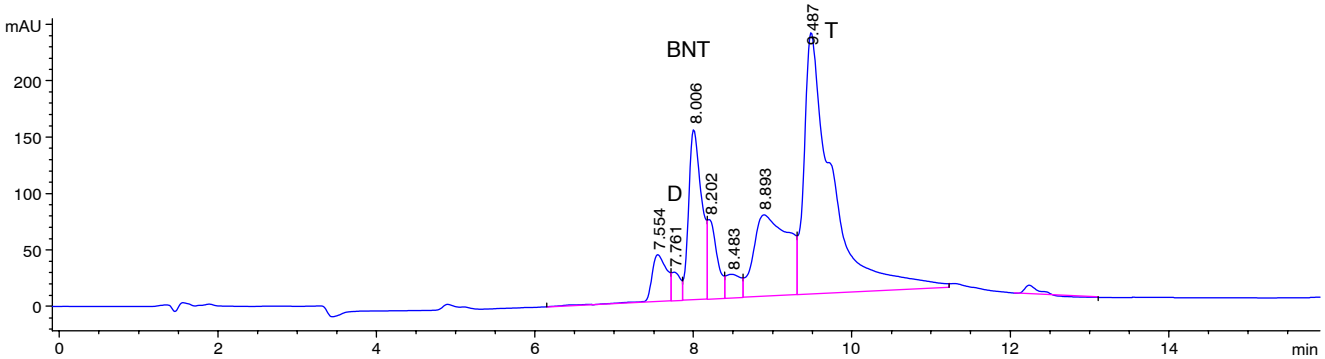
Annulohypoxylon squamulosum (Y.M. Ju, J.D. Rogers & H.M. Hsieh) Y.M. Ju, J.D. Rogers & H.M. Hsieh is likewise similar in ascospore size range, perithecial diameter and ostiolar discs diameter, but differs primarily in having a persistent reticulately cracked surface and slightly papillate ostioles with *bovei*-type discs. The type of this species [Taiwan, Taiwan Prov., I-lan Co., Fu-shan, on dead wood, 19 Aug 2001, Y.-M. Ju & H.-M. Hsieh 90081905 (HAST-holotype of *H. squamulosum*)] additionally revealed large amounts of daldinone A and traces of BNT and truncatone. It has a similar HPLC profile as the present fungus, which, however, contained no daldinone A but some unidentified compounds with characteristic truncatone-like UV-Vis spectra (Fig. 8).

Externally, *A. maeteangense* is well characterized by effused-applanate stromata with a matt, olivaceous-brown and tomentose surface strongly contrasting with the small black discs with broadly conical ostioles. The brown tomentum is progressively worn off with age and in mature stromata the ostiolar discs become less contrasted.

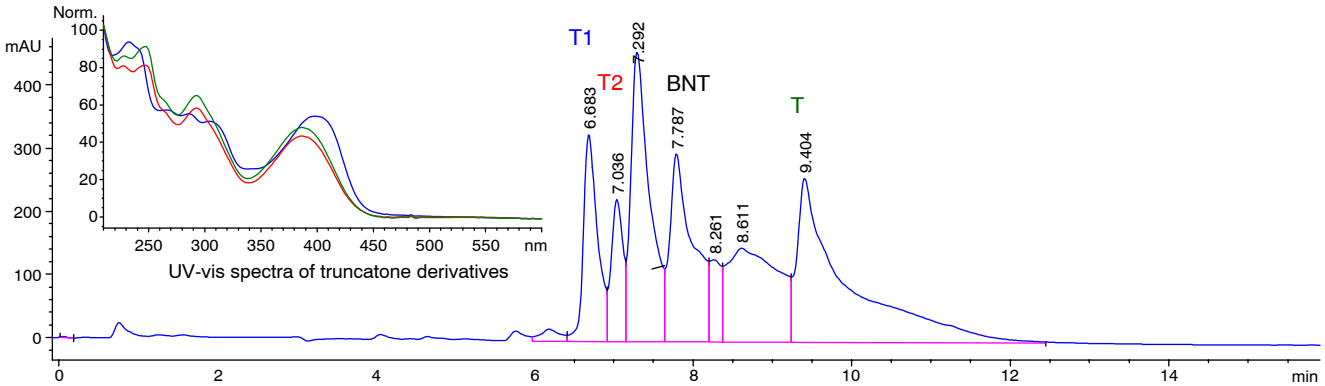
Fig. 8 HPLC-UV chromatograms of stromatal methanol extracts of several *Annulohypoxylon* spp (210 nm), and UV-vis spectra of some characteristic metabolite families



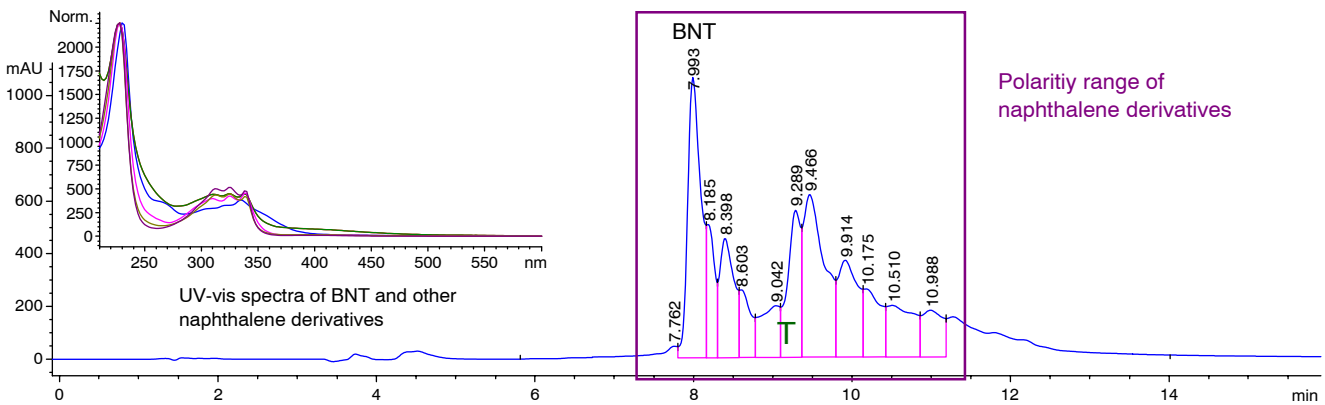
A. moriforme var. *microdiscum* JF-TH 26-02 and UV-vis spectra of cohaerins



A. moriforme var. *moriforme* JF-TH 19-03 (D = Daldinone A)



A. maeteangense JF-TH 05-01 (holotype) and UV-vis spectra of truncatone derivatives(T, T1, T2)



A. bahnphadengense (JF-TH 08-01) and UV-vis spectra of binaphthalene derivatives

Known distribution: Northern Thailand.

Habitat: Stromata on dead bark.

Specimens examined: THAILAND: Chiang Mai Province, Mae Teang District, Bahn Pha Deng, Mushroom Research Centre, N 19° 01′ 615″ E 98° 41′ 884″, 900 m, on a corticated branch, 5 Jun. 2005, J. Fournier **JF-TH 05-01** (MFLU-**holotype**); same location, 26 May 2005, J. Fournier **JF-TH 26-01** (BCC culture CBS 123835); same location, 5 Jun. 2005, J. Fournier **JF-TH 05-02**. (MFU08-1525).

Discussion

While the erection of new genera and species is based mainly on morphological and other phenetic data, in comparison with the definition of accepted *Xylariaceae* genera and species, we also wish to discuss the current situation with respect to the molecular phylogeny of *Xylariaceae*. All materials described here were found in Thailand, but the only available molecular study on hypoxyloid *Xylariaceae* (Suwannasai et al. 2005) unfortunately did not include any reference sequences derived from other studies. In any case, the authors did not accept *Annulohypoxylon*, even though the genus had already been erected at the time of publication. They used 5.8S/ITS nrDNA and only compared the species they exclusively found in Thailand with one another, except for two reference specimens from Taiwan. From their results, they found it difficult to establish a phylogeny that would justify the segregation of the species included in *Hypoxylon sensu* Ju and Rogers (1996). The new taxa they reported all belong to *Hyxpoxylon sensu* Hsieh et al. (2005) and do therefore not correspond to the fungi we have described and illustrated. In addition, Suwannasai et al. (2005) have summarised morphological data of six species of *Hypoxylon* sect. *Annulata* (i.e., *Annulohypoxylon*), including two taxa named *A.* cf. *archeri* and *A. atroroseum*, in a table. We have so far been unable to obtain the described specimens for comparison. Nonetheless, from the data presented in this table, it is not likely that one of their specimens corresponds to the new taxa described here. A concurrent preliminary molecular phylogenetic study based on similarity analyses of various different genes (Tang et al. 2009, "*Xylariaceae* sp. 1"), in which the holotype material of the new genus was included, clearly revealed that *R. terebratum* is nested inside the hypoxyloid *Xylariaceae*, with close affinities to the representatives of *Annulohypoxylon*. It is therefore likely that the inclusion of *Rostrohypoxylon* in molecular phylogenies using additional representatives of *Annulohypoxylon* would render the latter genus paraphyletic, as previously observed with other groups of the *Xylariaceae* (e.g. *Daldinia* vs. *Hypoxylon*; see Hsieh et al. 2005). However, we agree here with the authors of the latter study. They cited Brummit (2002), who convincingly explained that paraphyletic groups are inevitable in the Linnaean hierarchical classification system, to defend why they did not merge *Daldinia* with *Hypoxylon*.

A case could be made to integrate *Rostrohypoxylon* in *Annulohypoxylon*, but this would afford emending the latter, which is even now sometimes difficult to discriminate from *Hypoxylon*, based on morphological methods. A fungus like *Rostrohypoxylon* would not even match easily the concept of *Hypoxylon sensu* Ju and Rogers (1996), but could have been easily accommodated in the broad, outdated concept of *Hypoxylon sensu* Miller (1961). We think it is unwise to use molecular phylogenetic data to turn back the clock of fungal taxonomy by five decades, ignoring evidence that was meanwhile obtained from highly conclusive phenotype-based, descriptive taxonomy. Actually, several recent molecular phylogenetic studies (Peláez et al. 2008; Tang et al. 2009, and references cited therein) have shown in unison that the hypoxyloid and xylarioid *Xylariaceae* constitute two very distinct phylogenetic lineages. In scope of such data, it might be a better option to consider rearrangements at the suprageneric level, after a conclusive decision has been reached on the status of the higher taxa of the *Sordariomycetes* and the *Xylariales*. At this time, we fear that excessive lumping of genera that are well-defined by morphological traits may lead to further confusion and ultimately disguise the true diversity of the *Xylariaceae*.

Chemotaxonomic data proved to be informative as a means of "taxonomic quality control" at various different levels (Stadler and Hellwig 2005) in other taxa of *Xylariaceae* and may be useful in other families (Zhang et al. 2009). For *Rostrohypoxylon*, the limited evidence so far obtained on two specimens and a single culture appears to be insignificant, but this must be regarded in a broader context.

When a large number of hypoxyloid *Xylariaceae* was studied for the occurrence of characteristic metabolites, the results did not disagree with molecular data, thus proving phylogenetically informative (Bitzer et al. 2008; Stadler et al. 2008a). A comparison of such data revealed significant deviations of *Rostrohypoxylon* to *Annulohypoxylon* and other accepted genera. We admit that this evidence is so far mainly based on lack of characteristic extrolites and remains to be validated by identification of the characteristic unknown compounds that occur in *Rostrohypoxylon* and comparison of their potential biogenetic pathways.

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